Compass for Simple Western User Guide

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Compass for Simple Western User Guide

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Launching Compass for Simple Western

To open Compass for Simple Western, double-click the icon on the computer desktop.

Compass for Simple Western Overview

Compass for Simple Western has three main screens:

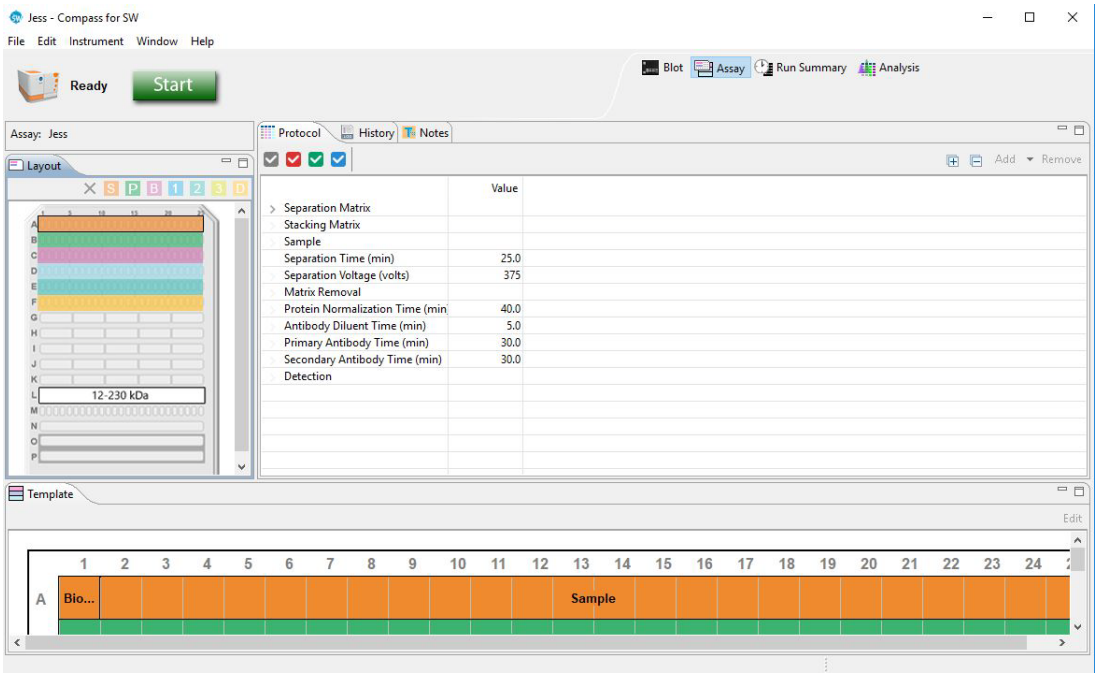
- **Assay** - You'll create and review your assay.
- **Run Summary** - Check out the status of your run.
- **Analysis** - Take a look at the data from your experiment.

Changing the Screen View

To move between the Assay, Run Summary and Analysis screens, just click the button in the screen tab located in the upper right corner of the main window.

Assay Screen

The Assay screen is used to create, view, and edit assays. You can assign well locations for assay plate reagents, modify assay protocol steps, enter assay notes and add annotations for individual wells on the assay plate.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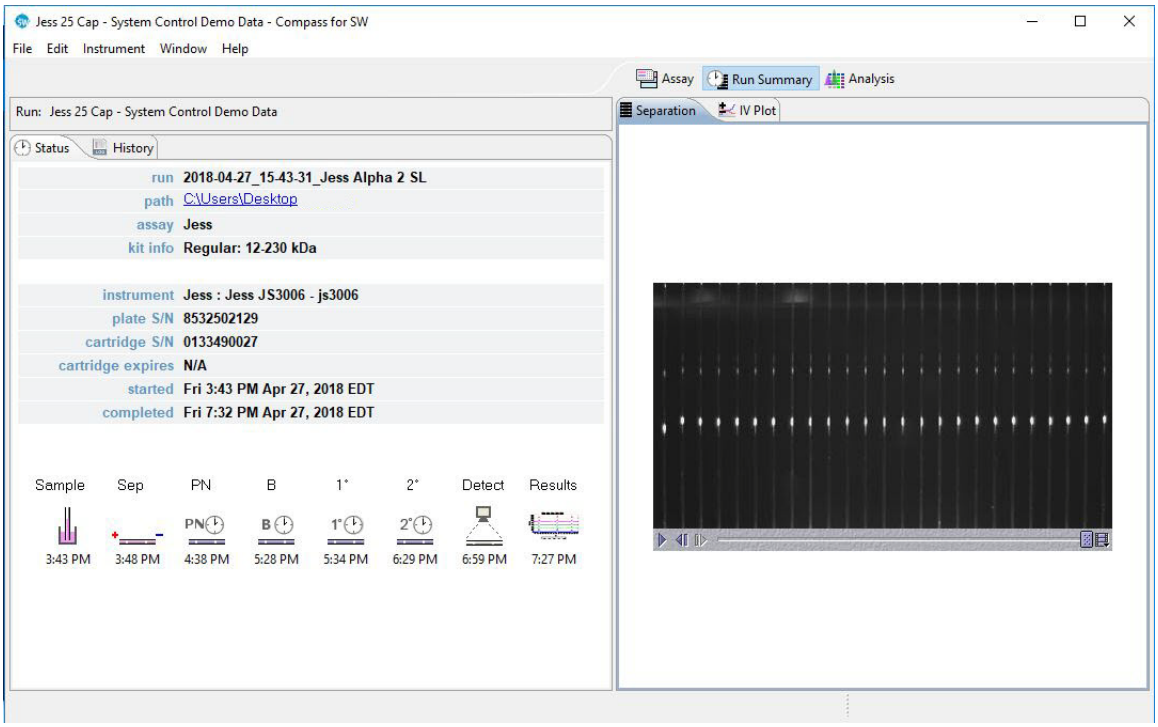
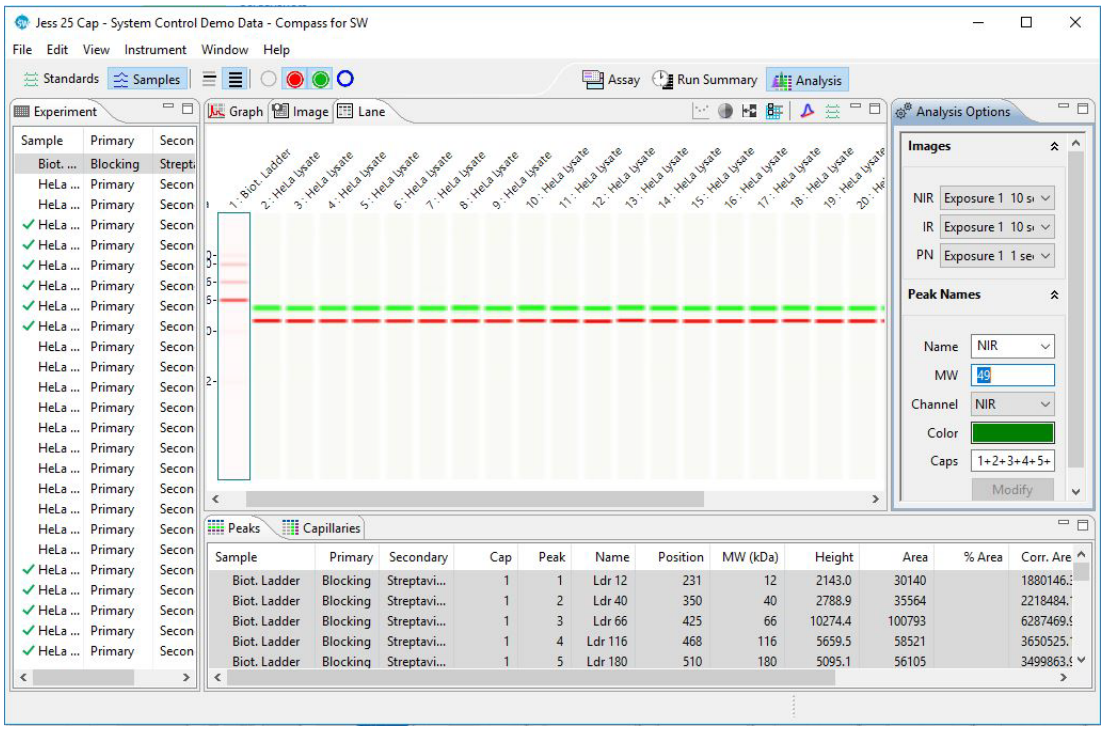
Run Summary Screen

The Run Summary screen is used to monitor status of a run in progress, watch movies of the separation in the capillaries, and view current and voltage plots for each run.



Analysis Screen

The Analysis screen is used to view data from your assay, including the graph view (electropherograms), lane view (shown below), capillary images, and a table with your results. You can also analyze your data here after the run is finished.



Screen Panes

Assay, Run Summary and Analysis screens all have multiple screen panes that let you view the individual components of a run, assay or data file. Each pane has a labeled tab and a unique icon. We'll describe panes specific to each screen later in the individual screen sections.

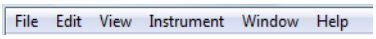
The active pane in a screen is blue. To view a pane, click in the pane or on its tab. The example below shows panes in the Analysis screen, and the Lane pane is active:

Title Bar

In the title bar you will see the run file name and the icons that allow the main Compass for Simple Western window to be minimized, maximized or closed.

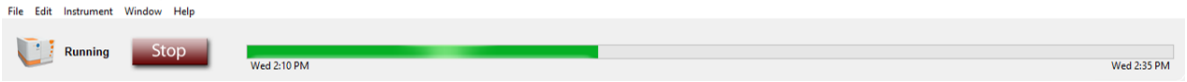
Main Menu

Access to various software, instrument and screen operations is available through the main menu. More details on menu commands can be found in "Software Menus" on page 7.



Instrument Status Bar

The instrument status bar is used to start runs and cleaning protocols, relay system status and show run progress. More details on instrument control and status can be found in Chapter 8, *"Controlling Jess, Wes, Sally Sue and Peggy Sue"*.



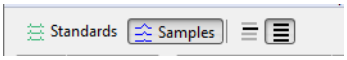
NOTE: You will only see the instrument status bar when Compass for Simple Western is connected to an instrument. There is no status bar on computer workstations that you're only using for data analysis.

Screen Tab

The screen tab lets you move between Assay, Run Summary or Analysis screens and is located in the upper right corner of the main window. Just click a button to view a screen.

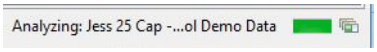
View Bar

The view bar is only displayed in the Analysis screen as part of the main menu bar and allows you to switch between displaying sample chemiluminescent, fluorescent or protein normalization data, fluorescent standards or capillary registration information, data for a single capillary or all capillaries in the run, or grouped capillary data. View bar options are detailed in "Switching Between Sample, Standards and Registration Data Views" on page 202 for size assays or page 349 for charge assays, and "Using Groups" on page 239 for size assays or page 375 for charge assays.



Compass Status Bar

The status bar is located in the lower right corner of the main window. It displays active software processes and their progress.

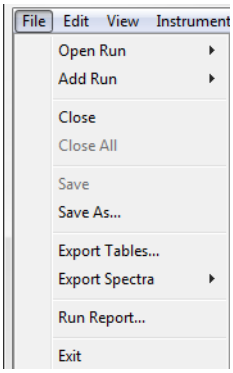


Software Menus

A brief description of the software menus in the main menu are described in this section. Not all menus are available in every screen, and menu commands change depending on what screen is active. The menus and commands available for each screen will be detailed in the individual screen sections.

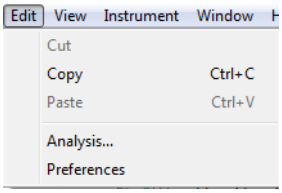
File Menu

The File menu contains basic file commands.



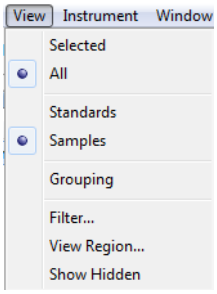
Edit Menu

The Edit menu contains basic editing commands, analysis and preferences options. Specific details on preferences are described in Chapter 11, *"Setting Your Preferences"*.



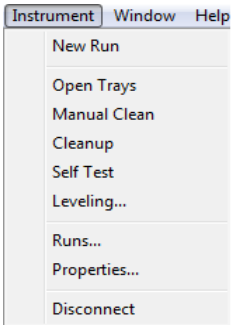
View Menu

The View menu can only be seen in the Analysis screen, and allows you to change how your data is displayed.



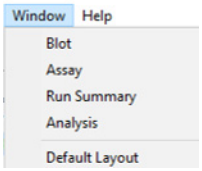
Instrument Menu

The Instrument menu is only available when Compass for Simple Western is connected to directly to your instrument. Instrument control options are explained in Chapter 8, *"Controlling Jess, Wes, Sally Sue and Peggy Sue"*.



Window Menu

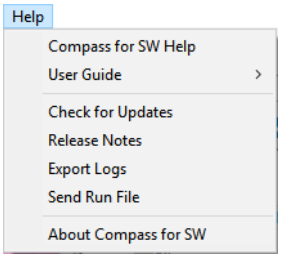
The Window menu allows you to switch between Blot, Assay, Run Summary or Analysis screens, and restore screens to the Compass for Simple Western default layout.



- **Blot** - Displays the Blot screen where you can view and enhance acquired blotting membrane images.
- **Assay** - Displays the Assay screen where you can create, view, and edit assays.
- **Run Summary** - Displays the Run Summary screen which tells you the status of a run in progress.
- **Analysis** - Displays the Analysis screen which is used to view sample electropherograms, lane data and results.
- **Default Layout** - Restores the individual panes in the current screen back to their default size and location.

Help Menu

The Help menu provides access to Help, software updates, release notes and other software information.



- **Compass for SW Help** - Displays the Compass for Simple Western User Guide.
- **User Guide** - Displays a menu of Simple Western instrument User Guides.
- **Check for Updates** - Automatically checks to see if a new version of Compass for Simple Western is available.
- **Release Notes** - Displays Compass for Simple Western release notes for the current and prior versions.
- **Export Logs** - In the event a technical support issue arises, the Export Logs option lets you gather instrument-specific logs into a .zip file and send it directly to ProteinSimple Technical Support. If you don't have an internet connection, you can also save the .zip file to the desktop.
- **Send Run File** - Lets you send a run file to ProteinSimple Technical Support directly from Compass for Simple Western.
- **About Compass for SW** - Displays the Compass for Simple Western software version and build information.

Changing the Compass for Simple Western Main Window Layout

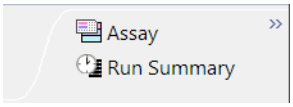
You can easily resize the Compass for Simple Western main window and the individual panes in each screen. Screen panes can also be moved outside of the main window.

Resizing the Main Compass for Simple Western Window

To resize the main window, roll the mouse over a corner or border until the sizing arrow appears. Then just click and drag to resize.

Resizing the Screen Tab

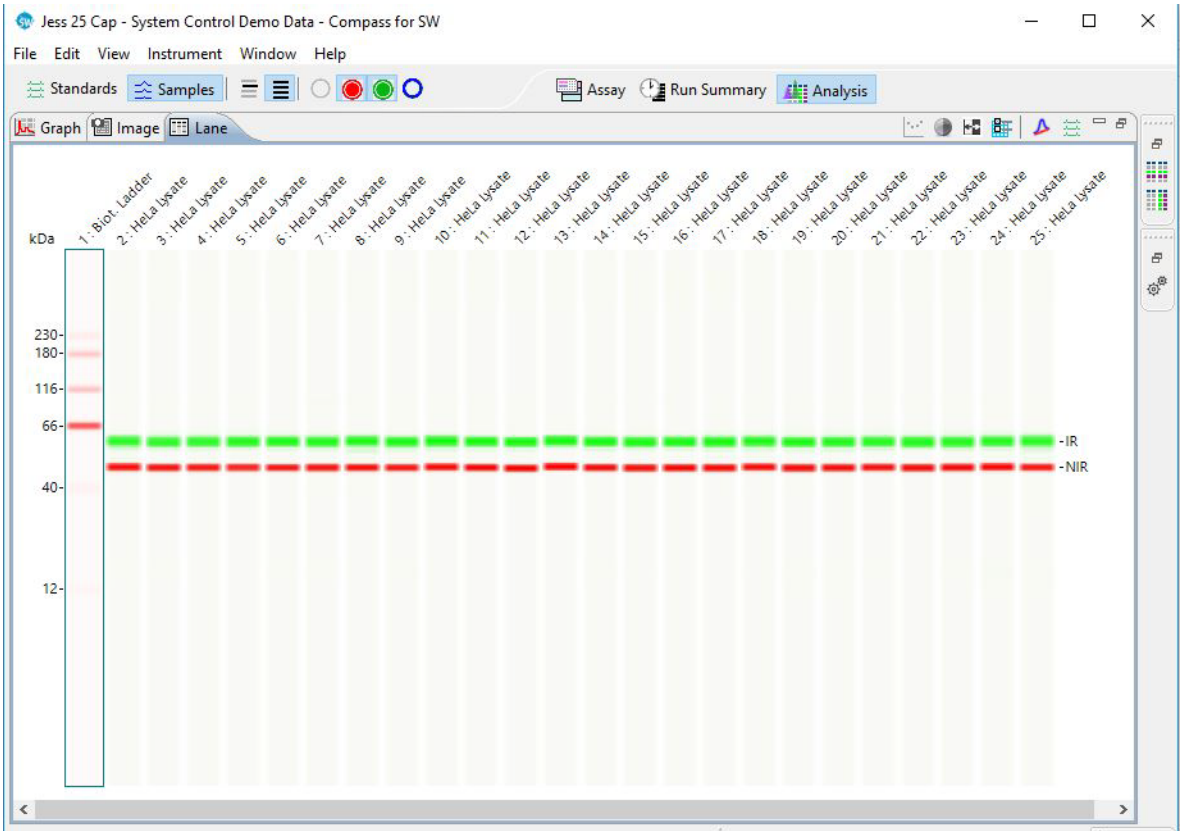
The screen tab can be sized to show all or just some of the screen buttons. To resize, roll the mouse over the left edge of the tab until the sizing arrow appears, then click and drag to resize. If a screen button is hidden, a double arrow will display in the tab. Click to display and select the hidden screen.



Resizing Screen Panes

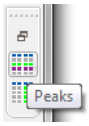
- **To resize a pane** - Roll the mouse over the pane border until the sizing arrow appears. Then just click and drag to resize.

- **To maximize a pane** - Click the maximize button in the upper right corner or double-click the tab. The other panes in the screen will automatically minimize to pane bars in the task area along the window border.

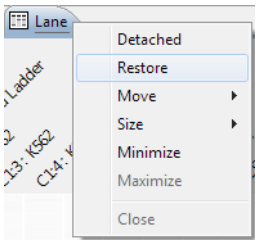


- **To restore all minimized panes** - Click **Restore** on the minimized pane bar.

- **To restore only one minimized pane** - Click the pane icon on the minimized pane bar.



- **To restore a maximized pane to its original size** - Double-click the tab or right click the tab and click **Restore**.



- **To restore all panes to their original sizes -** Select **Window** in the main menu and click **Default Layout**.

Changing the Location of Screen Panes

Panes can be moved to different locations within a screen.

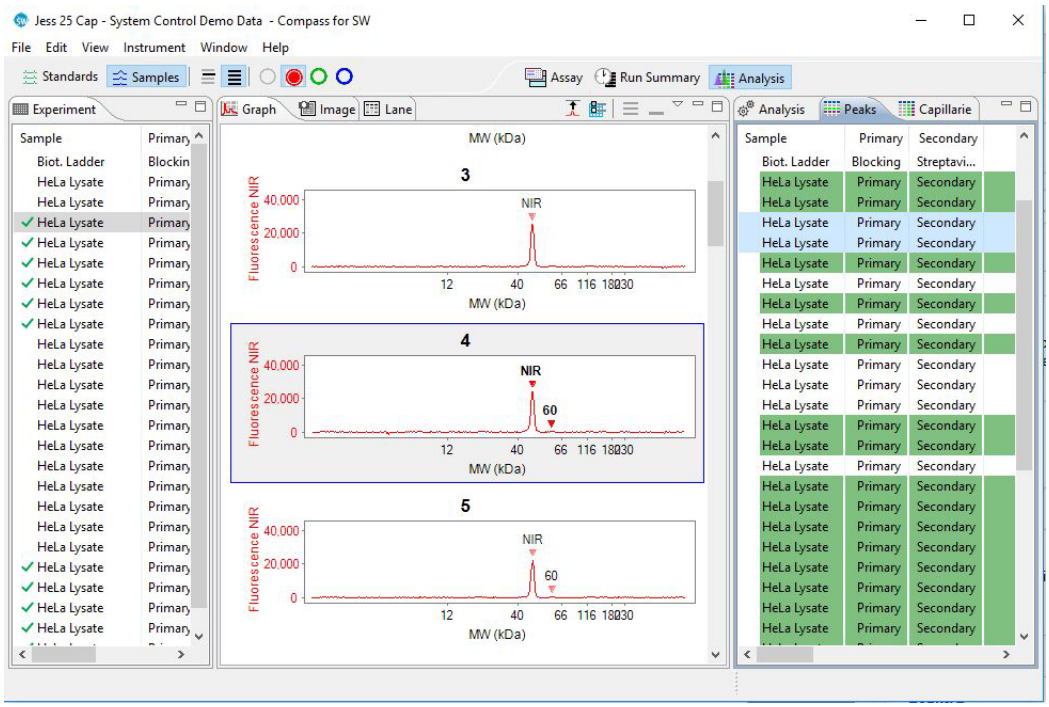
- **To move a pane** - Click on its tab and drag it to the new location. As the pane is moved, area guides will display to assist you in choosing a drop location.

Area guides with a black arrow let you know that if the pane is dropped at that location, it will be resized and relocated as an individual pane in that area of the screen.

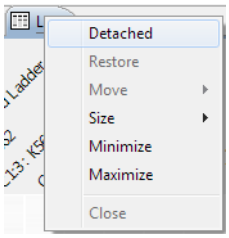
Area guides with a folder let you know that if the pane is dropped at that location, it will be added as a new tab in an area with one or more pane tabs.

Area guides with a window let you know that if the pane is dropped at that location, it will become a separate window outside the Compass for Simple Western main window.

The following figure shows the Analysis screen after moving the Graph pane.



- **To detach a pane from the main window -** Click on its tab and drag it outside the main Compass for Simple Western window or right click the tab and click **Detached**.



- **To move a detached pane back inside the main window -** Right click the tab and deselect **Detached**.
- **To restore all panes to their original locations -** Select **Window** in the main menu and click **Default Layout**.

Restoring the Main Window to the Default Layout

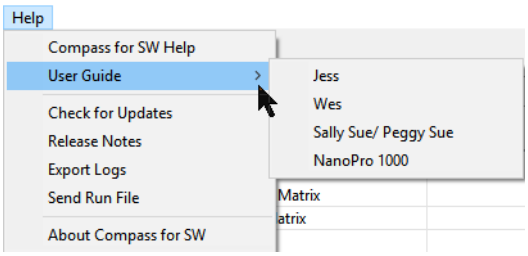
To restore screen pane sizes and locations to the original Compass for Simple Western layout, select **Window** from the main menu and click **Default Layout**.

Software Help

Select **Help** and click **Compass for SW Help** to view the Compass for Simple Western User Guide. If the computer you're using has an internet connection, the latest online version of the User Guide PDF will display. When an internet connection isn't available, the User Guide PDF shipped with the original installer for the software will open instead.

Viewing Instrument User Guides

Select **Help** and click **User Guide** to display a menu of Simple Western instrument User Guides. If the computer you're using has an internet connection, the latest online version of the User Guide PDF will display. When an internet connection isn't available, the User Guides PDF shipped with the original installer for the software will open instead. The latest versions of all User Guides are also available online at https://www.proteinsimple.com/technical_library.html.

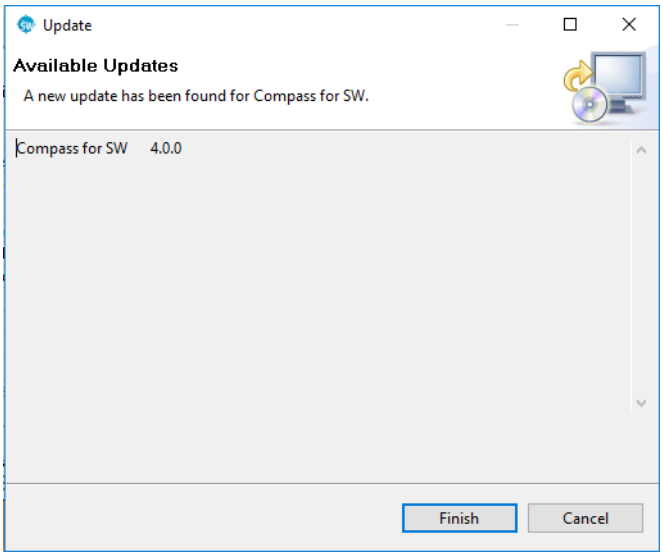


Checking for and Installing New Versions of Compass for Simple Western

Compass for Simple Western can automatically check to see if a newer version of software is available. To do this:

1. Make sure the computer being used has an active internet connection.

2. Select **Help** and click **Check for Updates**. If an update is found, the following screen will display:

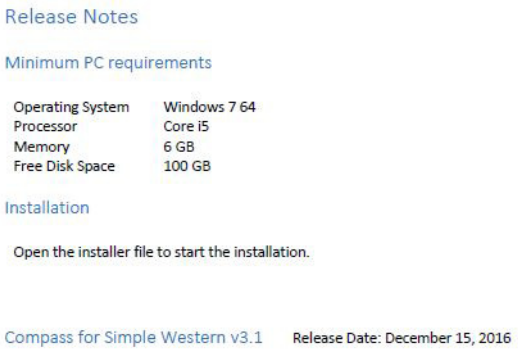


3. Click **Finish** to start the download and install the update.
4. Follow the on-screen instructions to complete the software installation.
5. Reboot the computer before using the new version of Compass for Simple Western.

Viewing Release Notes

Select **Help** and click **Release Notes** to view feature updates and bug fixes for new and past versions of Compass for Simple Western. If the computer you're using has an internet connection, the latest online version of the release notes PDF will display. When an internet connection isn't available, the release notes PDF

shipped with the original installer for the software will open instead. We recommend you review these notes whenever a software update is installed. The latest release notes are also available online at https://www.proteinsimple.com/technical_library.html



Release Notes

Minimum PC requirements

Operating System	Windows 7 64
Processor	Core i5
Memory	6 GB
Free Disk Space	100 GB

Installation

Open the installer file to start the installation.

Compass for Simple Western v3.1 Release Date: December 15, 2016

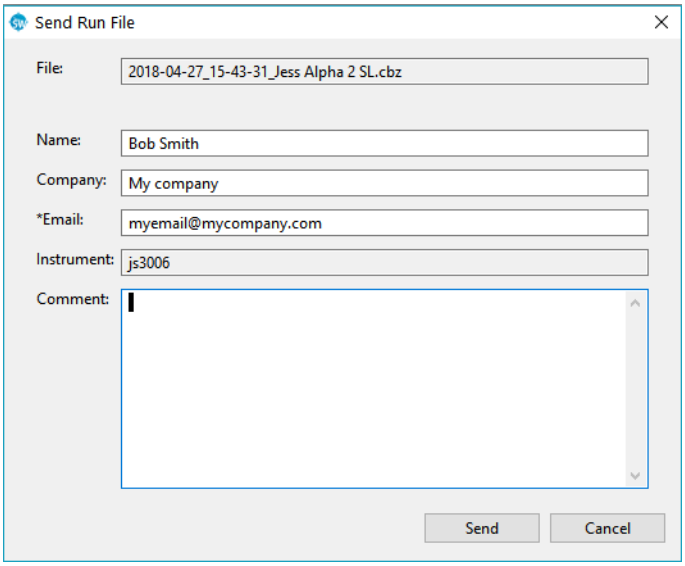
Exporting the Software Log

Select **Help** and click **Export Logs** to view the software log file.

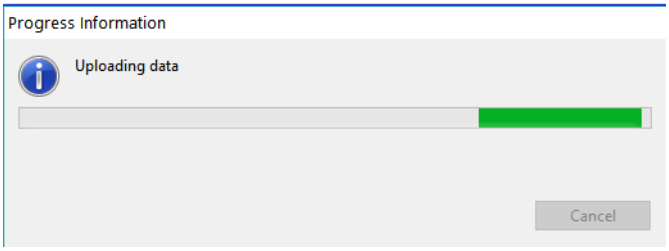
Sending Run Files to Technical Support

If the computer you're using has an internet connection, Compass for Simple Western can zip and send a run file directly to ProteinSimple Technical Support.

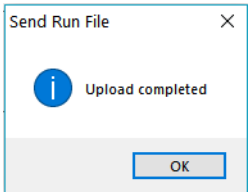
1. Open the run file you'd like to send in the Analysis screen.
2. Select **Help** and click **Send Run File**:
3. Enter your **Name**, **Company** and **E-mail** address, and any details in the **Comments** section:



4. Click **Send**. The progress window displays:

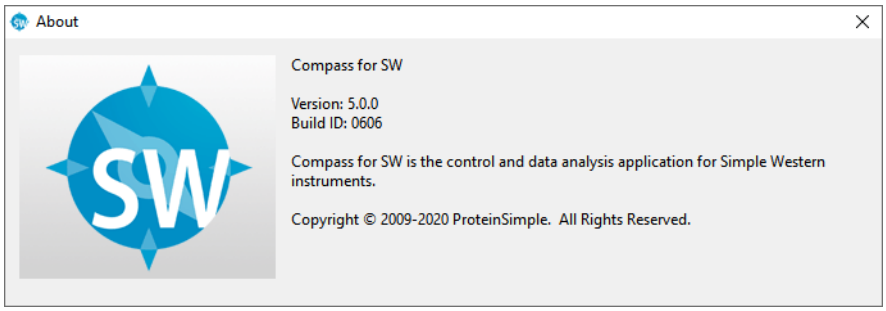


5. When the file upload is complete, the following message displays. Click **OK**.



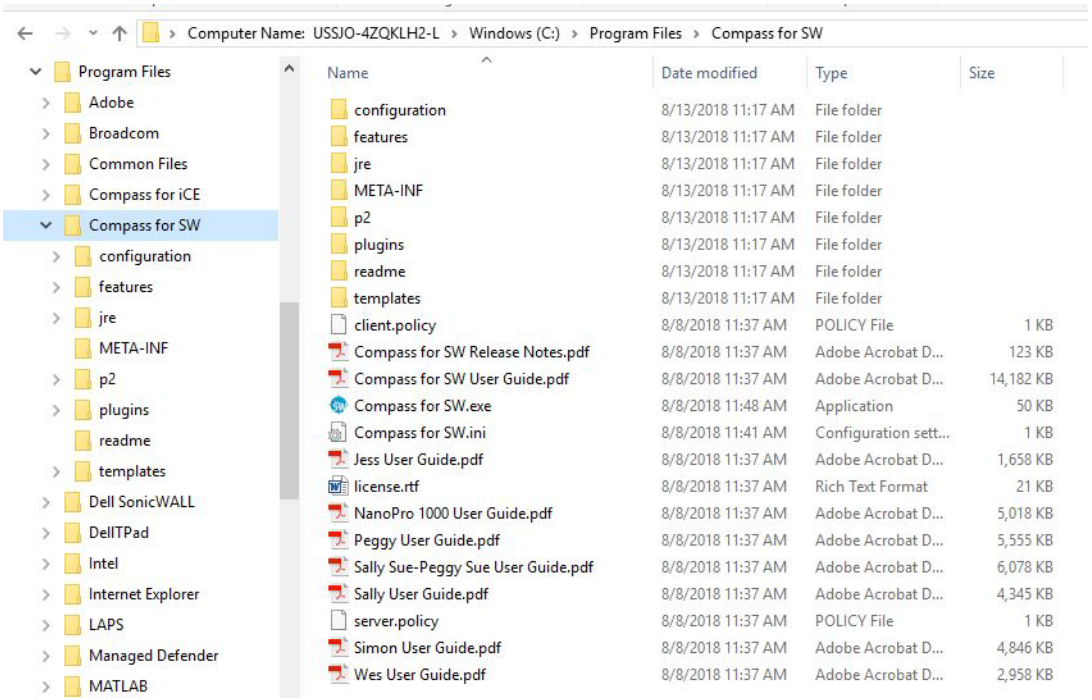
Compass for Simple Western Version Information

Select **Help** and click **About Compass for SW** to view the software version and build number information.

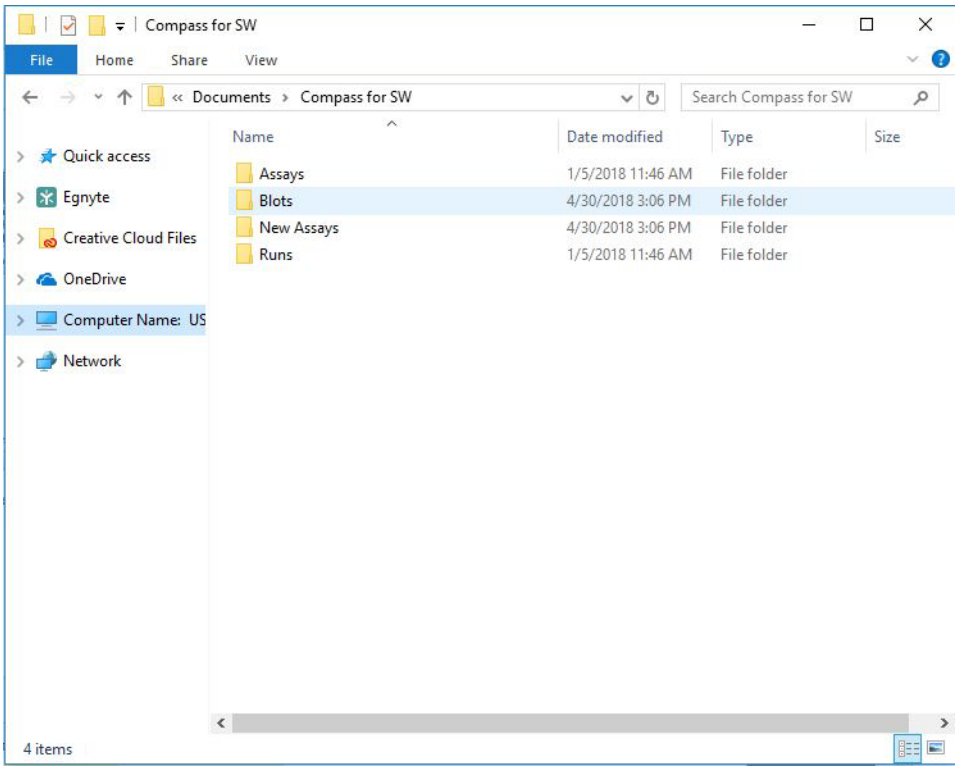


Directory and File Information

The main Compass for Simple Western directory is located in the **Program Files** folder, and also contains PDF files of the Jess, Wes and Sally Sue/Peggy Sue User Guides.



Compass for Simple Western assay and run files are located in the **Documents** folder in the User directory on your computer:



- **Assays Folder** - Contains all assay files that you've saved.
- **New Assays Folder** - Contains Simple Western assay template files.
- **Runs Folder** - Contains all run files. Run data is automatically written to this folder.
- **Blots Folder** - Contains all Western blot image files that you've saved.

NOTE: When a Compass for Simple Western software update is performed, the template assays in the New Assays folder are overwritten. If you have customized these assays, we recommend saving them in a unique subfolder prior to updating the software, then transferring them back to the New Assays folder after the update to avoid losing your assay customizations.

File Types

The following file types are used by Compass for Simple Western:

- **Assay Files** - Use an *.assay file extension.
- **Run Files** - Use a *.cbz file extension. The default file format for run files is Date_Time_AssayName. An example run file name would be 2012-09-28_18-50-53_Simple Western.cbz.
- **Blot Files** - Use a *.btz file extension.
- **Protocol Files** - Exported protocol files use a *.protocol file extension.
- **Template Files -** Exported template files use a *.template file extension.
- **Analysis Settings Files** - Exported analysis settings files use a *.settings file extension.

Chapter 2:

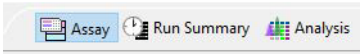
Size Assays

Chapter Overview

- Assay Screen Overview
- Reagent Color Coding
- Opening an Assay
- Immunoassays: Creating a New Assay
- Total Protein Assays: Creating a New Assay
- Protein Normalization on Jess: Creating a New Assay
- RePlex Assays on Jess: Creating a New Assay
- Making Changes to an Existing Assay
- Switching Between Open Assays
- Creating a Template Assay
- Viewing and Changing the Detection Exposures
- Copying Protocols and Templates
- Printing Protocols and Templates
- Importing and Exporting Protocols and Templates

Assay Screen Overview

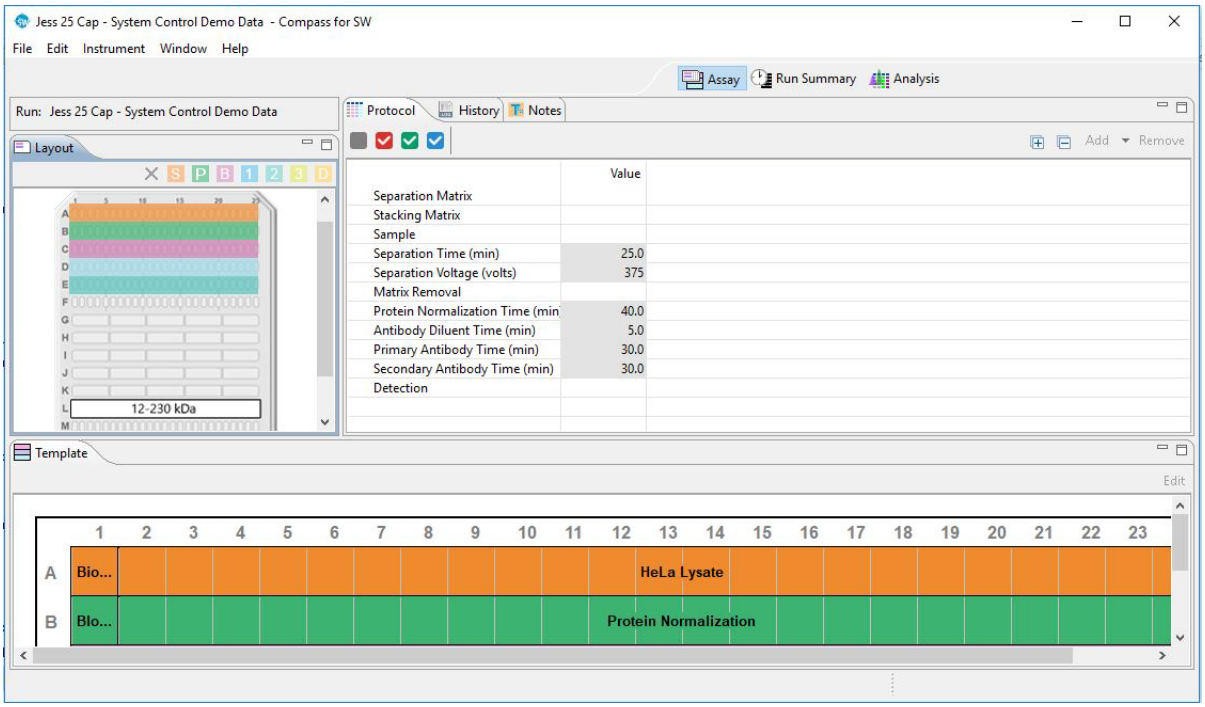
The Assay screen is used to create, view, and edit assays. To access this screen, click **Assay** in the screen tab:



Assay Screen Panes

The Assay screen has four panes:

- **Layout** - Displays a map of the assay plate and shows where assay reagents will be located.
- **Protocol** - Lists individual assay protocol steps and parameters that Jess, Wes, Sally Sue or Peggy Sue will execute for each of the capillaries.
- **Notes** - Lets you enter specific assay information that is saved with the assay and can be used for future reference.
- **Template** - Enter annotations for the individual well and row reagents in the assay plate.



Software Menus Active in the Assay Screen

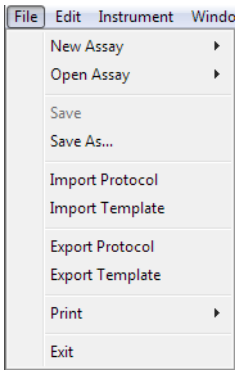
You can use the following software menus:

- File
- Edit
- Instrument (when Compass for Simple Western is connected to Jess, Wes, Sally Sue or Peggy Sue)
- Window
- Help

The File and Edit menu options specific to the Assay screen are described next.

File Menu

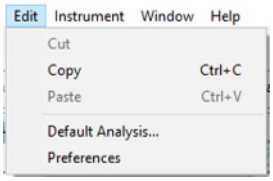
The following File menu options are active:



- **New Assay** - Creates a new assay from a starter template.
- **Open Assay** - Opens an existing assay.
- **Save** - Saves the open assay.
- **Save As** - Saves the open assay under a different file name.
- **Import Protocol** - Imports a saved protocol file into an assay.
- **Import Template** - Imports a saved template file into an assay.
- **Export Protocol** - Exports the current protocol file for future use.
- **Export Template** - Exports the current template file for future use.
- **Print** - Prints the information in the Protocol or Template panes.
- **Exit** - Closes Compass for Simple Western.

Edit Menu

The following Edit menu options are active:

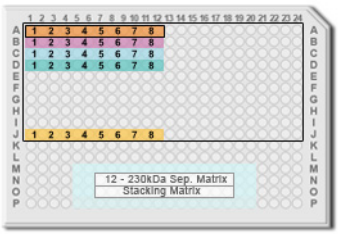


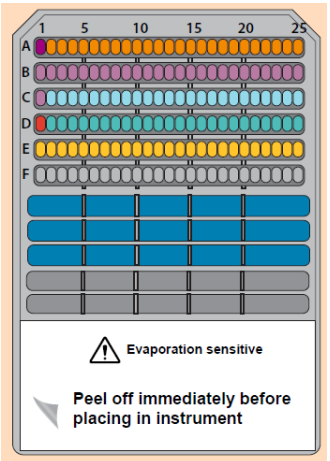
- **Copy** - Copies the information in the Protocol or Template panes into other documents.
- **Default Analysis** - Displays the default settings that will be used to analyze the run data generated with an assay.
- **Preferences** - Set and save your preferences for data export, plot colors in the graph and Twitter settings. See Chapter 11, *"Setting Your Preferences"* for more information.

Reagent Color Coding

Immunoassays

The Assay screen uses color coding to identify various assay reagents in all panes. The following example shows the Layout pane for chemiluminescence assays on the left and fluorescence assays (Jess only) on the right:



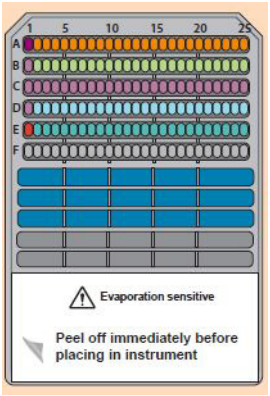


- **Orange** - Samples and Ladder
- **Magenta** - Antibody Diluent (2 or Milk-Free)
- **Light Teal** - Primary antibody
- **Teal** - Secondary HRP conjugate
- **Gold** - Luminol/Peroxide mix
- **Light Blue** - Water dispensed around Separation and Stacking Matrices

- **Orange** - Samples and Ladder
- **Magenta** - Antibody Diluent (2 or Milk-Free)
- **Light Teal** - Primary antibody
- **Red** - Streptavidin-NIR (fluorescence only) or HRP (chemiluminescence only)
- **Teal** - Secondary antibody
- **Gold** - Luminol/Peroxide mix (chemiluminescence only)

Protein Normalization on Jess

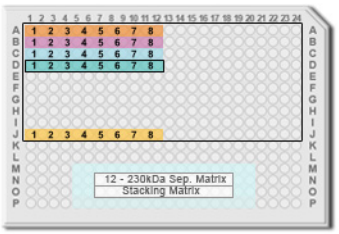
The Assay screen uses color coding to identify protein normalization reagent (green), which can be included with every size assay plate setup. There's no need to run protein normalization as a separate assay.



- **Orange** - Samples and Ladder
- **Green** - Protein Normalization reagent
- **Magenta** - Antibody Diluent (2 or Milk-Free)
- **Light Teal** - Primary antibody
- **Red** - Streptavidin-NIR
- **Teal** - Secondary NIR/IR Conjugate

Total Protein Assays

The Assay screen uses color coding to identify various assay reagents in all panes. The Layout pane is shown in the following example:



- **Orange** - Samples and Ladder
- **Magenta** - Labeling reagent
- **Light Teal** - Antibody Diluent
- **Teal** - Total Protein Streptavidin-HRP
- **Gold** - Luminol/Peroxide mix
- **No color coding** - Separation Matrix (clearly designated)

- **No color coding** - Stacking Matrix (clearly designated)
- **Light Blue** - Water dispensed around Separation and Stacking Matrices

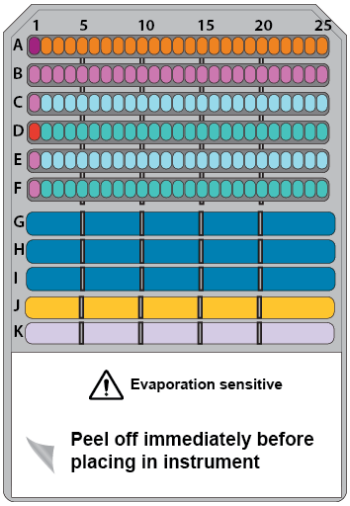
RePlex Assays on Jess

Jess lets you run two immunoassays or an immunoassay with Total Protein in a single run. This is done in two different probing cycles. These assay combinations are currently validated on Jess:

- Chemiluminescence + chemiluminescence
- Chemiluminescence + Total Protein
- Chemiluminescence/NIR fluorescence + Total Protein
- NIR fluorescence+ NIR fluorescence
- NIR fluorescence + chemiluminescence

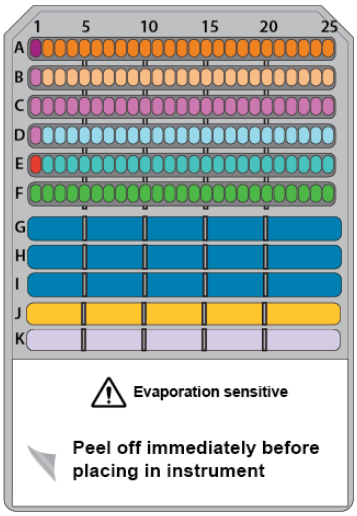
The Assay screen uses color coding to identify various assay reagents in all panes. The following examples show the Layout pane for a few of the RePlex Assay combinations listed above:

Chemiluminescence + Chemiluminescence



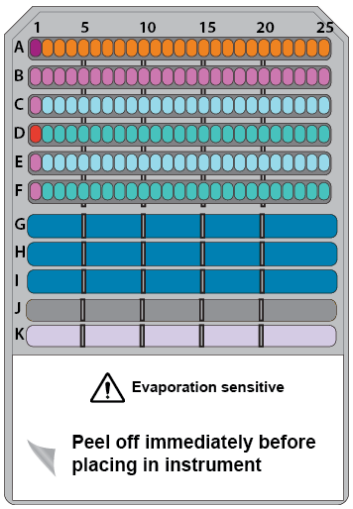
- **Orange** - Ladder and samples
- **Magenta** - Antibody Diluent (2 or Milk-Free)
- **Light Teal** - Primary antibody for Probe 1
- **Red -** Streptavidin-HRP
- **Teal** - Secondary antibody for Probe 1
- **Light Teal** - Primary antibody for Probe 2
- **Teal** - Secondary antibody for Probe 2
- **Gold** - Luminol/Peroxide mix
- **Purple** - RePlex reagent mix

Chemiluminescence/NIR fluorescence + Total Protein



- **Orange** - Ladder and samples
- **Peach -** Total Protein labeling reagent
- **Magenta** - Antibody Diluent (2 or Milk-Free)
- **Light Teal** - Primary antibody for Probe 1
- **Red -** Streptavidin-NIR
- **Teal** - Secondary antibody for Probe 1
- **Green** - Total Protein Streptavidin-HRP for Probe 2
- **Gold** - Luminol/Peroxide mix
- **Purple** - RePlex reagent mix

NIR fluorescence + NIR fluorescence

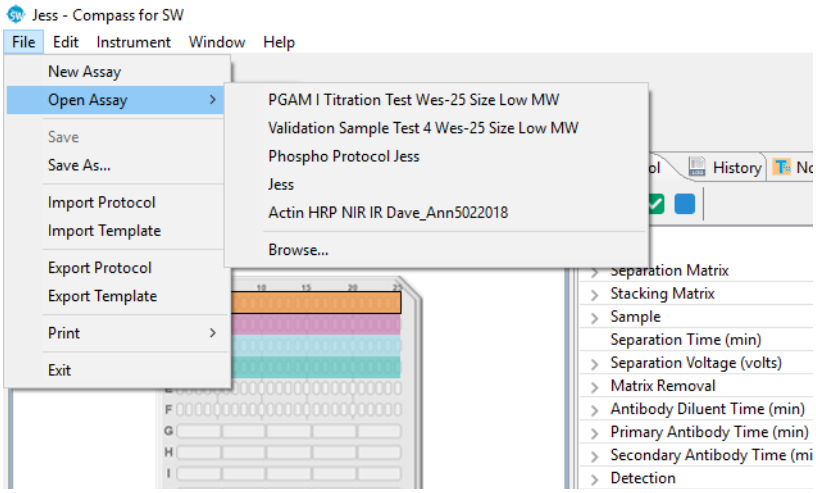


- **Orange** - Ladder and samples
- **Magenta** - Antibody Diluent (2 or Milk-Free)
- **Light Teal** - Primary antibody for Probe 1
- **Red -** Streptavidin-NIR
- **Teal** - Secondary antibody for Probe 1
- **Light Teal** - Primary antibody for Probe 2
- **Teal** - Secondary antibody for Probe 2
- **Purple** - RePlex reagent mix

Opening an Assay

To open an existing assay:

1. Select **File** in the main menu and click **Open Assay**.



2. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different assay.
3. To make changes to the assay, follow the instructions under "Immunoassays: Creating a New Assay" on page 32 for changing the assay parameters. Select **File** from the main menu and click **Save**.

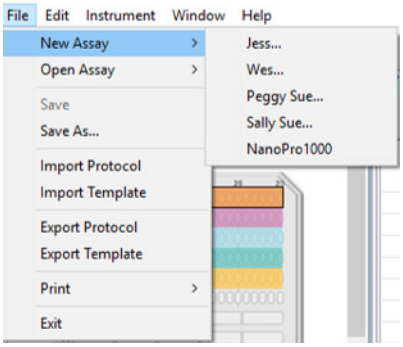
Immunoassays: Creating a New Assay

To create a new assay, we recommend using one of the template assays and making any necessary modifications from there.

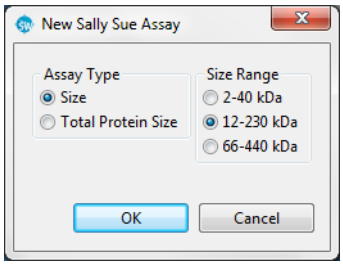
NOTE: This section provides details on how to create a standard immunoassay on Jess, Wes, Peggy Sue and Sally Sue. To create a RePlex Assay on Jess that includes an immunoassay, see "RePlex Assays on Jess: Creating a New Assay" on page 60.

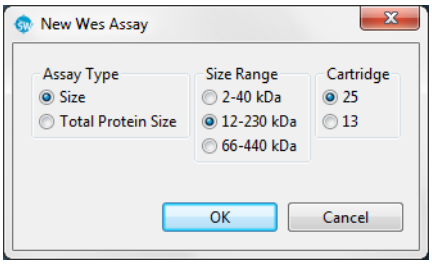
Step 1 - Open a Template Assay

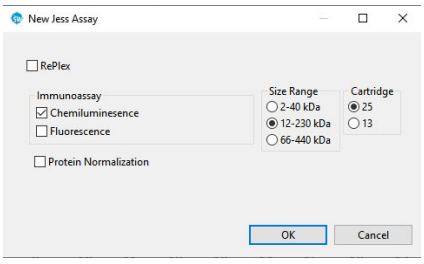
1. Select **File** in the main menu and click **New Assay**:



2. Select the template assay for your instrument by selecting the instrument-appropriate **Assay Type** or **Immunoassay**, **Size Range** and **Cartridge Type** (if running Jess or Wes), or choose **Open Assay** to select from the menu of saved assays.







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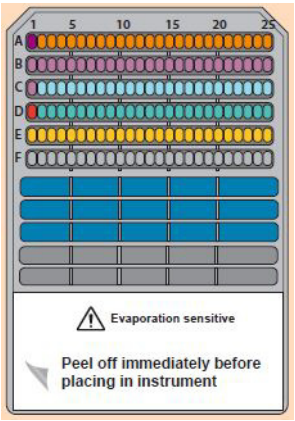
*NOTE: If you make changes to the assay, such as defining the wells in the plate, be sure to select **File** and **Save** before proceeding.*

Step 2 - Assign Assay Plate Reagents (Optional)

1. Click on the Layout tab. Displayed are the default row locations where each reagent should be placed on the assay plate.

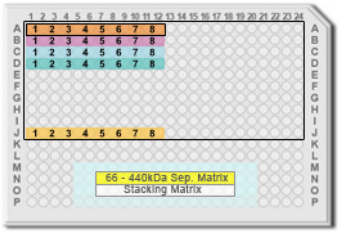
NOTE: For Sally Sue and Peggy Sue, up to 96 different samples or conditions (lysates, proteins, antibodies or incubation times) can be assayed using the full eight cycles to accommodate experimental needs.

Jess or Wes plate layout:



- **Row A** - Biotinylated Ladder (A1) and Sample (A2-25)
- **Row B** - Blocking (Milk-Free Antibody Diluent) or Protein Normalization Reagent
- **Row C** - Milk-Free Antibody Diluent (C1) and Primary antibody (C2-C25)
- **Row D** - Streptavidin-HRP or NIR (D1) and Secondary conjugate (D2-D25)
- **Row E** - Luminol-S/Peroxide mix (chemiluminescence only)
- **Row F** - Empty

Sally Sue/Peggy Sue plate layout:



- **Row A** - Biotinylated Ladder (A1) and Sample (A2-12)
- **Row B** - Blocking (Antibody Diluent)
- **Row C** - Antibody Diluent (C1) and Primary antibody (C2-C12)
- **Row D** - Streptavidin-HRP (D1) and Secondary HRP conjugate (D2-D12)
- **Row J** - Luminol-S/Peroxide mix
- **Row M** - Water (M5-M20)
- **Row N** - Water (N5-N6 and N19-N20) and Separation Matrix (N7-N18)
- **Row O** - Water (O5-O6 and O19-O20) and Stacking Matrix (O7-O18)
- **Row P** - Water (P5-P20)

NOTE: For details on sample, reagent and assay plate preparation, please refer to the product insert provided with the Simple Western kits.

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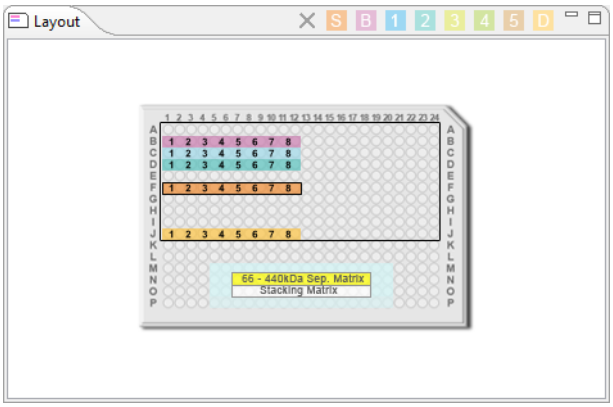
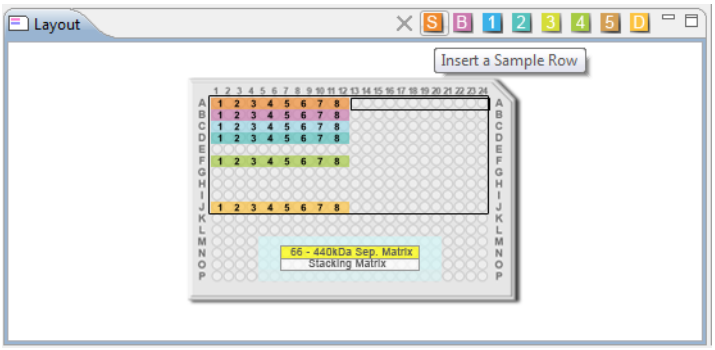
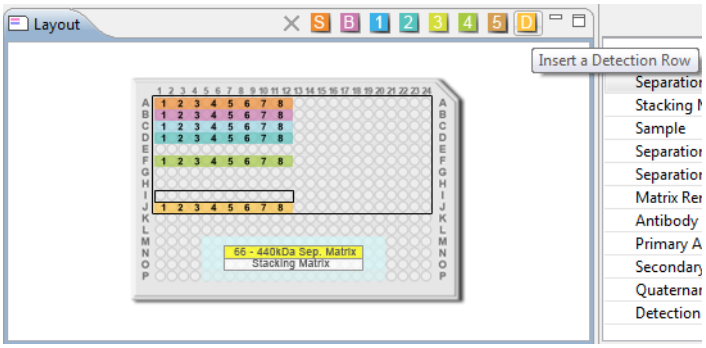
2. If needed, well assignments can be modified as described below. Any row assignments changed in the **Layout** pane are updated in the Protocol pane automatically.
   - **To move a reagent row to another location** - Click the row in the **Layout** pane, then drag and drop it on the new location. The row from which it was moved will be reassigned as empty.



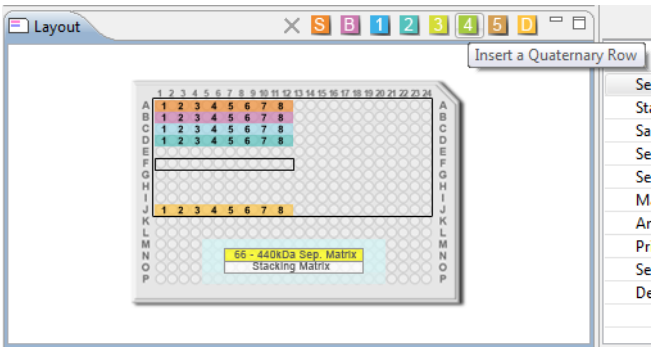
   - **To insert a sample row -** Click an empty row or the row below where the new sample row should be inserted, then click the **S** icon (Insert a sample row) in the **Layout** pane toolbar. A new sample row will be added in the empty row or inserted above the selected row.



- **To insert a detection row -** Click an empty row or the row below where the new detection row should be inserted, then click the **D** icon (Insert a detection row) in the **Layout** pane toolbar. A new detection row will be added in the empty row or inserted above the selected row.



- **To insert a fourth or fifth incubation reagent** - Click an empty row or the row below where the new incubation reagent should be inserted, then click the **4** icon (Insert a quaternary row) in the **Layout** pane toolbar. A new incubation reagent row will be added in the empty row or inserted above the selected row.

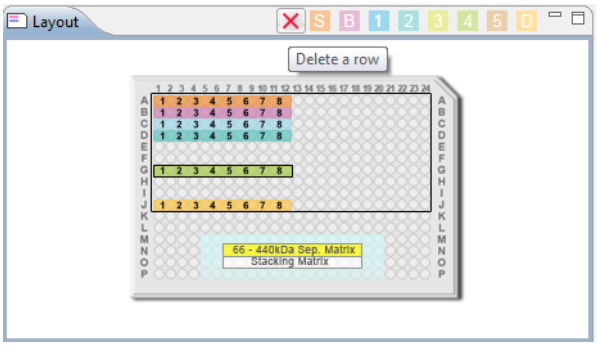


A fifth incubation reagent can now be added by repeating the above and clicking the **5** icon (Insert a Quinary row) instead.

NOTES:
When using the fourth and fifth incubation steps, only five cycles can typically be performed per run as more water is needed per cycle for the additional wash steps.

Row J is the last row assignment that can be used on the assay plate. New, sample, incubation or luminol rows cannot be inserted if row J already has an assigned reagent. To insert a row in this case, you must first move the contents of row J to another area on the plate.

- **To delete a row** - Click the row to be deleted, then click the red **X** icon (Delete a row) in the tool-bar. Only empty rows and fourth incubation rows can be deleted. Rows for required assay reagents cannot be deleted.



NOTES:
For Sally Sue and Peggy Sue, you can put samples, antibodies and blocking buffer in Rows A-J and in columns 1-12 or 13-24.

For chemiluminescence assays, we recommend keeping two rows empty between the substrate and the closest dispensed HRP conjugate to avoid any interaction with the substrate.

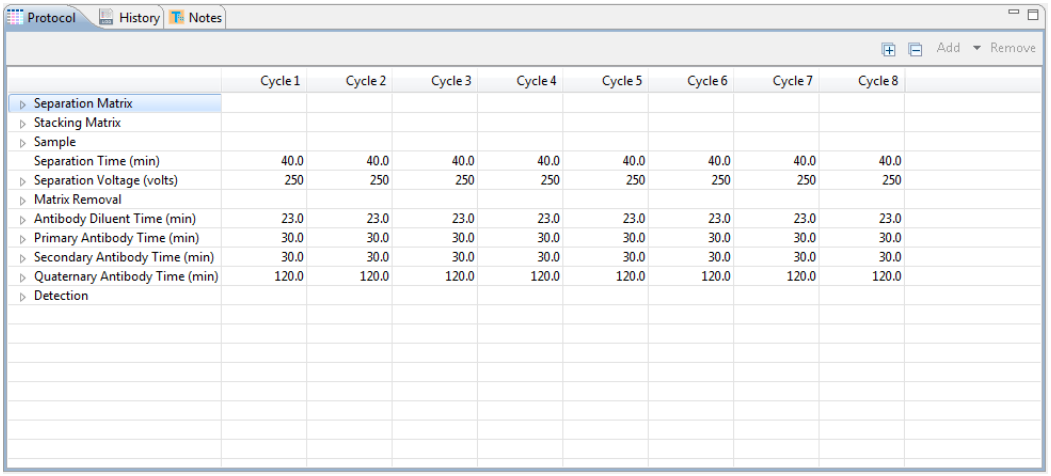
Rows K and L are purposely left empty, don't put anything into these wells.

Separation and Stacking matrices can only be pipetted into the designated wells.

Water is used in the wells to reduce the potential of evaporation of the matrices.

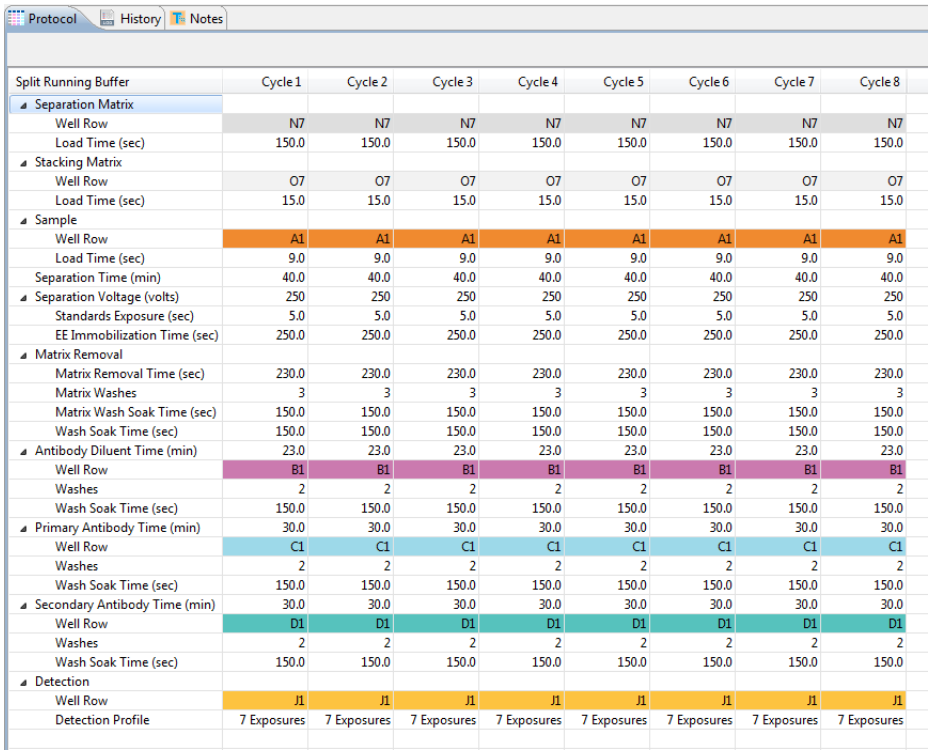
Step 3 - Modifying the Assay Protocol (Optional for All Instruments)

1. Click on the Protocol tab. This pane shows the individual steps of the assay protocol and allows you to change parameters. When creating a new assay, the default protocol automatically assigns all reagent locations for the assay. The Sally Sue assay is shown as an example:



	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Antibody Diluent Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Primary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Secondary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Quaternary Antibody Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Detection								

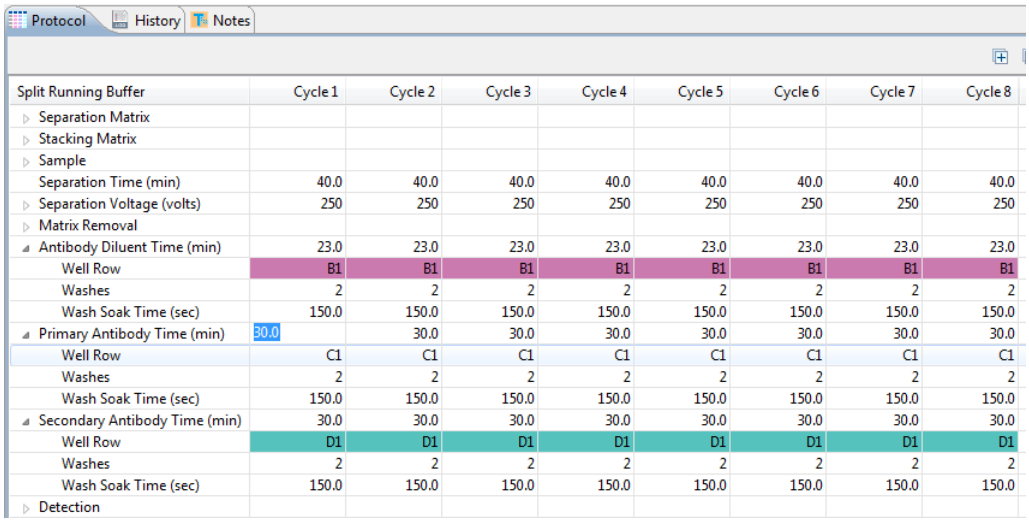
Each row contains an assay protocol step. Each step can contain a reagent assay plate row assignment and one or more parameter settings. To view the details for a step, click on the white arrow next to the step name. We recommend using the default protocol settings for the assays. An expanded list of the default protocol step parameters is shown below:



Protocol | History | Notes

Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Well Row	N7	N7	N7	N7	N7	N7	N7	N7
Load Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Stacking Matrix								
Well Row	O7	O7	O7	O7	O7	O7	O7	O7
Load Time (sec)	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Sample								
Well Row	A1	A1	A1	A1	A1	A1	A1	A1
Load Time (sec)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Standards Exposure (sec)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
EE Immobilization Time (sec)	250.0	250.0	250.0	250.0	250.0	250.0	250.0	250.0
Matrix Removal								
Matrix Removal Time (sec)	230.0	230.0	230.0	230.0	230.0	230.0	230.0	230.0
Matrix Washes	3	3	3	3	3	3	3	3
Matrix Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Antibody Diluent Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Well Row	B1	B1	B1	B1	B1	B1	B1	B1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Primary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	C1	C1	C1	C1	C1	C1	C1	C1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Secondary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	D1	D1	D1	D1	D1	D1	D1	D1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Detection								
Well Row	J1	J1	J1	J1	J1	J1	J1	J1
Detection Profile	7 Exposures	7 Exposures	7 Exposures	7 Exposures	7 Exposures	7 Exposures	7 Exposures	7 Exposures

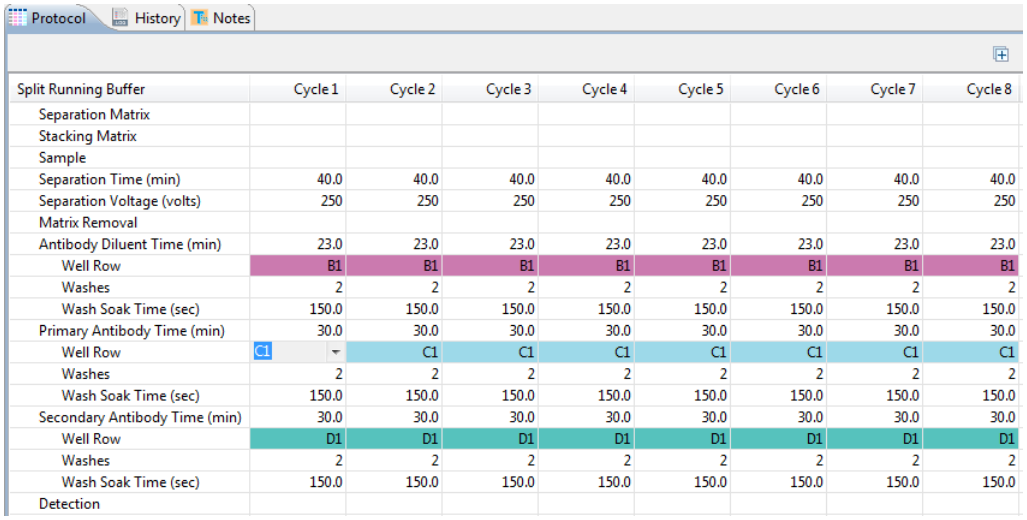
2. You can change the primary or secondary antibody incubation time. Click the cell in the value column next to Primary Ab Time (min) or Secondary Ab Time (min) and enter a new value in minutes:



Protocol | History | Notes

Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Antibody Diluent Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Well Row	B1	B1	B1	B1	B1	B1	B1	B1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Primary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	C1	C1	C1	C1	C1	C1	C1	C1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Secondary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	D1	D1	D1	D1	D1	D1	D1	D1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Detection								

3. You can change the primary and secondary antibody incubation reagent row location. Click the cell in the value column next to Well Row and select a different row on the assay plate:



Protocol | History | Notes

Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Antibody Diluent Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Well Row	B1	B1	B1	B1	B1	B1	B1	B1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Primary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	C1	C1	C1	C1	C1	C1	C1	C1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Secondary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	D1	D1	D1	D1	D1	D1	D1	D1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Detection								

NOTE: Only rows you've designated as primary antibody in the Layout tab can be selected in the Well Row drop-down menu.

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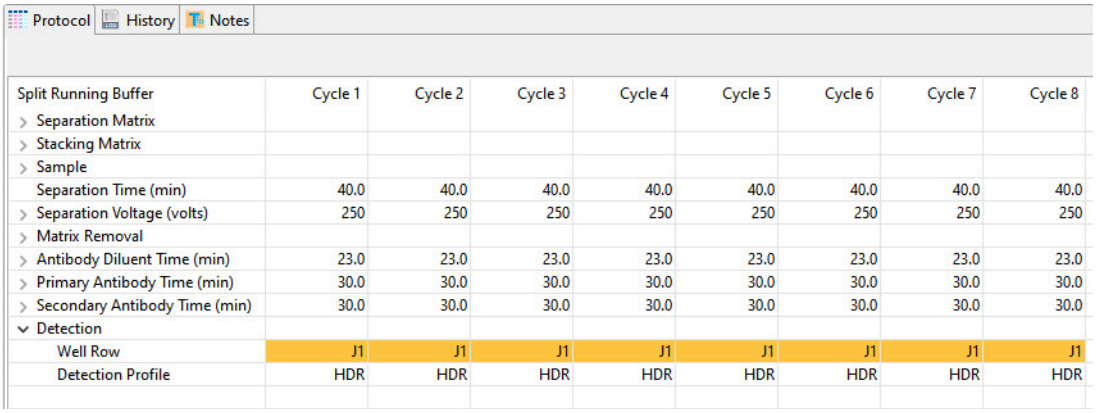
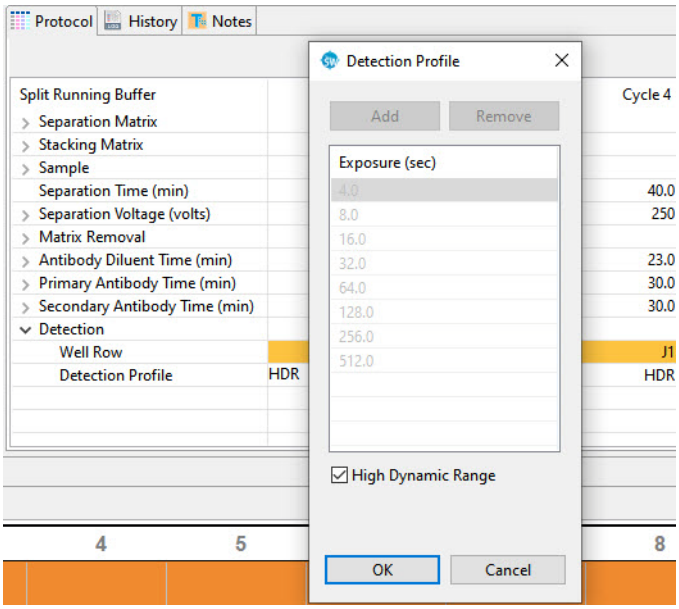
4. The default detection mode is High Dynamic Range (HDR). See "High Dynamic Range Detection Profile" on page 71 for more information.



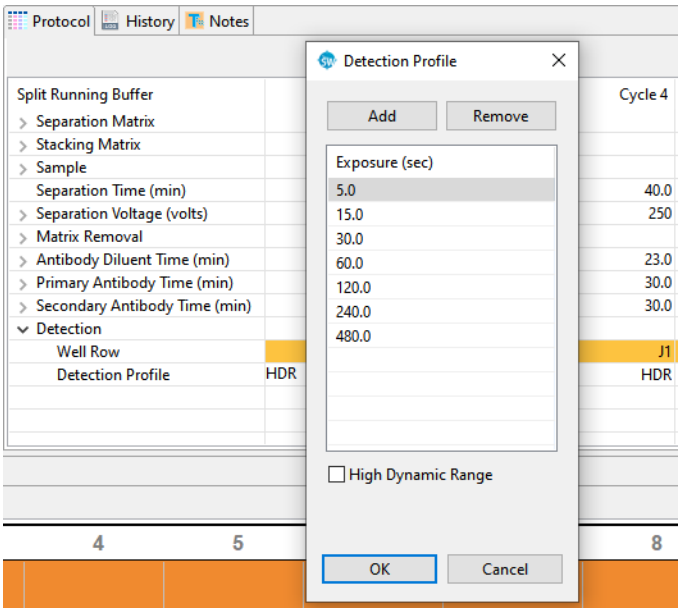
Protocol | History | Notes

Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Antibody Diluent Time (min)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Primary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Secondary Antibody Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Detection								
Well Row	J1	J1	J1	J1	J1	J1	J1	J1
Detection Profile	HDR	HDR	HDR	HDR	HDR	HDR	HDR	HDR

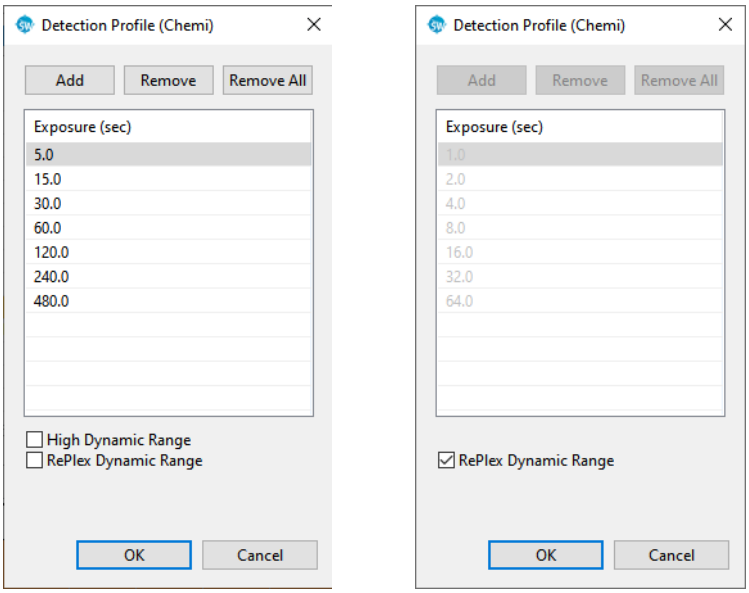
Additional exposures can be collected in the assay if desired. To do this, click the white arrow next to Detection to expand the row. Click the cell in the exposure column next to Detection Profile to open the Detection Profile window.



Deselect the High Dynamic Range checkbox. Additional times can be added to the protocol by clicking the **Add** button, entering the values and selecting **OK.**



For non-RePlex assays on Jess, when the High Dynamic Range box is unchecked, you also have the option of selecting the RePlex Dynamic Range:



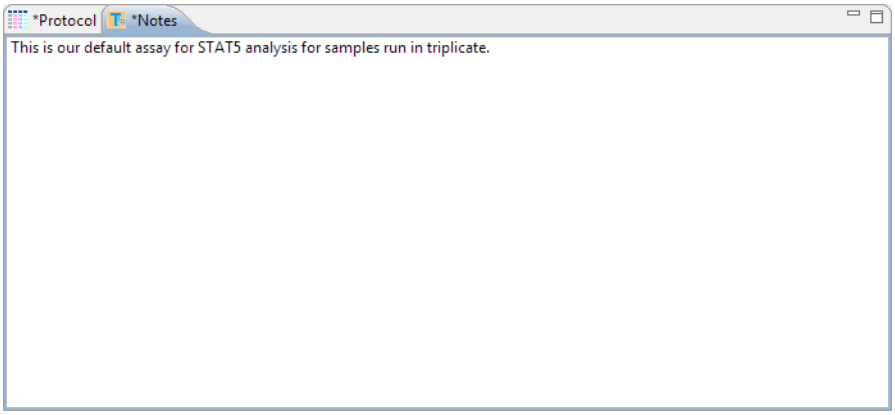
5. You can modify any other protocol parameters as needed.

NOTES:
For more information on changing protocol step parameters other than incubation times, contact ProteinSimple Technical Support toll-free in the US and Canada at 1-888-607-9692 (option 3) or your local Field Application Specialist.

When a protocol is being edited, an asterisk will appear in the Protocol tab to indicate changes have been made and should be saved.

Step 4 - Add Assay Notes (Optional)

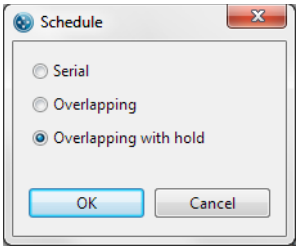
1. Click on the Notes tab.
2. Click in the pane and type any assay or protocol notes. This information will be stored with the assay and run data.



NOTE: When notes are being edited, an asterisk will appear in the Notes tab to indicate changes have been made and should be saved.

Step 5 - Select a Schedule (Optional for Sally Sue and Peggy Sue)

Sally Sue and Peggy Sue can execute cycles serially or in parallel. To choose a schedule option, select **Edit** and click **Schedule**.



- **Serial** - Each cycle is executed in sequential order, and each cycle completes before the next one starts. Selecting this option result in the longest run times.
- **Overlapping** - Cycle steps are executed in parallel if timing permits shared use of the separation tray and robot. Each cycle will run through to completion once started. This option offers a reduction in overall run time without affecting the individual run time for each cycle.
- **Overlapping with Hold (default)** - Each cycle executes through the separate step in sequence and then enters a variable length hold step where capillaries remain in the incubation tray. More sample stability is provided with this option as proteins are 'locked' to the capillary wall and therefore more stable than samples on the assay plate. This option also results in the most significant reduction in overall run time.

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*NOTE: We recommend using the **Overlapping with hold** option. For more information on when to use other schedule options, please contact ProteinSimple Technical Support toll-free in the US and Canada at 1-888-607-9692 (option 3) or your local Field Application Specialist.*

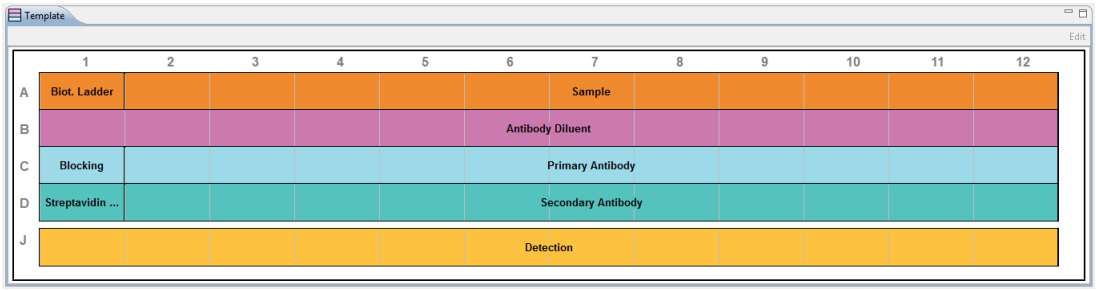
Step 6 - Add Assay Plate Annotations (Optional)

Custom reagent names, notes and annotations can be entered for individual rows and wells on the assay plate. This information will be stored with assay and run data. During post-run analysis, this information will be used to apply custom analysis settings to specific sample names, blocking reagent names, primary antibody names or sample and primary antibody attributes. This will be explained in more detail in “Analysis Settings Overview” on page 292.

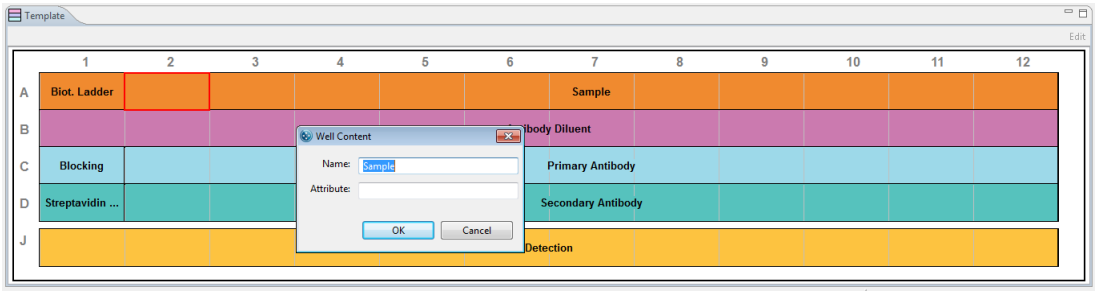
NOTE: Template pane information can also be added or updated after a run is complete.

To enter annotations:

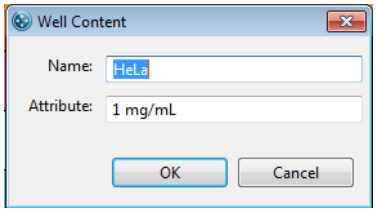
1. Click on the Template tab. The default annotations for reagent rows and individual wells on the assay plate will display:



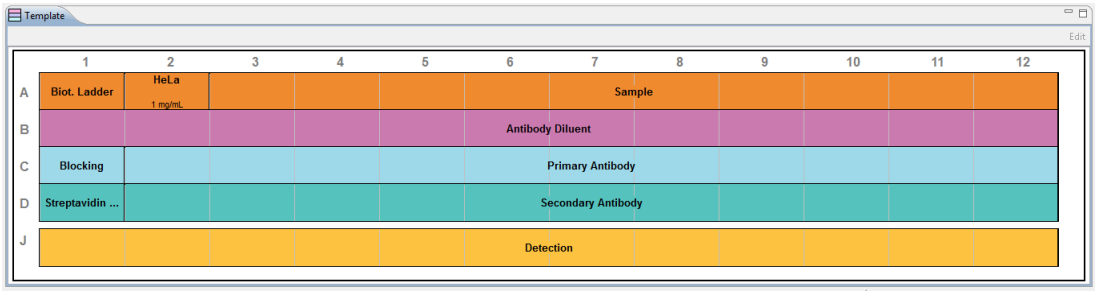
2. Change or add row and well annotations as needed. To do this:
   a. **To enter annotations for a specific well** - Right click the well and select **Edit** or click **Edit** in the upper right corner of the pane or double click the selected well. The following box will display:



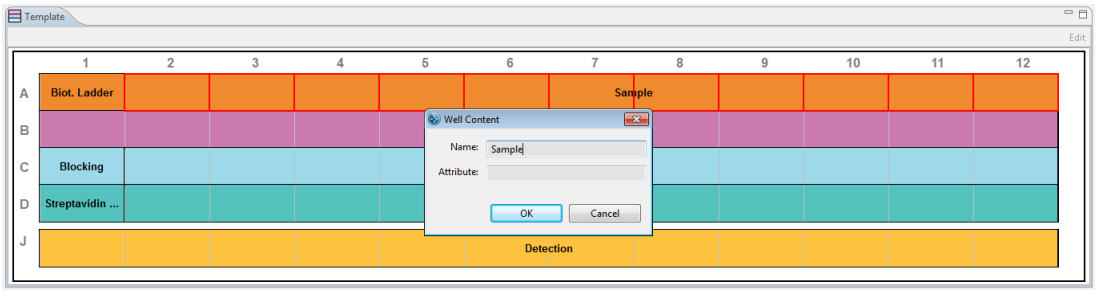
   Enter the reagent name, notes or specific attributes (for example, sample concentration or dilution ratio):



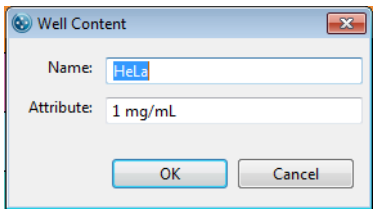
Click **OK**. The new information will display in the selected well:



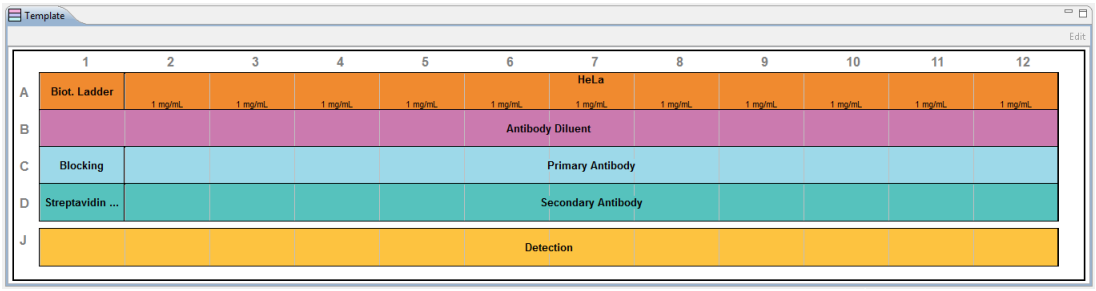
b. **To enter annotations for multiple wells or a row** - To select individual wells, press and hold the Control key and then select individual wells. To select a sequential set of wells or a full row, press and hold the Shift key, then select the first well and last well. Next, right click and select **Edit** or click **Edit** in the upper right corner of the pane. The following box will display:



Enter the reagent name, notes and specific attributes (for example, sample concentration or dilution ratio):



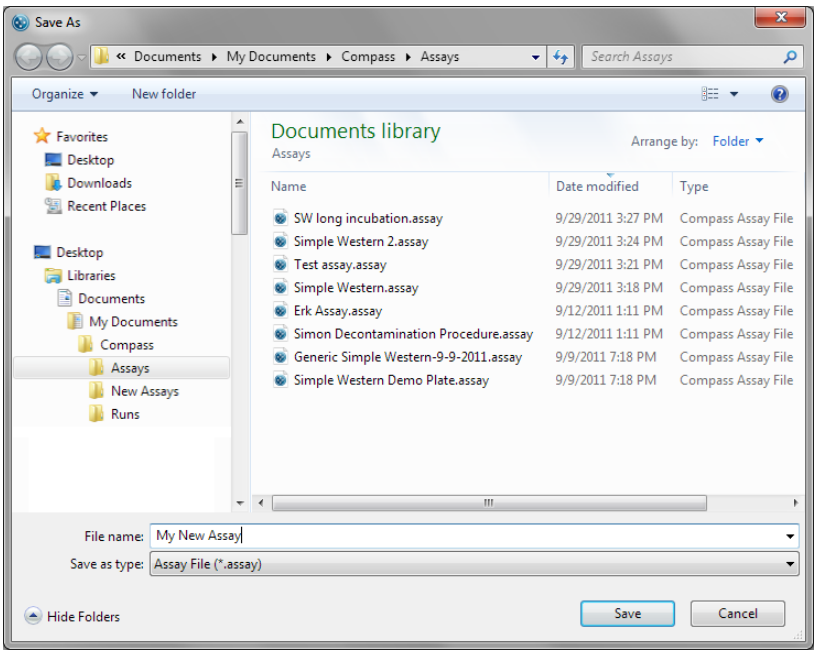
Click **OK**. The new information will display in the selected wells:



*NOTE: When annotations are being edited, an asterisk will appear in the **Template** tab to indicate changes have been made and should be saved. Only Name and Attribute will be used by Compass for Simple Western to annotate the data.*

Step 7 - Save the Assay

1. Select **File** from the main menu and click **Save As**. Enter the assay name and click **Save**.

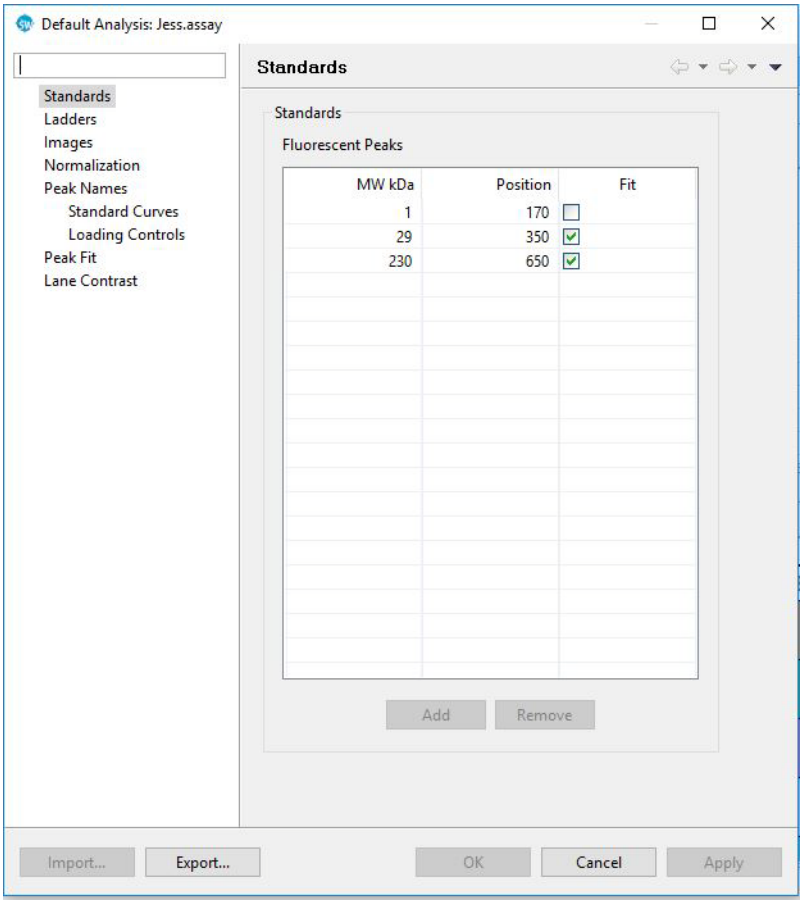


NOTE: New assays are saved in the Compass Assays directory.

Step 8 - Modify Default Analysis Parameters (Optional)

You can preset the parameters used to analyze run data generated with the assay.

1. Select **Edit** from the main menu and click **Default Analysis**. The following screen will display:



2. We recommend using the default parameters for Simple Western assays. However, you can modify any you want, then click **OK**. For detailed information on analysis parameters, please refer to "Analysis Settings Overview" on page 292.

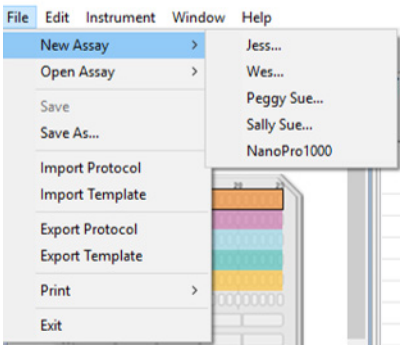
Total Protein Assays: Creating a New Assay

To create a new assay, we recommend using one of the template assays and making any necessary modifications from there.

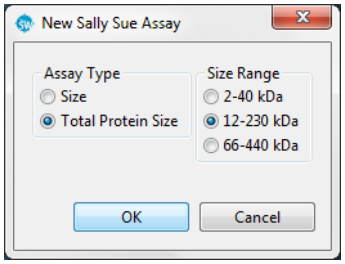
NOTE: This section provides details on how to create a standard Total Protein assay on Wes, Peggy Sue and Sally Sue. To create a RePlex Assay on Jess that includes a Total Protein assay, see "RePlex Assays on Jess: Creating a New Assay" on page 60.

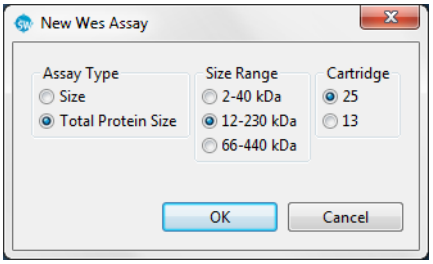
Step 1 - Open a Template Assay

1. Select **File** in the main menu and click **New Assay**:



2. Select the template assay for your instrument by selecting **Total Protein Size** as your assay type, **Size Range** and **Cartridge Type** if running Jess or Wes, or choose **Open Assay** to select from the menu of saved assays.





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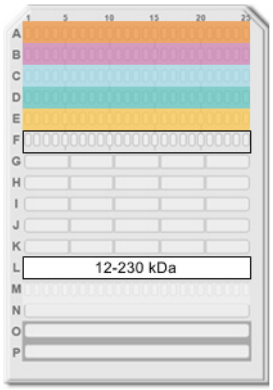
*NOTE: If you make changes to the assay, such as defining the wells in the plate, be sure to select **File** and **Save** before proceeding.*

Step 2 - Assign Assay Plate Reagents (Optional)

1. Click on the Layout tab. Displayed are the default row locations where each reagent should be placed on the assay plate.

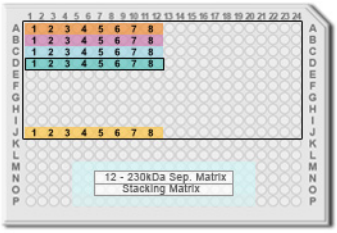
NOTE: For Sally Sue and Peggy Sue, up to 96 different samples or conditions (lysates, proteins, antibodies or incubation times) can be assayed using the full eight cycles to accommodate experimental needs.

Jess and Wes plate layout:



- **Row A** - Biotinylated Ladder (A1) and Sample (A2-A25)
- **Row B** - Antibody Diluent (B1) and Labeling Reagent (B2-25)
- **Row C** - Antibody Diluent
- **Row D** - Total Protein Streptavidin-HRP
- **Row E** - Luminol-S/Peroxide mix
- **Row G** - Wash buffer
- **Row H** - Wash buffer
- **Row I** - Wash buffer
- **Row M-P** - Pre-filled Separation Reagents

Sally Sue/Peggy Sue plate layout:



- **Row A** - Biotinylated Ladder (A1) and Sample (A2-A12)
- **Row B** - Antibody Diluent (B1) and Total Protein Labeling Reagent (B2-12)
- **Row C** - Antibody Diluent
- **Row D** - Total Protein Streptavidin-HRP
- **Row J** - Luminol-S/Peroxide mix
- **Row M** - Water (M5-M20)
- **Row N** - Water (N5-N6 and N19-N20) and Separation Matrix (N7-N18)
- **Row O** - Water (O5-O6 and O19-O20) and Stacking Matrix (O7-O18)
- **Row P** - Water (P5-P20)

NOTE: For details on sample, reagent and assay plate preparation, please refer to the product insert provided with the Simple Western kits.

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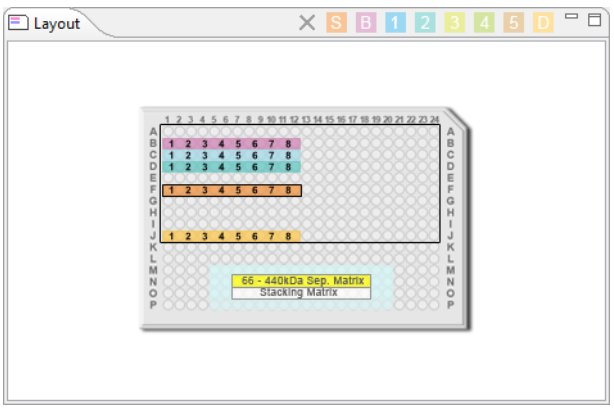
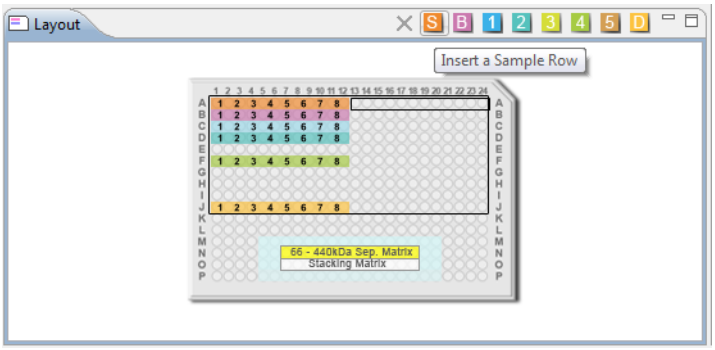
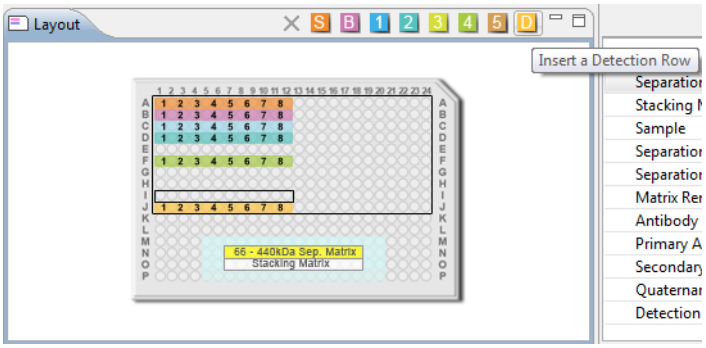
2. If needed, well assignments can be modified. Any row assignments changed in the **Layout** pane are updated in the Protocol pane automatically.
   - **To move a reagent row to another location** - Click the row in the **Layout** pane, then drag and drop it on the new location. The row from which it was moved will be reassigned as empty.



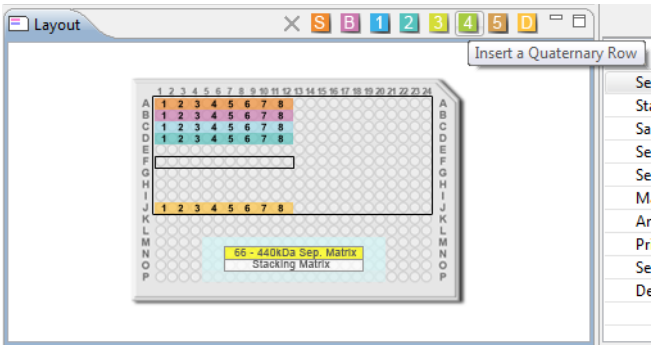
- **To insert a sample row -** Click an empty row or the row below where the new sample row should be inserted, then click the **S** icon (Insert a sample row) in the **Layout** pane toolbar. A new sample row will be added in the empty row or inserted above the selected row.



- **To insert a detection row -** Click an empty row or the row below where the new detection row should be inserted, then click the **D** icon (Insert a detection row) in the **Layout** pane toolbar. A new detection row will be added in the empty row or inserted above the selected row.



- **To insert a fourth or fifth incubation reagent** - Click an empty row or the row below where the new incubation reagent should be inserted, then click the **4** icon (Insert a quaternary row) in the **Layout** pane toolbar. A new incubation reagent row will be added in the empty row or inserted above the selected row.

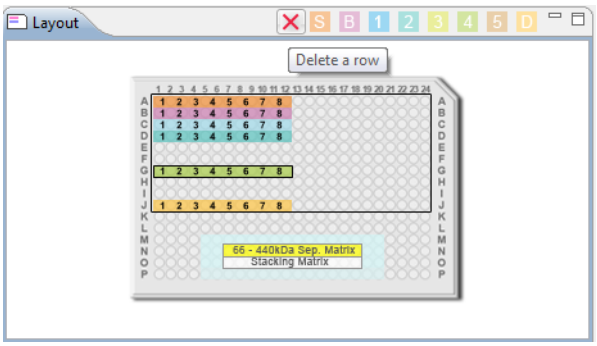


A fifth incubation reagent can now be added by repeating the above and clicking the **5** icon (Insert a Quinary row) instead.

NOTES:
When using the fourth and fifth incubation steps, only five cycles can typically be performed per run as more water is needed per cycle for the additional wash steps.

Row J is the last row assignment that can be used on the assay plate. New, sample, incubation or luminol rows cannot be inserted if row J already has an assigned reagent. To insert a row in this case, you must first move the contents of row J to another area on the plate.

- **To delete a row** - Click the row to be deleted, then click the red **X** icon (Delete a row) in the tool-bar. Only empty rows and fourth incubation rows can be deleted. Rows for required assay reagents cannot be deleted.



NOTES:
We recommend keeping two rows empty between the substrate and the closest dispensed HRP conjugate to avoid any interaction with the substrate.

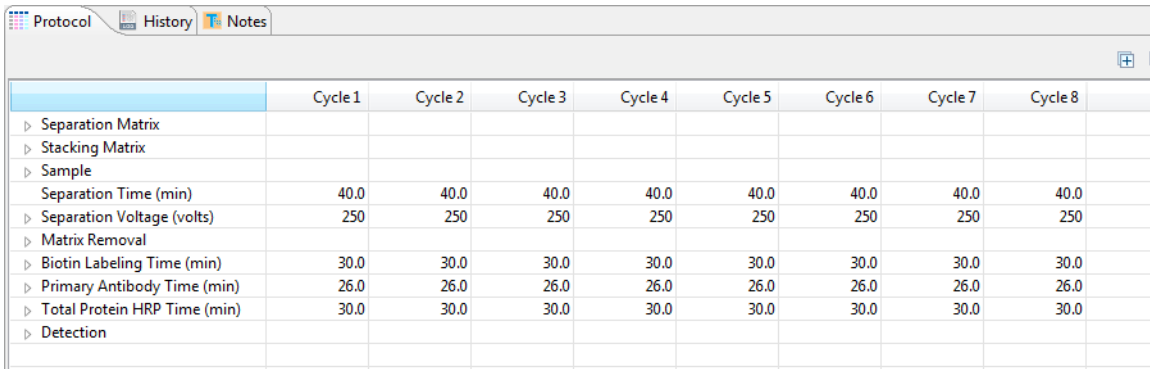
Rows K and L are purposely left empty, don't put anything into these wells.

Separation and Stacking matrices can only be pipetted into the designated wells.

Water is used in the wells to reduce the potential of evaporation of the matrices.

Step 3 - Modifying the Assay Protocol (Optional for All Instruments)

1. Click on the Protocol tab. This pane shows the individual steps of the assay protocol and allows you to change parameters. When creating a new assay, the default protocol automatically assigns all reagent locations for the assay. The Sally Sue assay is shown as an example:


Protocol History Notes

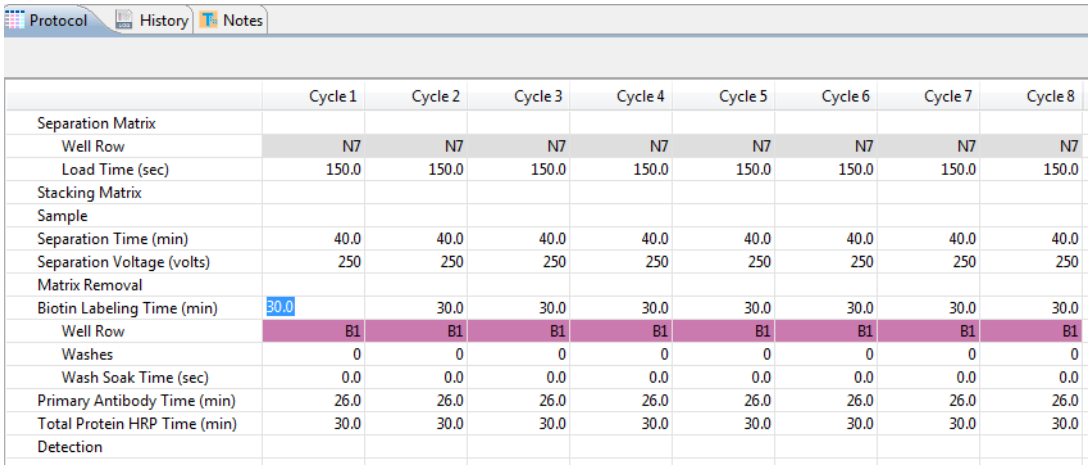
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Biotin Labeling Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Primary Antibody Time (min)	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0
Total Protein HRP Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Detection								

Each row contains an assay protocol step. Each step can contain a reagent assay plate row assignment and one or more parameter settings. To view the details for a step, click on the white arrow next to the step name. We recommend using the default protocol settings for the assays. An expanded list of the default protocol step parameters is shown:

Protocol | History | Notes

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
◢ Separation Matrix								
Well Row	N7	N7	N7	N7	N7	N7	N7	N7
Load Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
◢ Stacking Matrix								
Well Row	O7	O7	O7	O7	O7	O7	O7	O7
Load Time (sec)	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
◢ Sample								
Well Row	A1	A1	A1	A1	A1	A1	A1	A1
Load Time (sec)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
◢ Separation Voltage (volts)	250	250	250	250	250	250	250	250
Standards Exposure (sec)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
EE Immobilization Time (sec)	250.0	250.0	250.0	250.0	250.0	250.0	250.0	250.0
◢ Matrix Removal								
Matrix Removal Time (sec)	230.0	230.0	230.0	230.0	230.0	230.0	230.0	230.0
Matrix Washes	3	3	3	3	3	3	3	3
Matrix Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
◢ Biotin Labeling Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	B1	B1	B1	B1	B1	B1	B1	B1
Washes	0	0	0	0	0	0	0	0
Wash Soak Time (sec)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
◢ Primary Antibody Time (min)	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0
Well Row	C1	C1	C1	C1	C1	C1	C1	C1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
◢ Total Protein HRP Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	D1	D1	D1	D1	D1	D1	D1	D1
Washes	2	2	2	2	2	2	2	2
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
◢ Detection								
Well Row	J1	J1	J1	J1	J1	J1	J1	J1

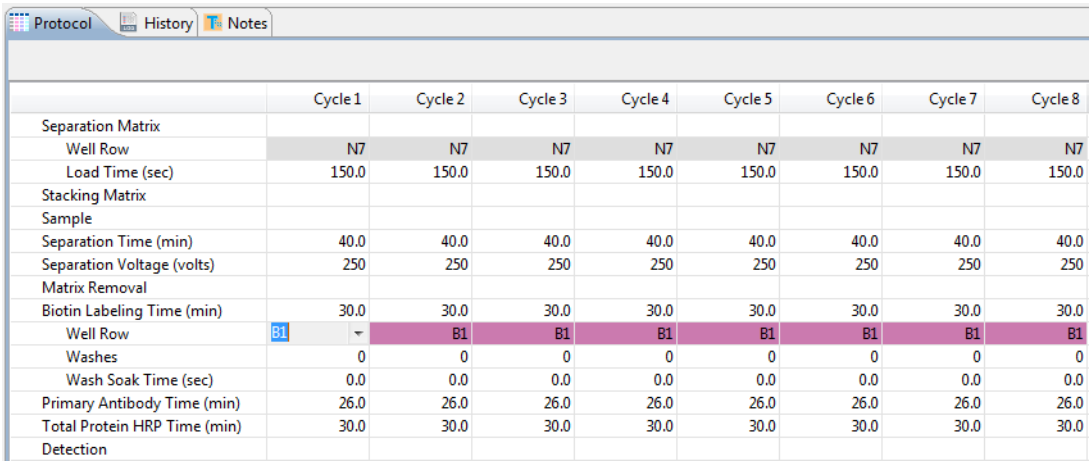
2. You can change the biotin labeling and Total Protein streptavidin-HRP incubation time. Click the cell in the value column next to Biotin Labeling Time (min) or Total Protein HRP Time (min) and enter a new value in minutes:



Protocol | History | Notes

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Well Row	N7	N7	N7	N7	N7	N7	N7	N7
Load Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Biotin Labeling Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	B1	B1	B1	B1	B1	B1	B1	B1
Washes	0	0	0	0	0	0	0	0
Wash Soak Time (sec)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Primary Antibody Time (min)	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0
Total Protein HRP Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Detection								

3. You can also change the biotin labeling and Total Protein streptavidin-HRP row location. Click the cell in the value column next to Well Row and select a different row on the assay plate:



Protocol | History | Notes

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Well Row	N7	N7	N7	N7	N7	N7	N7	N7
Load Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Biotin Labeling Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Well Row	B1	B1	B1	B1	B1	B1	B1	B1
Washes	0	0	0	0	0	0	0	0
Wash Soak Time (sec)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Primary Antibody Time (min)	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0
Total Protein HRP Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Detection								

NOTE: Only rows you've designated as labeling reagent in the Layout tab can be selected in the Well Row drop-down menu.

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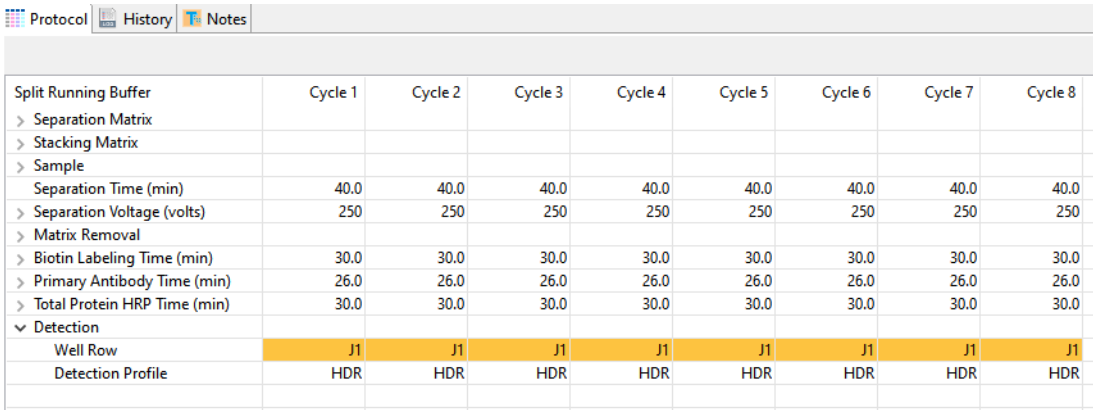
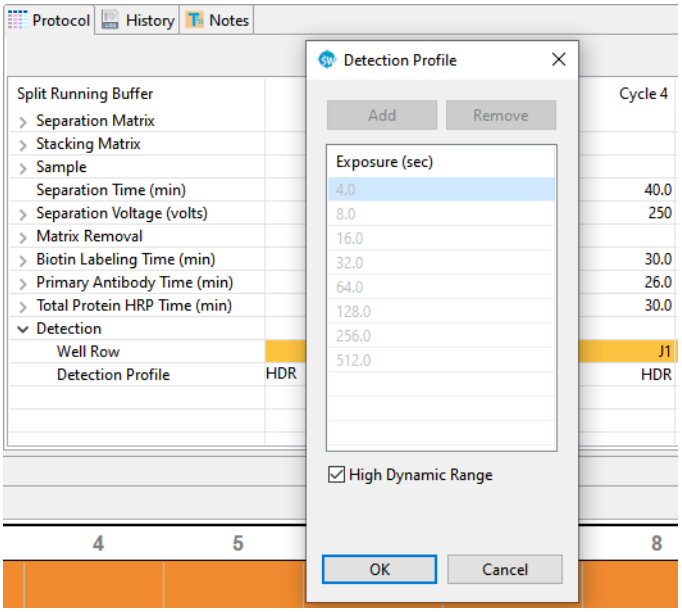
4. The default detection mode is High Dynamic Range (HDR). See "High Dynamic Range Detection Profile" on page 71 for more information.



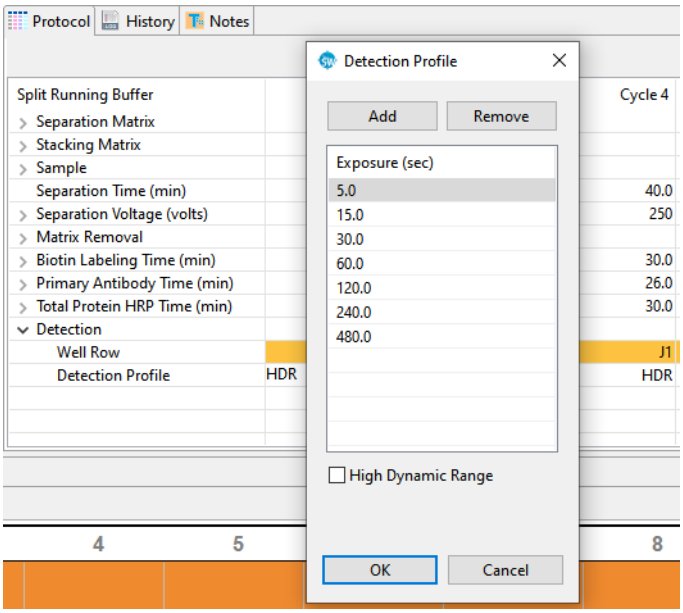
Protocol | History | Notes

Split Running Buffer	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Stacking Matrix								
Sample								
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Matrix Removal								
Biotin Labeling Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Primary Antibody Time (min)	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0
Total Protein HRP Time (min)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Detection								
Well Row	J1	J1	J1	J1	J1	J1	J1	J1
Detection Profile	HDR	HDR	HDR	HDR	HDR	HDR	HDR	HDR

Additional exposures can be collected in the assay if desired. To do this, click the white arrow next to Detection to expand the row. Click the cell in the exposure column next to Detection Profile to open the Detection Profile window.



Deselect the High Dynamic Range checkbox. Additional times can be added to the protocol by clicking the **Add** button, entering the values and selecting **OK.**



5. You can modify any other protocol parameters as needed.

NOTES:
For more information on changing protocol step parameters other than incubation times, contact ProteinSimple Technical Support toll-free in the US and Canada at 1-888-607-9692 (option 3) or your local Field Application Specialist.

When a protocol is being edited, an asterisk will appear in the Protocol tab to indicate changes have been made and should be saved.

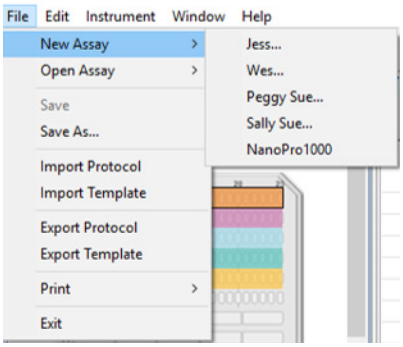
Steps 4 - 8

Steps 4 through 8 for creating a Total Protein Assay are the same as when you're creating an Immunoassay. Please go to *"Step 4 - Add Assay Notes (Optional),"* starting on page 42 to continue.

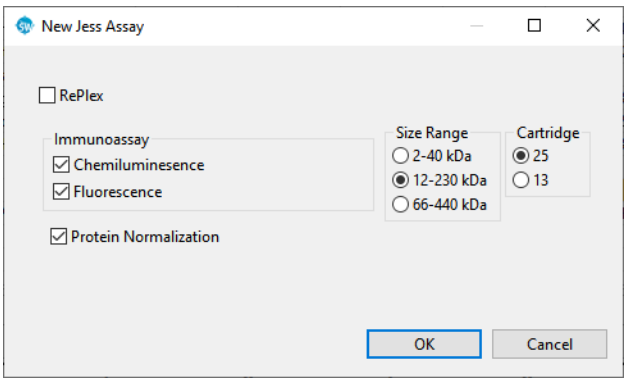
Protein Normalization on Jess: Creating a New Assay

Step 1 - Open a Template Assay

1. Select **File** in the main menu, click **New Assay** and choose **Jess**:



2. Select the template assay by selecting **Protein Normalization** in addition to **Fluorescence** and/or **Chemiluminescence** as your assay type, **Size Range** and **Cartridge Type**, or choose **Open Assay** from the **File** menu to select from the menu of saved assays.



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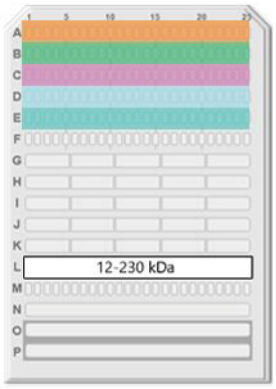
*NOTE: If you make changes to the assay, such as defining the wells in the plate, be sure to select **File** and **Save** before proceeding.*

Step 2 - Assign Assay Plate Reagents

1. Click on the Layout tab. Displayed is the default row location (green) where the protein normalization reagent should be placed on the assay plate.

Jess plate layout:



- **Row A** - Biotinylated Ladder (A1) and Sample (A2-A25)
- **Row B** - Protein Normalization Reagent
- **Row C** - Milk-Free Antibody Diluent
- **Row D** - Milk-Free Antibody Diluent (D1) and Primary Antibody Multiplex mix (D2-D25)
- **Row E** - Streptavidin-NIR (E1) and Secondary Antibody Multiplex mix (E2-E25)
- **Row F** - Empty
- **Row G** - Wash buffer
- **Row H** - Wash buffer
- **Row I** - Wash buffer
- **Row M-P** - Pre-filled Separation Reagents

NOTE: For details on sample, reagent and assay plate preparation, please refer to the product insert provided with the Simple Western kits.

Step 3 - Modifying the Assay Protocol (Optional for All Instruments)

Steps 3 for creating a Protein Normalization Assay on Jess are the same as when you're creating an Immunoassay. Please go to *"Step 3 - Modifying the Assay Protocol (Optional for All Instruments),"* starting on page 37 to continue.

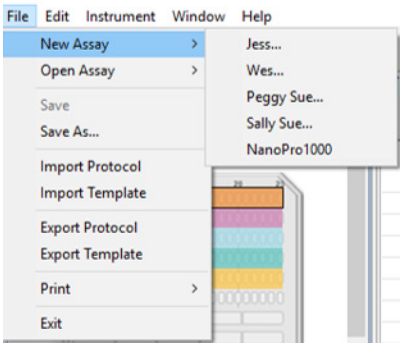
Steps 4 - 8

Steps 4 through 8 for creating a Protein Normalization Assay are the same as when you're creating an Immunoassay. Please go to *"Step 4 - Add Assay Notes (Optional),"* starting on page 42 to continue.

RePlex Assays on Jess: Creating a New Assay

Step 1 - Open a Template Assay

1. Select **File** in the main menu, click **New Assay** and choose **Jess**:



Alternatively you can choose **Open Assay** from the **File** menu to select from the menu of saved assays.

NOTES:
*If you make changes to the assay, such as defining the wells in the plate, be sure to select **File** and **Save** before proceeding.*

Creating a RePlex assay is currently only supported when Access Control is disabled. When Access Control is enabled in Preferences, the RePlex checkbox will not be available for selection.

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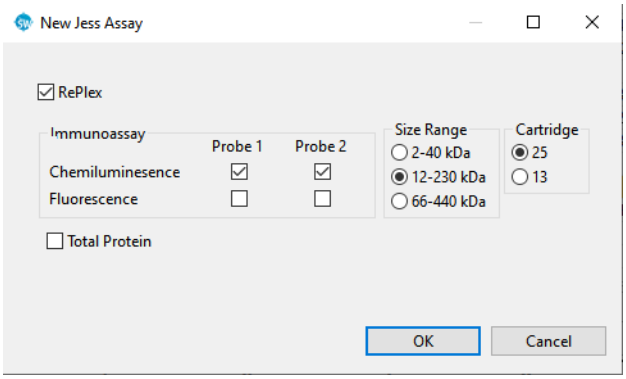
2. Select **RePlex.**



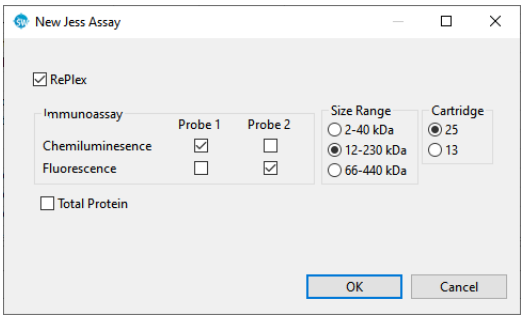
These RePlex Assay combinations are currently validated on Jess:

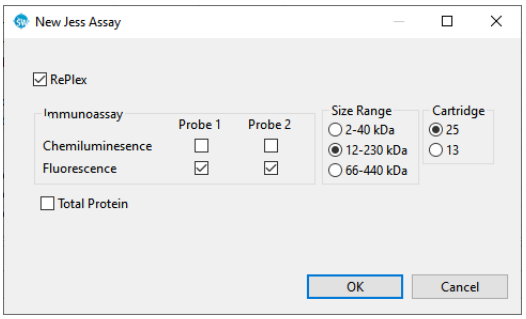
- Chemiluminescence + chemiluminescence
- Chemiluminescence + Total Protein
- Chemiluminescence/NIR fluorescence + Total Protein
- NIR fluorescence + NIR fluorescence
- NIR fluorescence + chemiluminescence

The assay selected for Probe 1 is run first. When Probe 1 is complete, Jess performs a wash, RePlex purge, and wash step combination prior to beginning the assay selected for Probe 2.

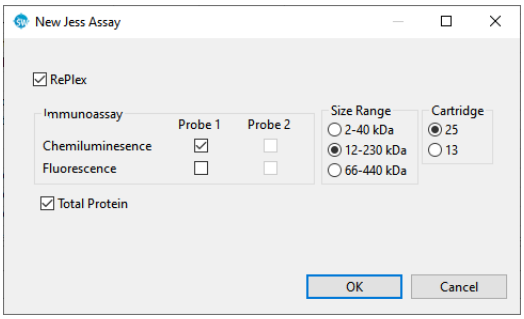
3. Select the assays to run in Probe 1 and 2. The default selection is chemiluminescence for both.

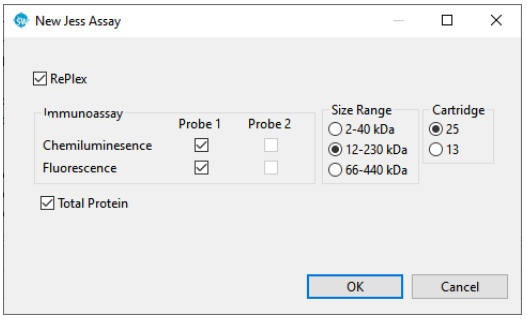
   To run two immunoassays: Select any combination of chemiluminescence or fluorescence for Probes 1 and 2. For example:





   To run one immunoassay and one Total Protein assay: Select **Total Protein**, then select any combination of chemiluminescence or fluorescence for Probe 1. Probe 2 will be used for the Total Protein assay. For example:



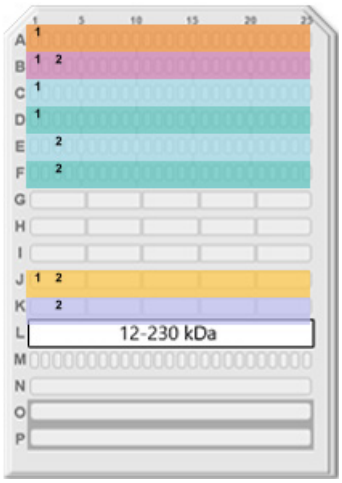


4. Select **Size Range** and **Cartridge Type**.

Step 2 - Assign Assay Plate Reagents

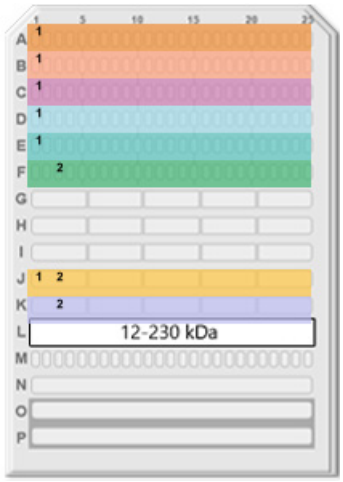
1. Click on the Layout tab. Default row locations where each reagent should be placed on the assay plate are displayed. Reagents used in Probe 1 are labeled '1' on the plate, those used in Probe 2 are labeled '2':

2 Immunoassays



- **Row A** - Biotinylated ladder (A1) and Samples (A2-A25)
- **Row B** - Blocking: Milk-Free Antibody Diluent
- **Row C** - Primary Probe 1: Milk-Free Antibody Diluent (C1) and Primary antibody for Probe 1(C2-C25)
- **Row D** - Secondary Probe 1: Streptavidin-HRP or NIR (D1) and Secondary antibody for Probe 1 (D2-D25)
- **Row E** - Primary Probe 2: Milk-Free Antibody Diluent (E1) and Primary antibody for Probe 2 (E2-E25)
- **Row F**- Secondary Probe 2: Milk-Free Antibody Diluent (F1) and Secondary antibody for Probe 2 (F2-F25)
- **Row J** - Luminol/Peroxide mix (chemiluminescence only)
- **Row K** - RePlex reagent mix

Immunoassay + Total Protein

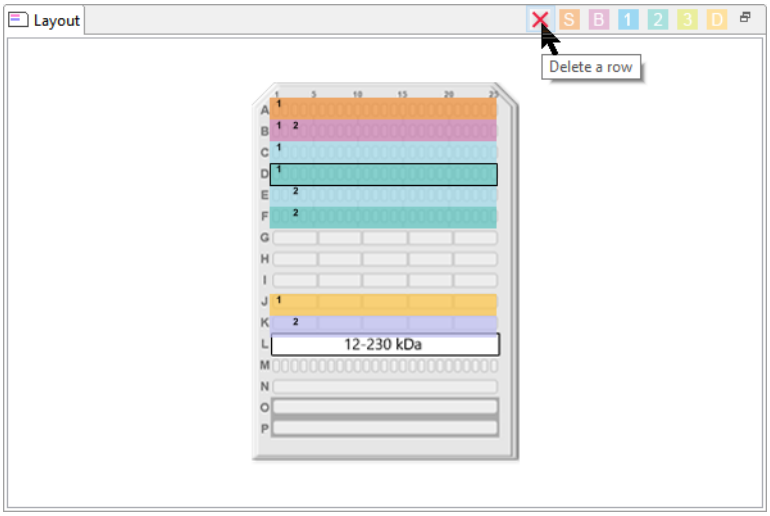


- **Row A** - Biotinylated ladder (A1) and Samples (A2-25)
- **Row B -** Total Protein biotin labeling reagent
- **Row C** - Blocking: Milk-Free Antibody Diluent
- **Row D** - Primary Probe 1: Milk-Free Antibody Diluent (D1) and Primary antibody for Probe 1 (D2-D25)
- **Row E** - Secondary Probe 1: Streptavidin-HRP or NIR (E1) and Secondary antibody for Probe 1 (E2-E25)
- **Row F** - Total Protein Streptavidin-HRP for Probe 2 (F1-F25)
- **Row J** - Luminol/Peroxide mix (chemiluminescence only)
- **Row K** - RePlex reagent mix

NOTE: For details on sample, reagent and assay plate preparation, please refer to the product insert provided with the Simple Western kits.

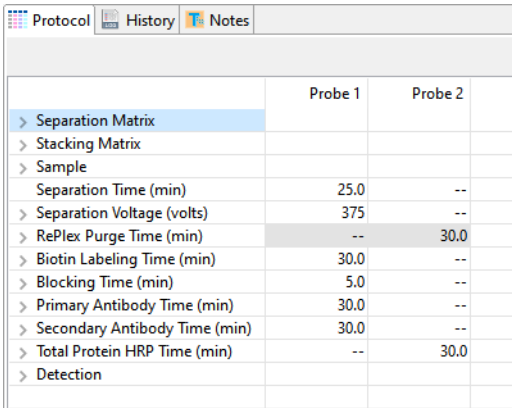
2. If needed, the Secondary antibody row assignments for either Probe 1 or 2 can be deleted for assays where the Primary antibody contains HRP. Any row assignments changed in the **Layout** pane are updated in the Protocol pane automatically.

   Click the Secondary antibody row you want to delete, then click the red **X** icon (Delete a row) in the toolbar. Only Secondary antibody rows can be deleted. Rows required for other assay reagents cannot be deleted.



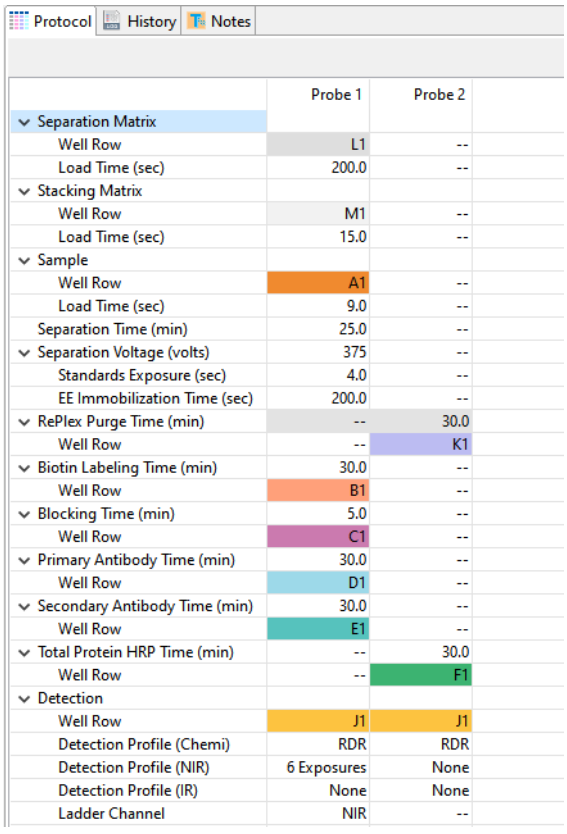
Step 3 - Modifying the Assay Protocol (Optional)

1. Click on the Protocol tab. This pane shows the individual steps of the assay protocol and allows you to change parameters. When creating a new assay, the default protocol automatically assigns all reagent locations for the assay. A chemiluminescence/NIR fluorescence + Total Protein RePlex Assay is shown in this example:



	Probe 1	Probe 2
Separation Matrix		
Stacking Matrix		
Sample		
Separation Time (min)	25.0	--
Separation Voltage (volts)	375	--
RePlex Purge Time (min)	--	30.0
Biotin Labeling Time (min)	30.0	--
Blocking Time (min)	5.0	--
Primary Antibody Time (min)	30.0	--
Secondary Antibody Time (min)	30.0	--
Total Protein HRP Time (min)	--	30.0
Detection		

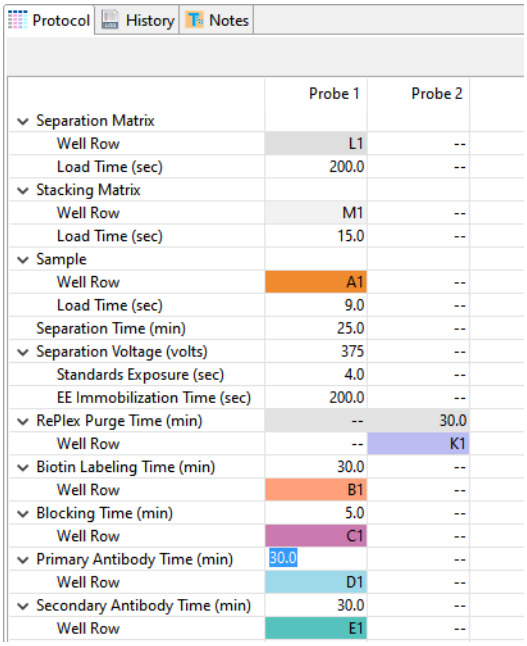
Each row contains an assay protocol step. Each step can contain a reagent assay plate row assignment and one or more parameter settings. To view the details for a step, click on the arrow next to the step name. We recommend using the default protocol settings for the assays. An expanded list of the default protocol step parameters is shown below:



	Probe 1	Probe 2
Separation Matrix		
Well Row	L1	--
Load Time (sec)	200.0	--
Stacking Matrix		
Well Row	M1	--
Load Time (sec)	15.0	--
Sample		
Well Row	A1	--
Load Time (sec)	9.0	--
Separation Time (min)	25.0	--
Separation Voltage (volts)	375	--
Standards Exposure (sec)	4.0	--
EE Immobilization Time (sec)	200.0	--
RePlex Purge Time (min)	--	30.0
Well Row	--	K1
Biotin Labeling Time (min)	30.0	--
Well Row	B1	--
Blocking Time (min)	5.0	--
Well Row	C1	--
Primary Antibody Time (min)	30.0	--
Well Row	D1	--
Secondary Antibody Time (min)	30.0	--
Well Row	E1	--
Total Protein HRP Time (min)	--	30.0
Well Row	--	F1
Detection		
Well Row	J1	J1
Detection Profile (Chemi)	RDR	RDR
Detection Profile (NIR)	6 Exposures	None
Detection Profile (IR)	None	None
Ladder Channel	NIR	--

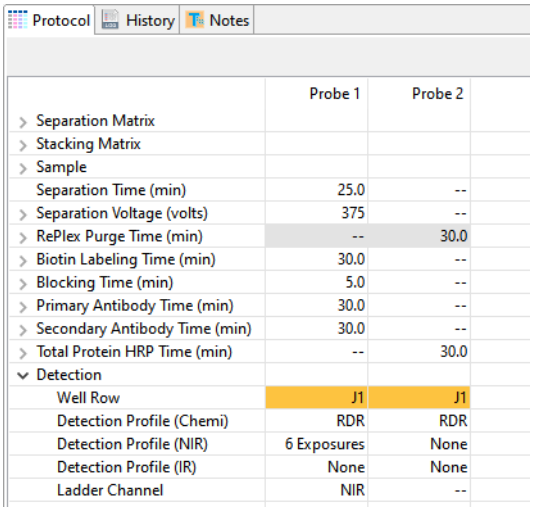
2. You can change the primary or secondary antibody incubation time. Click the cell in the value column next to Primary Antibody Time (min) or Secondary Antibody Time (min) and enter a new value in minutes:

NOTE: If you won't be using a Secondary antibody with Probe 1 or 2, set the Secondary Antibody incubation time to 0.



	Probe 1	Probe 2
Separation Matrix		
Well Row	L1	--
Load Time (sec)	200.0	--
Stacking Matrix		
Well Row	M1	--
Load Time (sec)	15.0	--
Sample		
Well Row	A1	--
Load Time (sec)	9.0	--
Separation Time (min)	25.0	--
Separation Voltage (volts)	375	--
Standards Exposure (sec)	4.0	--
EE Immobilization Time (sec)	200.0	--
RePlex Purge Time (min)	--	30.0
Well Row	--	K1
Biotin Labeling Time (min)	30.0	--
Well Row	B1	--
Blocking Time (min)	5.0	--
Well Row	C1	--
Primary Antibody Time (min)	30.0	--
Well Row	D1	--
Secondary Antibody Time (min)	30.0	--
Well Row	E1	--

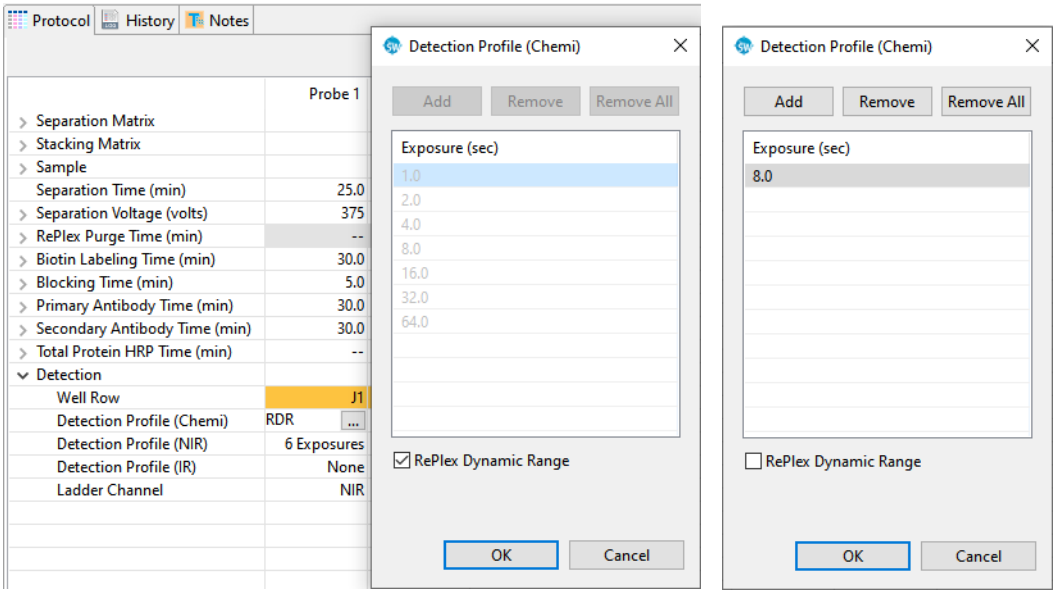
3. The default detection mode fore chemiluminescent RePlex Assays is RePlex Dynamic Range (RDR). See "RePlex Dynamic Range Detection Profile" on page 72 for more information. The default detection mode for fluorescent RePlex Assays is NIR.



Additional exposures can be collected in the assay, and IR exposures can be added if desired. To do this, click the white arrow next to Detection to expand the row. Click the cell in the exposure column next to Detection Profile in either the Probe 1 or Probe 2 column to open the Detection Profile window.

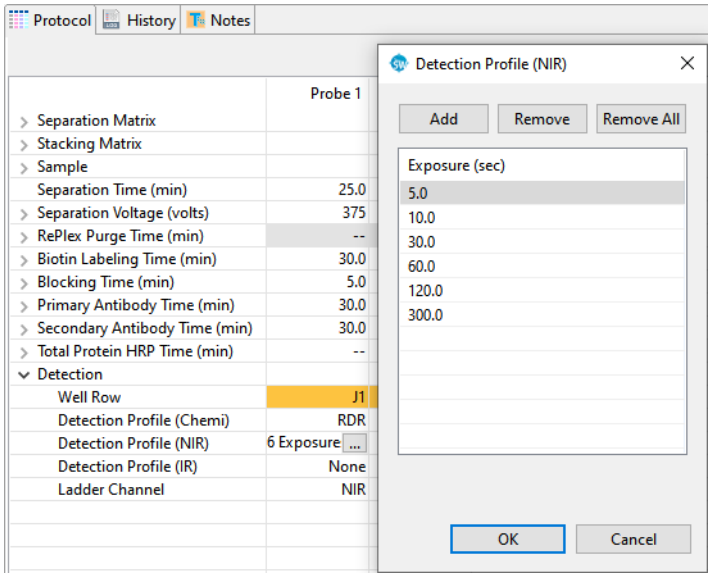
Chemiluminescent detection:

Deselect the RePlex Dynamic Range checkbox. Up to 6 additional times (for a total of 7 exposures) can be added to the protocol by clicking the **Add** button, entering the values and selecting **OK.**



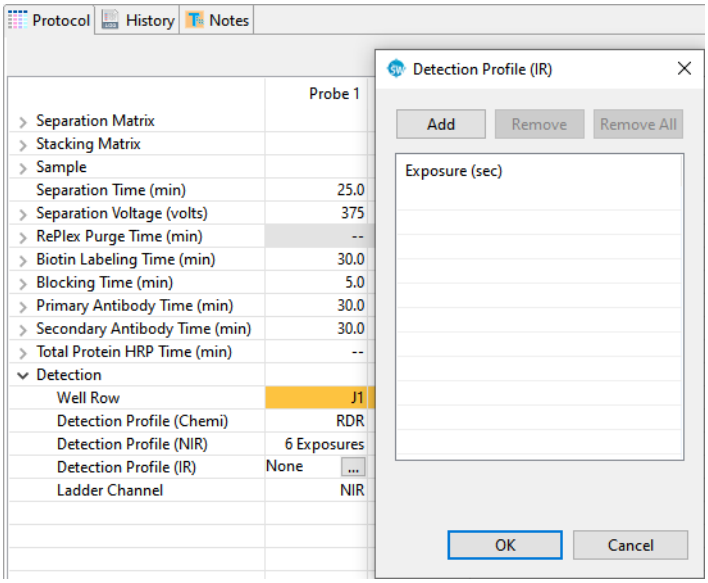
NIR Fluorescent detection:

Additional times can be added to the protocol by clicking the **Add** button, entering the values and selecting **OK.**



IR Fluorescent detection:

An IR detection profile can be added to the protocol by clicking the **Add** button, entering exposure values and selecting **OK.**



4. You can modify any other protocol parameters as needed.

NOTES:
For more information on changing protocol step parameters other than incubation times, contact ProteinSimple Technical Support toll-free in the US and Canada at 1-888-607-9692 (option 3) or your local Field Application Specialist.

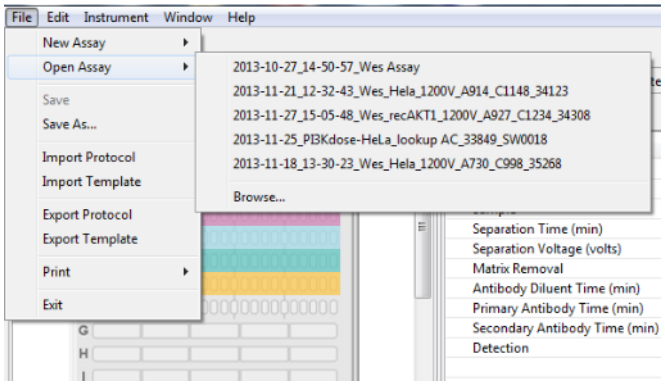
When a protocol is being edited, an asterisk will appear in the Protocol tab to indicate changes have been made and should be saved.

Steps 4 - 8

Steps 4 through 8 for creating a RePlex Assay are the same as when you're creating an Immunoassay. Please go to *"Step 4 - Add Assay Notes (Optional),"* starting on page 42 to continue.

Making Changes to an Existing Assay

1. Select **File** in the main menu and click **Open Assay**.

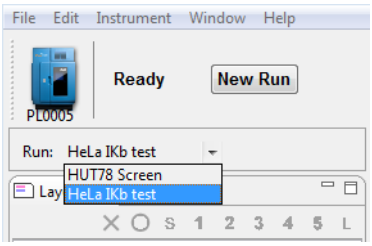


2. A list of the last assays opened will display. Select one of these assays or click **Browse** to open the assay folder and select a different assay.
3. Follow the steps in "Immunoassays: Creating a New Assay" on page 32 to make changes and save the assay.

Switching Between Open Assays

If more than one run file is open, you can switch between viewing the assays for each run. To do this:

1. Click the down arrow in the run box.

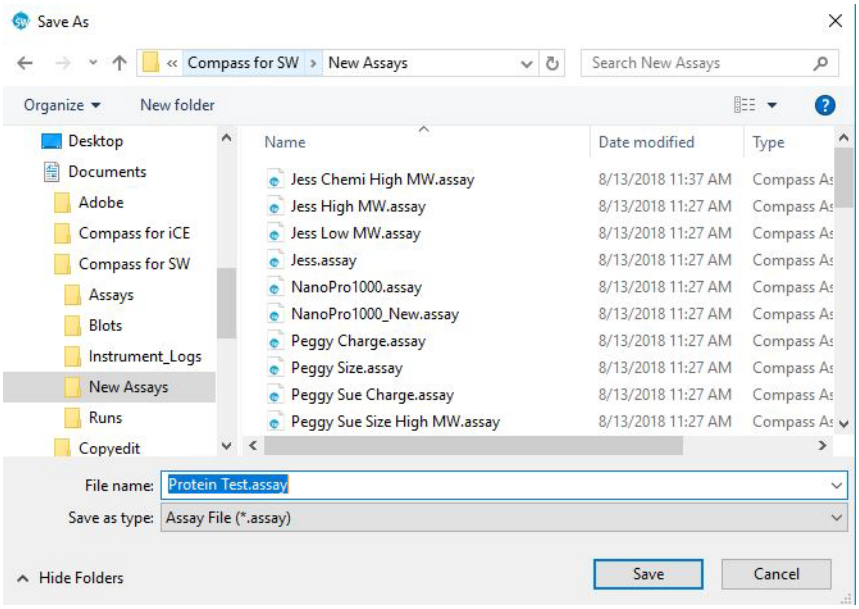


2. Select the run for the assay you want to view from the drop down list.

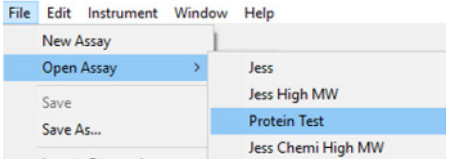
Creating a Template Assay

You can create and save template assays that can be used as a starting point for creating new assays. To do this:

1. Select **File** in the main menu. Click **Open Assay** to open an existing assay or click **New Assay** to open an existing template assay.
2. Follow the steps in "Immunoassays: Creating a New Assay" on page 32 to make changes to the assay.
3. When changes are complete, select **File** in the main menu and click **Save As**. Select the New Assays folder:

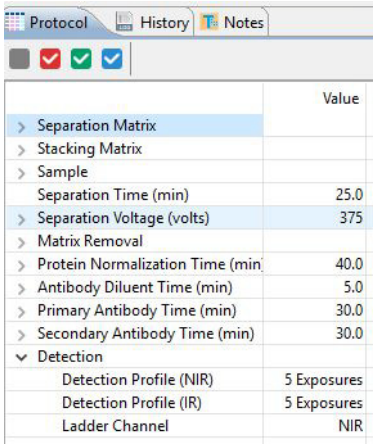


4. Type the name for the new template assay and click **Save**.
5. Select **File** in the main menu and click **Open Assay**. The new template assay will now be available in the drop down list:



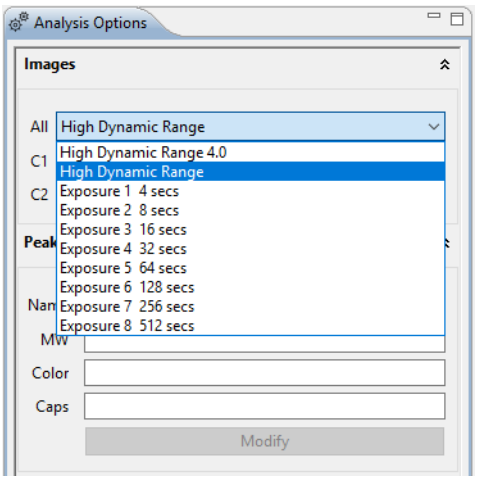
Viewing and Changing the Detection Exposures

To view the current detection profile, roll the cursor over the exposures cell in Protocol pane:



High Dynamic Range Detection Profile

Starting with Compass for Simple Western 4.0 and the Jess instrument, HDR (high dynamic range) is the default detection profile for chemiluminescent detection. Exposure times for HDR have been optimized for best performance and can't be edited. These exposures differ for Jess, Wes and Peggy Sue or Sally Sue, and they were selected for maximum performance for each instrument when HDR mode is selected. Below is an example of different exposure settings:



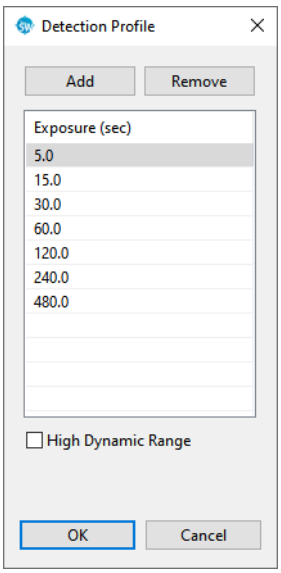
To achieve a broader detection dynamic range, you need Compass for Simple Western v4.0. To download the latest version of the software go to **Help** > **Check for Updates**.

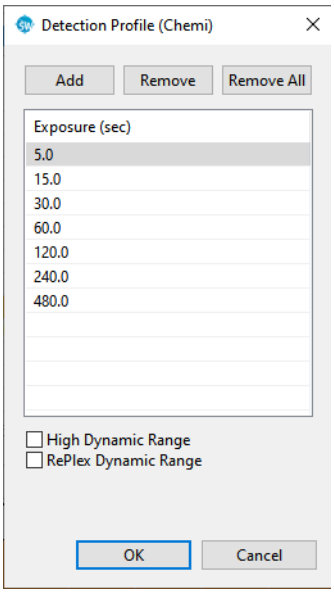
For Jess and Wes, the higher dynamic range is achieved by using a more effective algorithm to create the High Dynamic Range multi-image analysis, refreshing substrate from the assay plate prior to each exposure and minimizing the time between substrate aspiration and Image.

For Peggy Sue and Sally Sue only the new algorithm is used. This constitutes a divergence of Peggy Sue/Sally Sue vs Jess/Wes performance and you can expect a wider dynamic range on Jess and Wes. Note that for Peggy Sue/Sally Sue, you can select HDR for some cycles and uncheck it for others, giving you great flexibility for data analysis later.

NOTE: See "Images Analysis Settings" on page 293 to find out more about how data sets are analyzed under HDR and standard detection profiles.

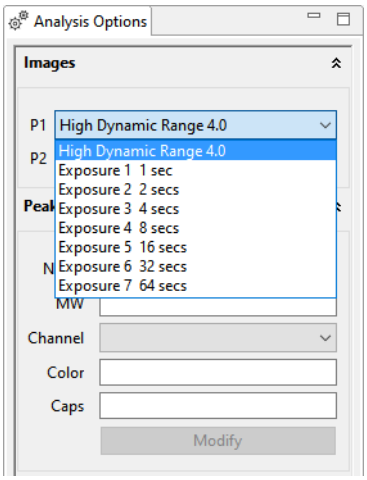
If you need to switch to an older detection profile for data generated on another Simple Western instrument or if you'd rather not use HDR detection profile, uncheck the box next to HDR. Doing so reverts all instrument functions to a pre-Compass 4.0 state. Under this detection profile, the exposure times can be edited. For non-RePlex assays on Jess, when the High Dynamic Range box is unchecked, you also have the option of selecting RePlex Dynamic Range:





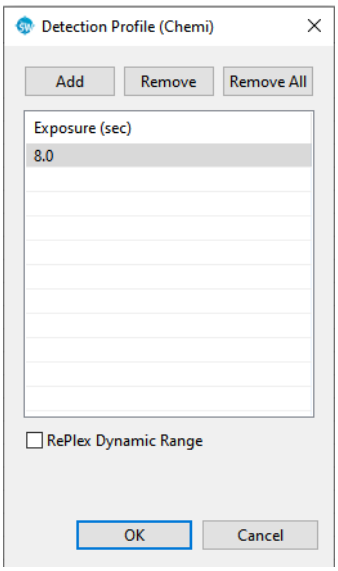
RePlex Dynamic Range Detection Profile

For RePlex assays on the Jess instrument that use chemiluminescent detection, RePlex dynamic range (RDR) is the default detection profile. Exposure times for RDR use the short exposure times from HDR, eliminating the 128 and 512 second exposures. Below is an example of different exposure settings:



NOTE: See "Images Analysis Settings" on page 293 to find out more about how data sets are analyzed under HDR and standard detection profiles.

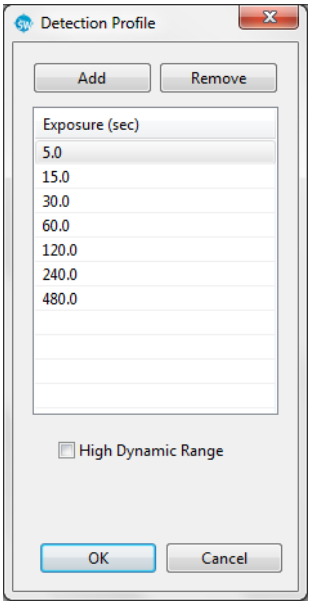
If you'd rather not use the RDR detection profile, uncheck the box. For more information on RDR, please refer to Simple Western assay optimization guidelines. The exposure times can then be manually added and edited. For RePlex assays, up to 7 exposures may be added to the Detection Profile.



Changing the Detection Profile

While we recommend using the default detection profile, you can change the profile, exposure times and exposure sequences if needed. To do this:

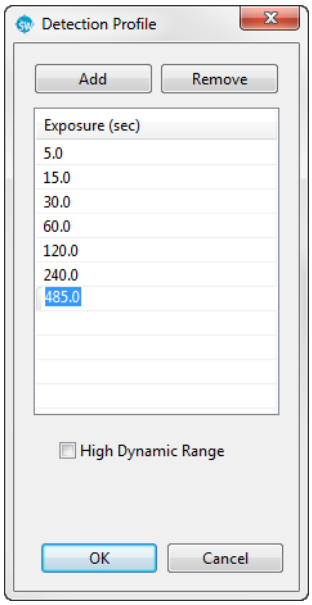
1. Select the exposures cell in the Protocol pane and click the button, or double-click in the cell. The following screen displays:



NOTE: Assays created and saved with Compass v2.7 or Compass for Simple Western v3.0 will have HDR unchecked by default. If you want HDR to be the default detection profile for that assay, check the box next to HDR in the Detection Profile, then re-save the assay.

---

a. **To change an existing exposure time (applies for NIR and IR, and when HDR or RDR is unchecked for chemiluminescent detection)** - Click in the exposure cell and enter a new time in seconds:



b. **To delete an existing exposure** - Select a type or exposure cell and click **Remove**.

c. **To add a new exposure** - Select **Add**. A new exposure will be added to the end of the list. Click in the exposure cell and enter an exposure time in seconds.

2. Click **OK** to save and exit.

Copying Protocols and Templates

The steps and parameters in the Protocol pane can be copied and pasted into other documents, as can the graphic image of the annotations in the Template pane.

Copying an Assay Protocol

1. Click on the Protocol tab.
2. Select **Edit** in the main menu and click **Copy**.
3. Open a document (Microsoft® Word®, Excel®, etc.). Right click in the document and select **Paste**. All assay protocol steps and parameters will be copied into the document as a list in the same format shown in the Protocol pane.

Copying an Assay Template

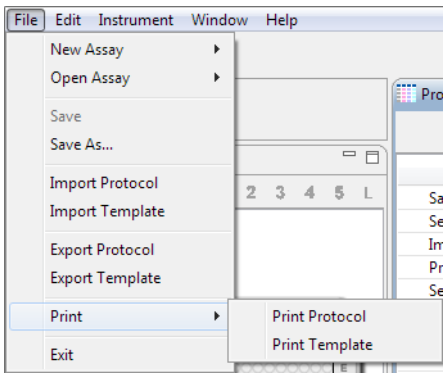
1. Click on the Template tab.
2. Select **Edit** in the main menu and click **Copy**.
3. Open a document (Microsoft® Word®, Excel®, etc.). Right click in the document and select **Paste**. The assay template will be copied into the document as an image, exactly as it is shown in the Template pane.

Printing Protocols and Templates

The information in the Protocol pane can be printed, as can a graphic image of the information in the Template pane.

Printing an Assay Protocol

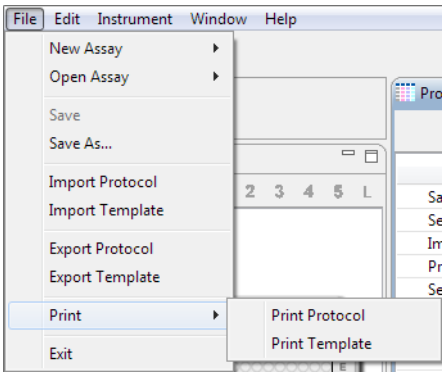
1. Click on the Protocol tab.
2. Select **File** in the main menu, click **Print**, and then click **Print Protocol**.



All assay protocol steps and parameters will be printed as a list in the same format shown in the Protocol pane.

Printing an Assay Template

1. Click on the Template tab.
2. Select **File** in the main menu, click **Print**, and then click **Print Template**.



The assay template will print as an image, exactly as it is shown in the Template pane.

Importing and Exporting Protocols and Templates

The assay protocol in an open assay or run file can be exported as a separate file, as can the assay template annotation information. This allows the same assay protocol and template information to be imported into another assay at a later time, rather than having to re-enter assay protocol and template information manually.

Importing an Assay Protocol

NOTE: Importing an assay protocol imports information into the Protocol pane only.

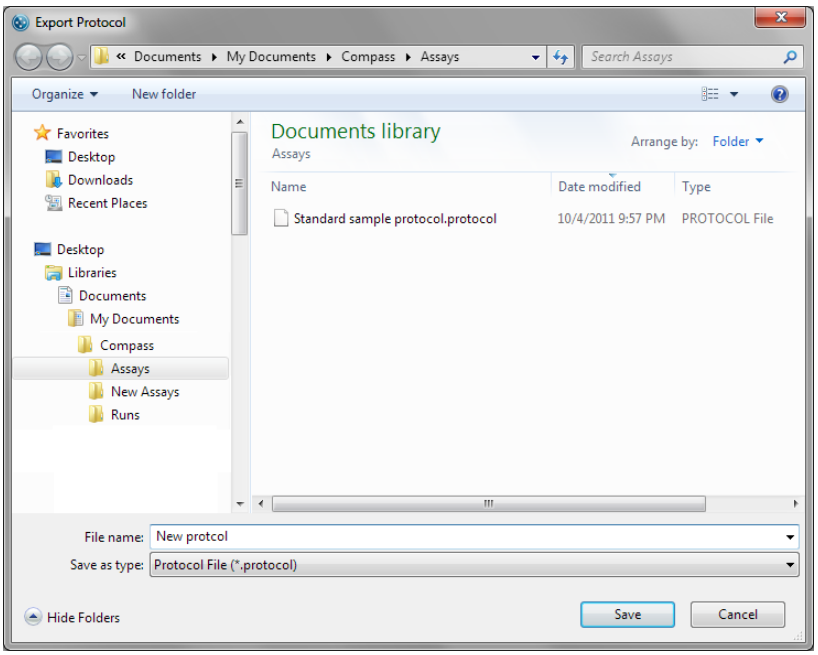
---

1. Open the assay you want to import the assay protocol in to.
2. Select **File** in the main menu and click **Import Protocol**.
3. Select a protocol file (*.protocol) and click **OK**. The imported information will display in the Protocol pane.

Exporting an Assay Protocol

NOTE: Exporting an assay protocol exports information in the Protocol pane only.

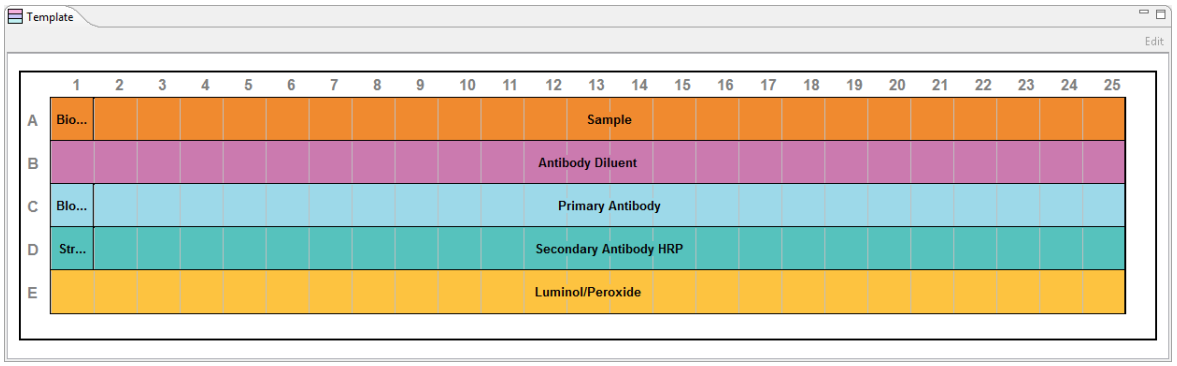
1. Open the assay you want to export the assay protocol from.
2. Select **File** in the main menu and click **Export Protocol**. The following window displays:



3. The default directory is Compass/Assays. Change the directory if needed.
4. Enter a protocol name and click **Save**. The protocol will be saved as a *.protocol file.

Template Export and Import

The Template information including the sample and reagent names can be exported to a file. As an example, a default Jess/Wes Template is displayed in the Assay view.



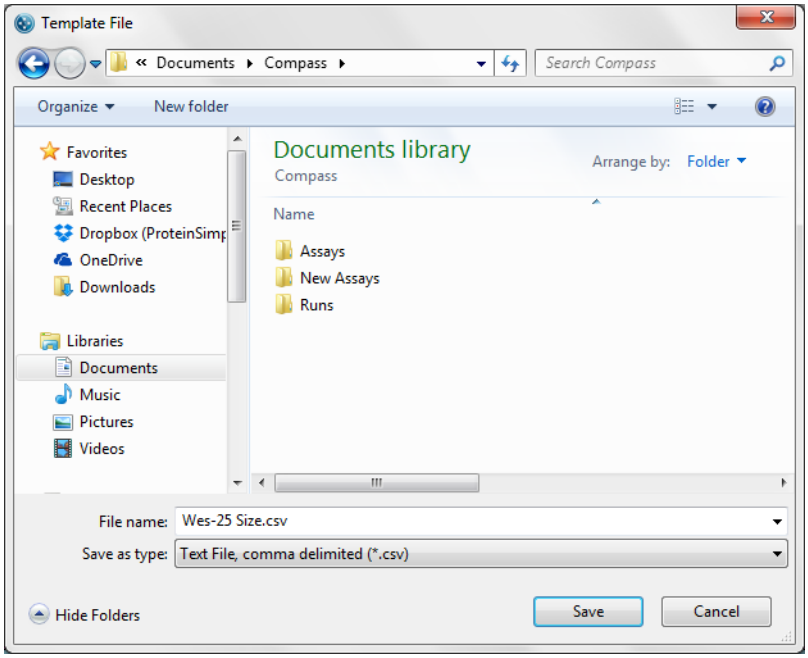
There are three file format options:

- A comma separated CSV file that is best opened in a spreadsheet.
- An XML template file that has full control over the sample and reagent names and layout.
- A tab delimited TXT file that can be opened in a spreadsheet even when the decimal separator is set to a comma.

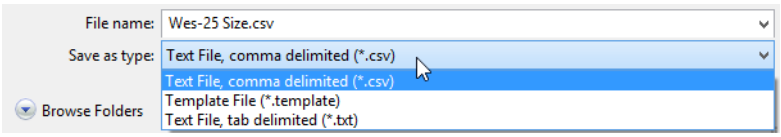
Exporting the Template to a CSV file

Follow the steps below to easily import a template into Compass for Simple Western, populating it with reagent names (sample, antibody, etc.) and their corresponding attributes (concentration, dilution factor, etc.). This example uses a Jess/Wes Template, but the same steps can be followed for a Sally Sue or Peggy Sue plate template.

1. Select **File** in the main menu and click **Export Template**. The following window displays:



Make sure the Save as type is set to CSV.



2. Enter a protocol name and click **Save**. The protocol will be saved as a *.csv file.
3. Open the CSV file in a spreadsheet program like Microsoft® Excel®.

	A	B	C	D	E	F
1	Biot. Ladder	Sample	Sample	Sample	Sample	Sample
2						
3	Antibody Diluent	Antibody Diluent	Antibody Diluent	Antibody Diluent	Antibody Diluent	Antibody Diluent
4						
5	Blocking	Primary Antibody	Primary Antibody	Primary Antibody	Primary Antibody	Primary Antibody
6						
7	Streptavidin HRP	Secondary Antibody HRP	Secondary Antibody HRP	Secondary Antibody HRP	Secondary Antibody HRP	Secondary Antibody HRP
8						
9	Luminol/Peroxide	Luminol/Peroxide	Luminol/Peroxide	Luminol/Peroxide	Luminol/Peroxide	Luminol/Peroxide

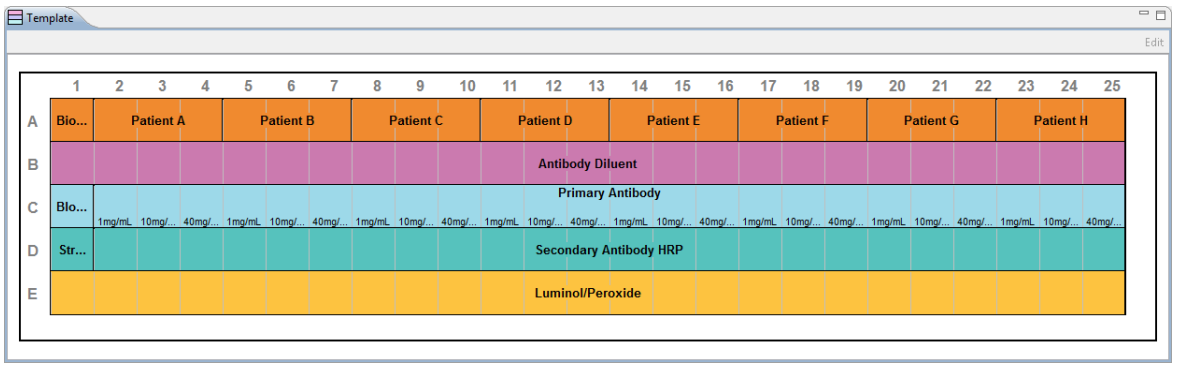
The names in the spreadsheet are arranged in the same order as the Compass Template, and the rows alternate between names and attributes.

NOTE: The default assay has no attributes so these rows will be empty.

4. Edit the names and add attributes, then save the spreadsheet as a CSV file.

NOTE: Make sure not to edit the first column of the spreadsheet, this corresponds to the ladder wells.

5. To import the edited CSV file into Compass for Simple Western, select **File** in the main menu, click **Import Template** and then browse to the .cvs file you just saved. Once imported, the edited CSV file displays the edited Sample names and Primary Antibody with attributes in the Template.

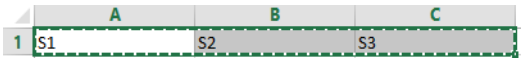


Template Cut and Paste

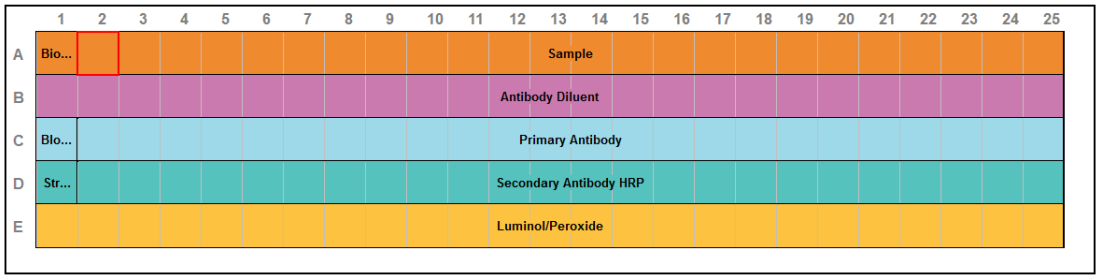
The names and attributes in the Template can be copied and pasted within Compass for Simple Western, between two copies of Compass, and between Compass and a spreadsheet like Microsoft® Excel®.

All you need to do is enter names in a spreadsheet row, then copy to the clipboard. Next, select a well in the Template and paste from the clipboard. The new names will be pasted into the row at the selected column and columns to the right. Here's a quick example of how to do it:

1. Copy these three rows from a spreadsheet:

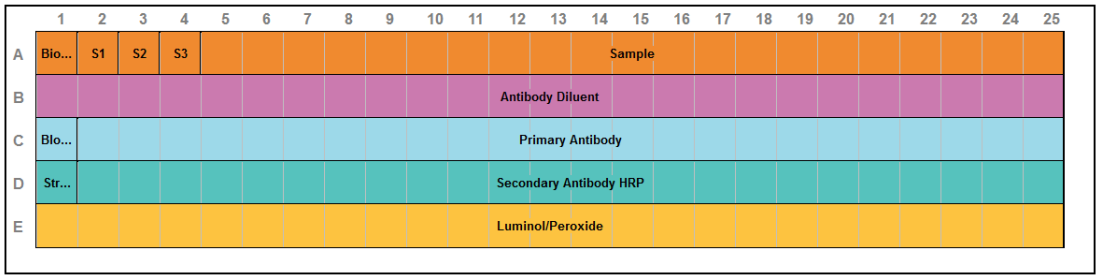


2. Select a well in the Template, A2 is selected for this example:

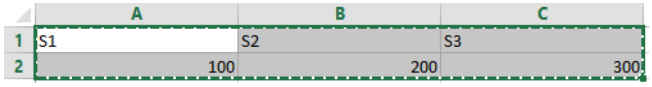


3. Paste the names from the clipboard.

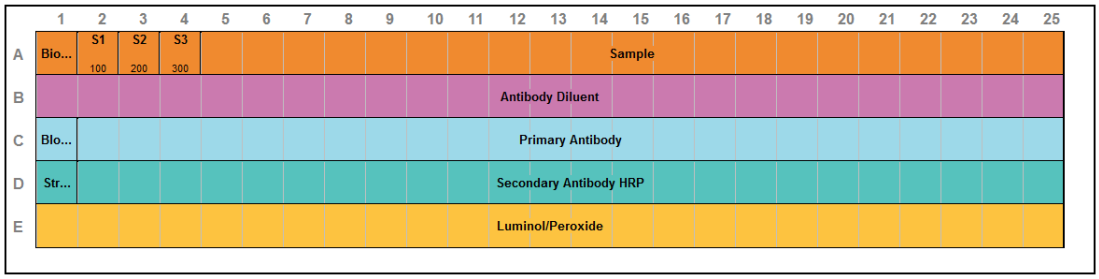
Note: Make sure not to select any of the ladder wells (A1, B1, C1, etc.)



4. To include attributes, add them in the next row:



5. Pasting into the Template will update the names and attributes.

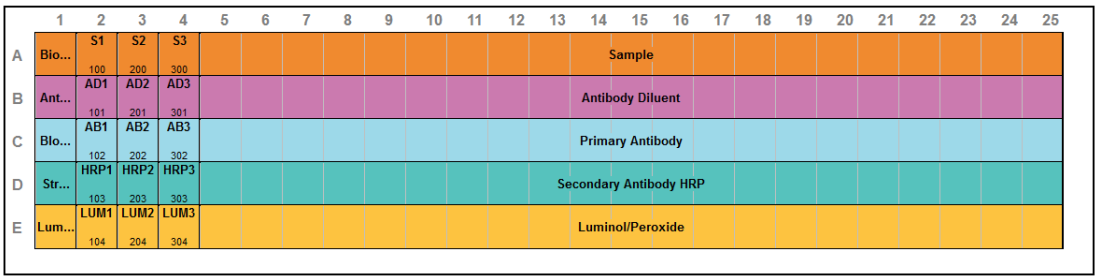


Multiple rows can also be copied and pasted with names and attributes on alternate rows:

	A	B	C
1	S1	S2	S3
2	100	200	300
3	AD1	AD2	AD3
4	101	201	301
5	AB1	AB2	AB3
6	102	202	302
7	HRP1	HRP2	HRP3
8	103	203	303
9	LUM1	LUM2	LUM3
10	104	204	304

Only wells in the same row can be selected, but multiple rows will be pasted into the rows below.

Note: Make sure not to select any of the ladder wells (A1, B1, C1, etc.)



Chapter 3:

Running a Size Assay

Chapter Overview

- Starting a Run
- Stopping a Run

Starting a Run

Step 1 - Get Ready

1. Create or open an assay file in Compass for Simple Western.
2. Prepare the assay plate using the information provided in the product insert.

IMPORTANT

To prevent well evaporation and ensure best results, keep a lid on the assay plate until ready to use.

---

3. Prepare the instrument following the procedure described in the User Guide.

IMPORTANT

Capillaries are light sensitive. Keep them covered until you are ready to transfer the capillary cartridge or box to the instrument.

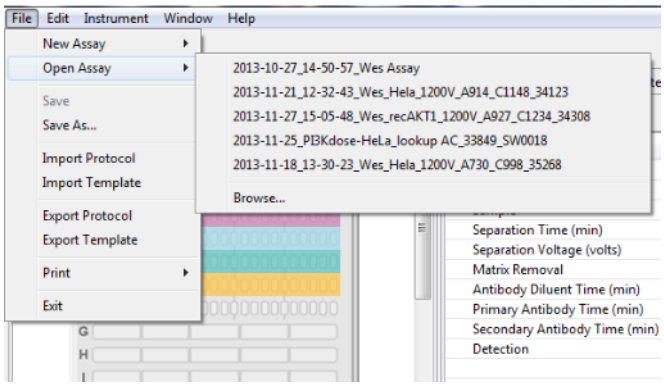
---

4. Place assay plate into the sample tray of the instrument.

Step 2 - Start the Run

You can start a run in one of two ways depending on whether you want to run an assay using existing parameters or set up a new assay.

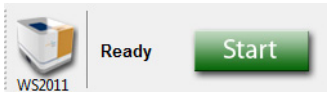
1. New run of an existing assay:
   a. Select **File** in the main menu and click **Open Assay**.



b. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different assay.

2. Alternatively, choose **New Assay** and select one of the size assays to get the default assay conditions for using Jess, Wes, Sally Sue or Peggy Sue.

   a. The **Start** button will display. This indicates that an assay has been loaded.

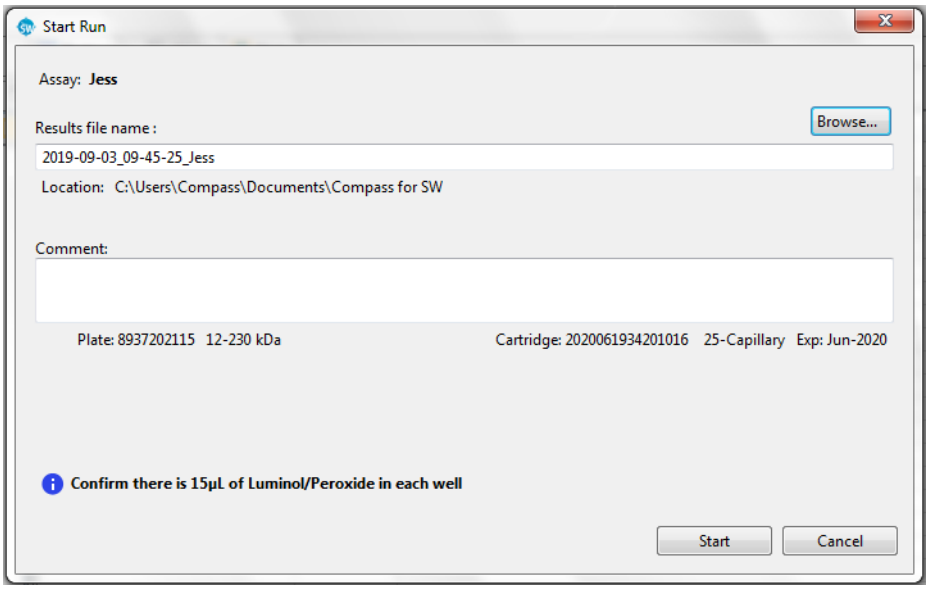


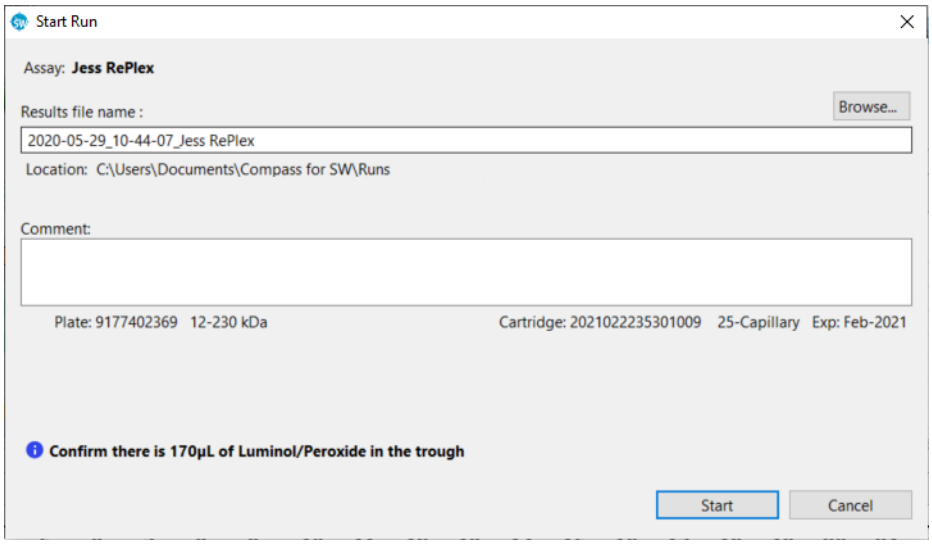
   a. Go to the Assay screen and verify this is the assay you want to use. If not, select **File** in the main menu, click **Open Assay**, and select another assay.

3. Click **Start** to begin the run.

For Jess and Wes:

The Start Run window for immunoassays displays with the default run file name and location. In the example below, Start Run windows for a standard Immunoassay and a RePlex Assay are shown. You can edit the file name and/or click **Browse** if you want to save it in a different location:





---

*NOTE: For Jess runs, if the cartridge type or plate installed in the instrument doesn't match the assay selected for the run messages indicating this will display in the Start Run window. If this happens, click **Cancel** and adjust your assay settings before starting the run again.*

Click **Start** to begin the run. Instrument status will change to Running, the Start button will change to Stop and the Run Progress bar will display:

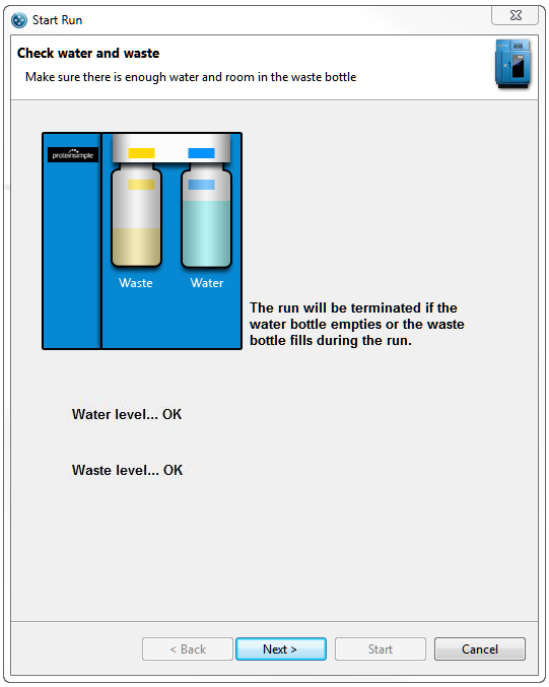
For Sally Sue/Peggy Sue:
This will launch the **Start Run Wizard** (continue on to the next step).

---

*NOTE: If you didn't clean the manifold in Sally Sue or Peggy Sue prior to starting the run, a message will appear. If you see this message, click **Yes** to cancel the run and perform the manifold cleaning.*

---

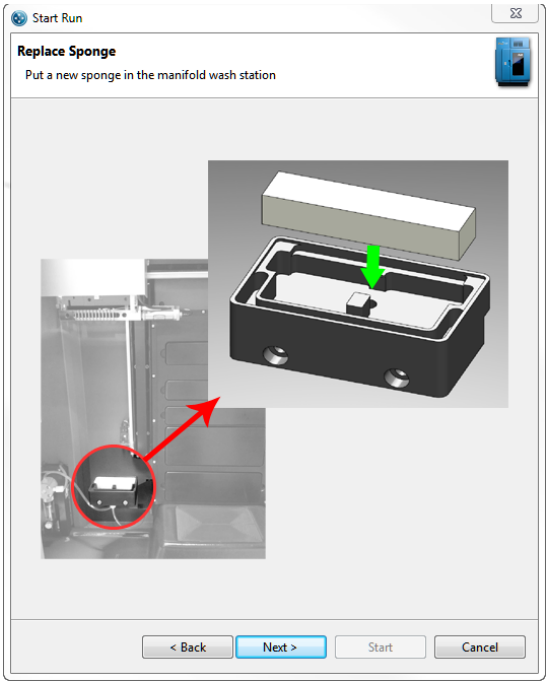
4. **Check Water and Waste.** The fluid levels in the accessory module bottles will be checked by the software. If the level in both bottles will allow Sally Sue an Peggy Sue to complete the run, the wizard screen will display **Water Level OK** and **Waste Level OK** messages. Click **Next** to proceed.



NOTE: If the waste level is too high or the water level is too low to complete the run, messages to indicate one or both will be presented in this screen. If this occurs, fill or empty each bottle as indicated using the procedures outlined in the Sally Sue/Peggy Sue User Guide. When this is complete, the error status will be automatically updated and allow the ***Start Run Wizard*** *to proceed.*

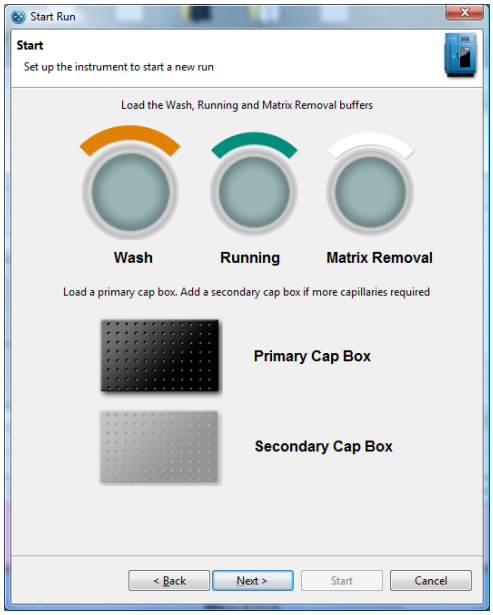
---

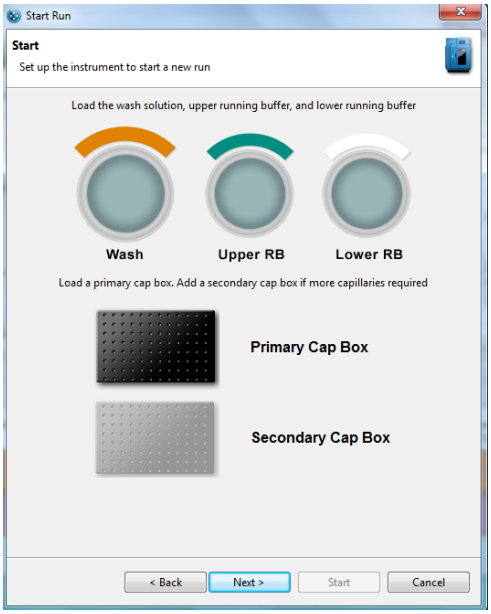
5. **Replace Sponge.** You should use a new sponge each time a new experimental run is started. Discard the old sponge and put a new sponge in the manifold wash station. Click **Next** to proceed.



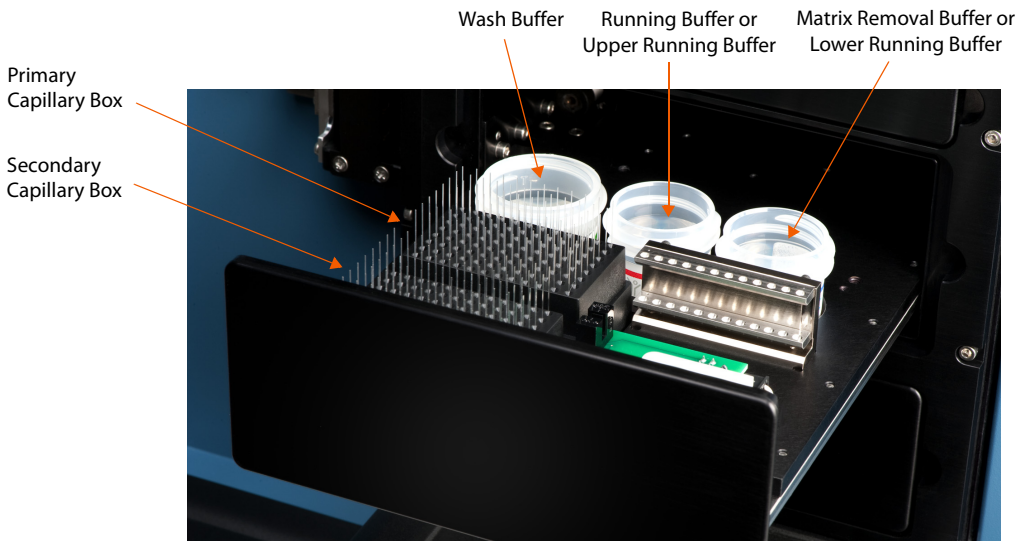
6. **Start.** The resource tray will automatically open. Fill the Wash Buffer, Running Buffer and Matrix Removal Buffer cups and insert the capillary box in the primary capillary box position. Add a secondary capillary box if more capillaries are required. Click **Next** when finished. The resource tray will close.

NOTE: Make sure enough capillaries are loaded to complete the run. A capillary box should be inserted in the primary position first and if additional capillaries are needed, use the secondary position. When the primary box is depleted, Sally Sue or Peggy Sue will automatically move to the secondary box. Discard left-over Running Buffer, Wash Buffer and Matrix Removal Buffer prior to refilling bottles for new run.

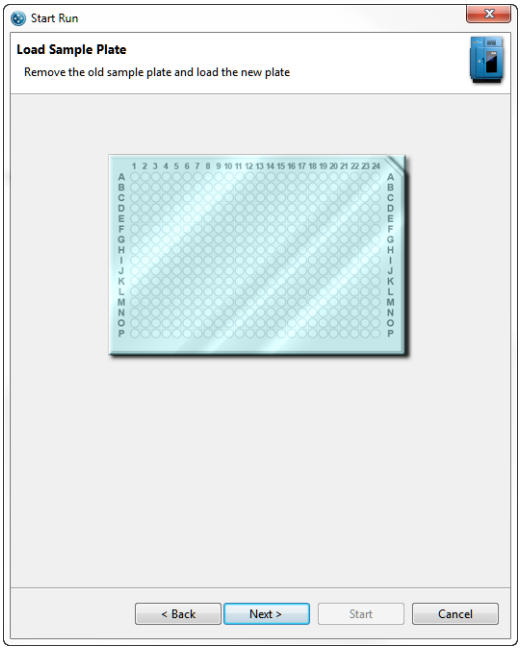


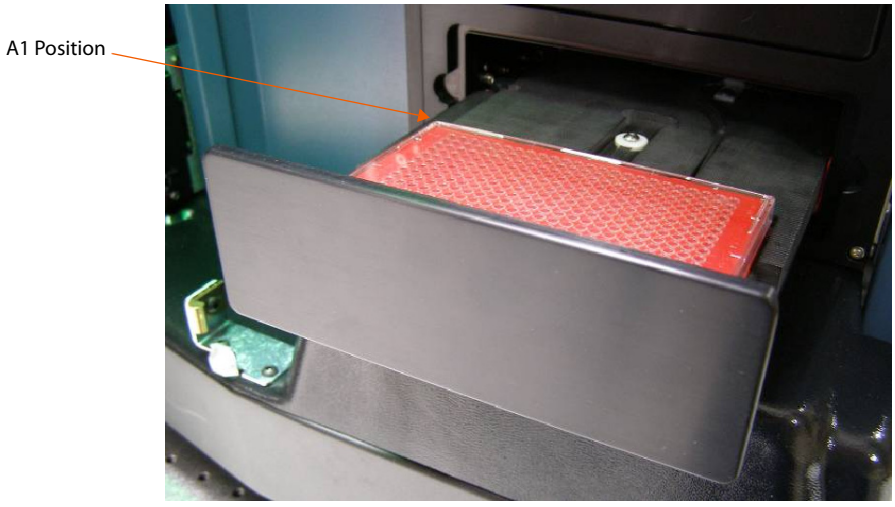


NOTE: You can also refer to the labels on the resource tray for proper insertion of reagents.



7. **Load Sample Plate.** The sample tray will automatically open. Place the lidded 384-well plate on the cooling block in the tray, ensuring that the A1 well position is aligned with the upper left corner of the cold block. When this is complete, click **Next**. The sample tray will close.



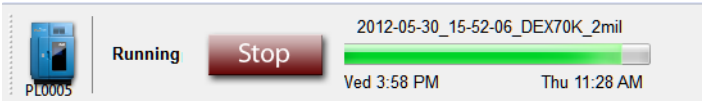


NOTES:
Plate lids must be used on sample plates for Sally Sue and Peggy Sue. If a lid is not detected, a message will be displayed in the Start Run Wizard. Compass for Simple Western will reopen the sample tray to allow you to place the lid on the sample plate.

When inserting the sample plate, ensure that the plate is firmly seated and level on the cold block. Plates that are not level can interfere with the movement of the sample tray.

8. **Begin the Automated Run.** The data file name will automatically default to the assay name appended with the current date and time. To change the file name, begin typing in the text box. To change the directory where the data file will be stored, click **Browse**:

Click **Start** to begin the run. Instrument status will change to **Running**, the **Start** button will change to **Stop** and the **Run Progress bar** will display:



The run will continue until complete (~14-19 hours depending on the assay).

Step 3 - Post-Run Procedures

1. Remove the capillary cartridge (Jess/Wes) or empty the capillary discard tray (Sally Sue/Peggy Sue).
2. Remove the assay plate.
3. Dispose of the assay plate and capillaries. Disposal will depend on the samples that have been assayed. If sample origins are unknown, we recommend that used capillaries cartridges, capillaries and plates be disposed of as biohazard waste.

!WARNING! SHARPS HAZARD

The capillaries may present a potential sharps hazard. Dispose of used capillaries in biomedical waste sharps containers.

!WARNING! BIOHAZARD

Samples and waste bottle contents should be handled by procedures recommended in the CDC/NIH manual: Biosafety in Microbiological and Biomedical Laboratories (BMBL). The manual is available from the U.S. Government Printing Office or online at http://www.cdc.gov/biosafety/publications/bmbl5/.

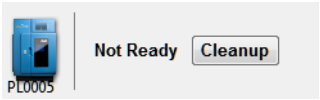
Depending on the samples used, waste bottle contents may constitute a biohazard. Use precaution when emptying the waste bottle. Dispose of waste bottle contents in accordance with good laboratory practices and local, state/provincial and national environmental and health regulations. Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste vial before you store, handle or dispose of chemical waste.

Stopping a Run

1. To stop a run, click **Stop**.

   a. **Jess/Wes:** When the run stops, the Start button with reappear and the instrument will start a process that plugs the capillaries in the cartridge. Once that's done, you can then remove the capillary cartridge and plate and discard them. The stopped status, date and time will display in the Run Summary screen.

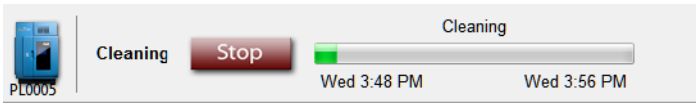
   b. **Sally Sue/Peggy Sue:** When the run stops, Sally Sue's or Peggy Sue's status will go to **Not Ready** and a **Cleanup** button displays:



NOTE: If a run is stopped prior to completion, Sally Sue and Peggy Sue must perform a cleaning protocol to remove used capillaries from system trays. This is required to prepare the system for the next run.

---

2. Click **Cleanup**.



Allow Sally Sue and Peggy Sue to complete the cleaning protocol which takes about ten minutes. When complete, instrument status will change to **Ready** and a new run can be started.

Chapter 4:

Imaging a Blot on Jess

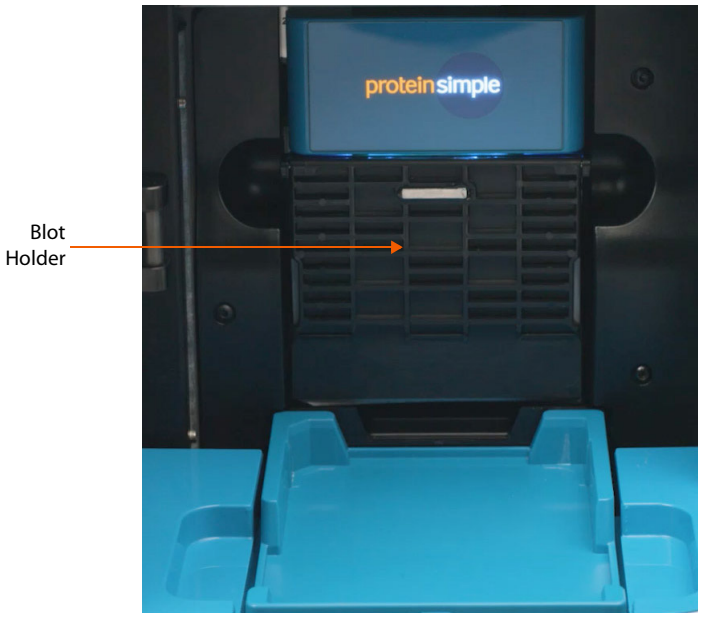
Chapter Overview

- Starting a Run

Starting a Run

Step 1 - Get Ready

1. Prepare the membrane and assemble the blot cartridge following the product insert.
2. Open Compass for Simple Western software on your desktop.
3. Make sure that the software is connected to the instrument.
4. Insert the blot cartridge into the blot holder. The light in the blot holder will change from orange to blue when correctly seated.
5. Close the instrument door by gently pushing it until you feel Jess pull it closed.

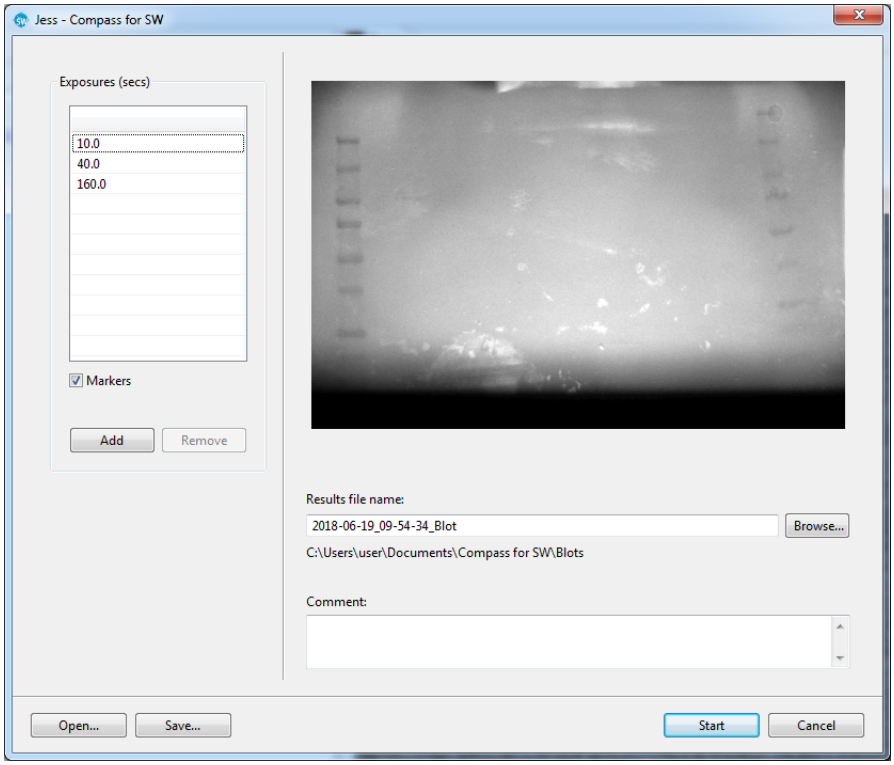


The software will automatically change to the Blot screen:

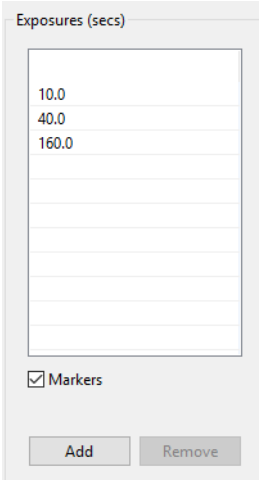
Step 2 - Start the Imaging Run

NOTE: Jess's door must be closed before starting the imaging run.

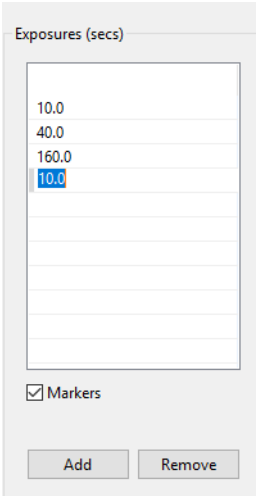
Once the blot cartridge is installed and Jess's door is closed, Compass for Simple Western will automatically provide a preview image.



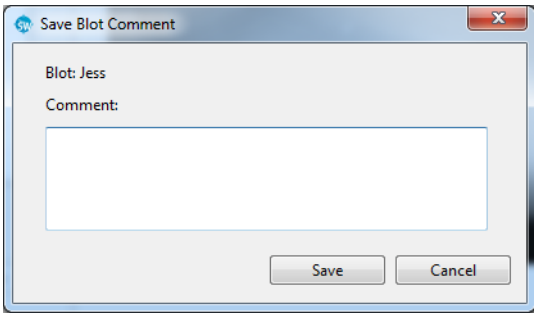
1. Edit the exposure settings in the Exposures table.
   - **To add an exposure:** Click **Add** then select the exposure time to change it.
   - **To remove an exposure:** Select the exposure and click **Remove**.
   - **To edit exposure time:** Click on an exposure and enter a new time.



2. Check the **Markers** box if the blot contains a molecular weight ladder.



3. To load the saved settings from an existing blot imaging protocol file (*.blot), click **Open**. Select the protocol file and click **Open**.
4. You can change the Results File name and location if desired. Imaging file names are automatically generated as date_time_blot.btz and saved in the Compass for SW\Blots folder of your My Documents folder.
5. Optional: Add any comments you would like saved with your imaging run in the **Comments** box and click **Save**.

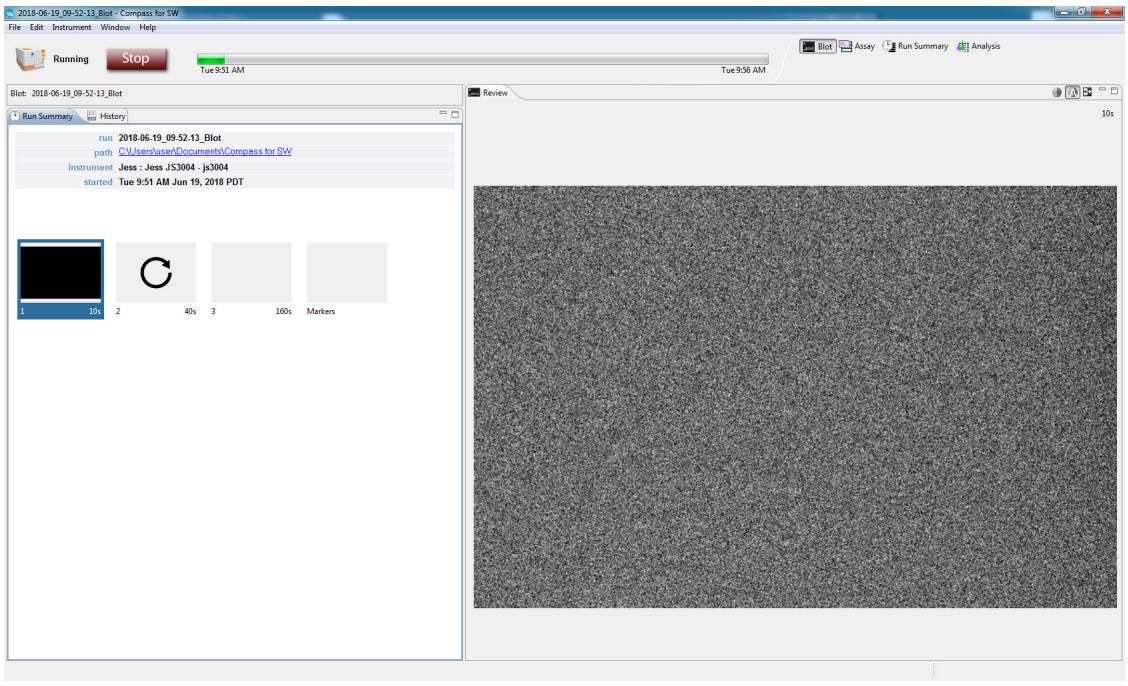


6. Optional: To save the settings associated with this blot imaging protocol for future use, click **Save**.
7. Click **Start** to begin imaging. .

Step 3 - Imaging Run Summary and Analysis

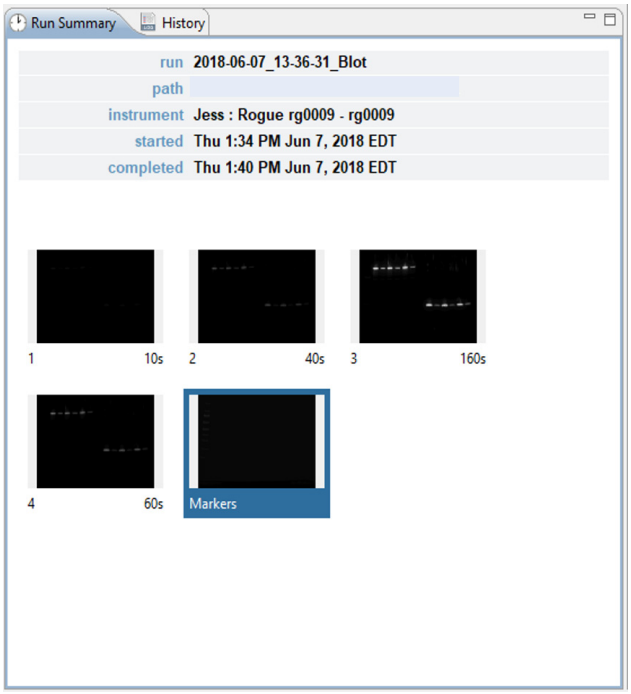
Reviewing Imaging Data

When the imaging run starts, thumbnails are shown in the Run Summary screen.



As images are acquired, they populate the predefined thumbnail boxes and also display in the Review pane. Unless you click on a thumbnail, the most recently acquired image displays in the Review pane. Images that haven't been taken yet will display blank until the image is acquired.

The Run Summary pane will display thumbnail images for each exposure configured in the imaging protocol:



NOTE: The Markers image is taken at the end of the imaging run. If you click Stop before all exposures are taken, the software will prompt you to confirm that you want to stop the run before the Markers image is taken.

Adjusting Blot Images

You can adjust the blot image using the Review pane toolbar.

The toolbar has the following options:

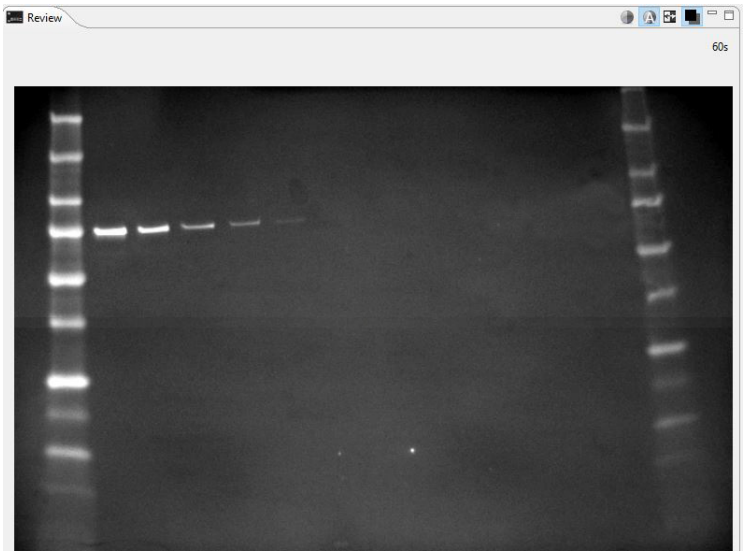
 Contrast Adjustment

 Auto Contrast

 Invert

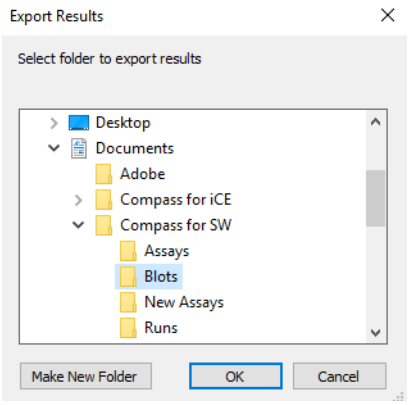
 Overlay Markers

An unadjusted blot image is shown in the following example:



Exporting Image Files

To export raw images for further analysis, from the main menu, select **File** > **Export Images**. Then select a folder to export the files to.



Compass for Simple Western will automatically launch a Windows Explorer window for you to access the saved files. Images are saved in .png format, and both raw and adjusted images are saved in the folder.

- **Raw chemiluminescence images are saved as:** <Results File Name>_Chemi_<Exposure Number>_<Exposure Time>.
- **Processed images, as shown in the Review pane, are saved as:** <Results File Name>_<Exposure Time>_view.
- **Raw and processed marker images are saved as:** <Results File Name>_Markers and <Results File Name>_Markers_view, if selected during the blot imaging protocol.

Step 4 - Post-Imaging Procedures

When imaging is done, you'll need to:

1. Remove the blot cartridge.
2. Remove your membrane and clean the blot cartridge by rinsing it with water. Dry with a lint-free wipe.
3. Wipe down the area underneath the blot cartridge in the instrument with a dry lint-free wipe.
4. Keep the blot cartridge in a dry, clean area protected from dust.

Stopping a Run

To stop an imaging run, click **Stop** and confirm your choice. When the run stops, the Start button will reappear so you can start another run when you're ready.

Chapter 5:

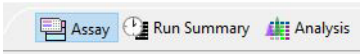
Charge Assays

Chapter Overview

- Assay Screen Overview
- Reagent Color Coding
- Opening an Assay
- Creating a New Assay
- Making Changes to an Existing Assay
- Switching Between Open Assays
- Creating a Template Assay
- Viewing and Changing the Detection Exposures
- Copying Protocols and Templates
- Printing Protocols and Templates
- Importing and Exporting Protocols and Templates

Assay Screen Overview

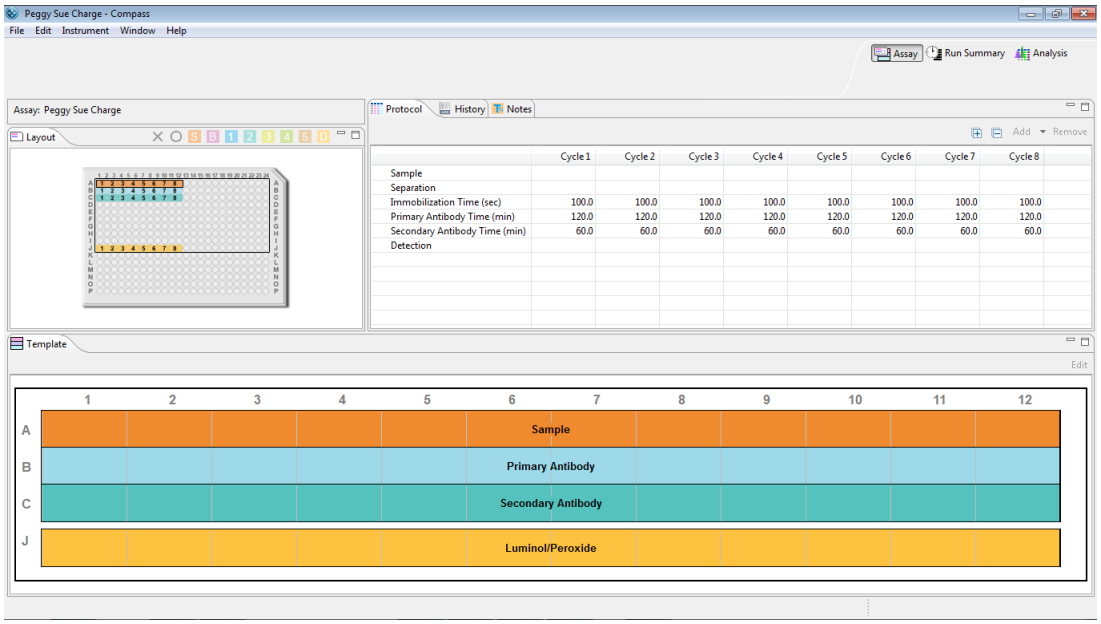
The Assay screen is used to create, view and edit assays. To access this screen, click **Assay** in the screen tab:



Assay Screen Panes

The Assay screen has four panes:

- **Layout** - Displays a map of the assay plate and shows where assay reagents will be located.
- **Protocol** - Lists individual assay protocol steps and parameters that Peggy Sue will execute for each of the 12 capillaries simultaneously.
- **Notes** - Lets you enter specific assay information that is saved with the assay and can be used for future reference.
- **Template** - Enter annotations for the individual well and row reagents in the assay plate.



Software Menus Active in the Assay Screen

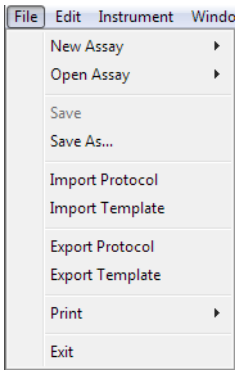
The following software menus are available:

- File
- Edit
- Instrument (when Compass for Simple Western is connected to Peggy Sue)
- Window
- Help

The File and Edit menu options specific to the Assay screen are described next.

File Menu

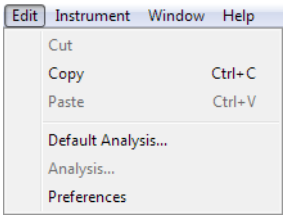
The following File menu options are active:



- **New Assay** - Creates a new assay from a starter template.
- **Open Assay** - Opens an existing assay.
- **Save** - Saves the open assay.
- **Save As** - Saves the open assay under a different file name.
- **Import Protocol** - Imports a saved protocol file into an assay.
- **Import Template** - Imports a saved template file into an assay.
- **Export Protocol** - Exports the current protocol file for future use.
- **Export Template** - Exports the current template file for future use.
- **Print** - Prints the information in the Protocol or Template panes.
- **Exit** - Closes Compass for Simple Western.

Edit Menu

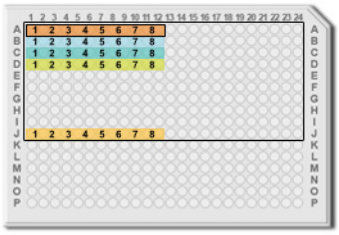
The following Edit menu options are active:



- **Copy** - Copies the information in the Protocol or Template panes into other documents.
- **Default Analysis** - Displays the default settings that will be used to analyze the run data generated with an assay.
- **Analysis** - Not active in this screen.
- **Preferences** - Set and save custom preferences for data export, plot colors in the graph and Peggy Sue's Twitter settings. See Chapter 11, *"Setting Your Preferences"* for more information.

Reagent Color Coding

The Assay screen uses color coding to identify various assay reagents in all panes. The Layout pane is shown in the following example:

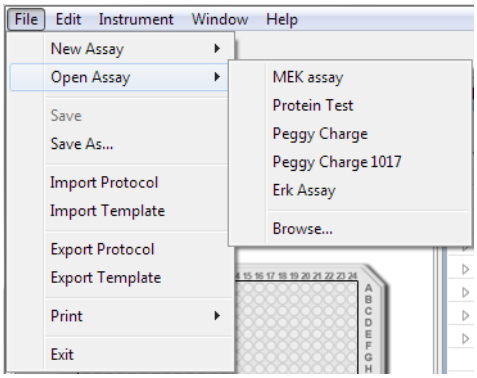


- **Orange** - Samples
- **Light Blue** - Primary antibody
- **Teal** - Tertiary antibody
- **Yellow** - Secondary HRP conjugate
- **Gold** - Luminol/Peroxide mix

Opening an Assay

To open an existing assay:

1. Select **File** in the main menu and click **Open Assay**.



2. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different assay.

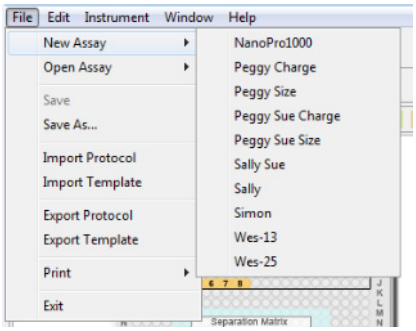
Creating a New Assay

To create a new assay, we recommend using the Peggy Sue template assay and modifying from there as needed.

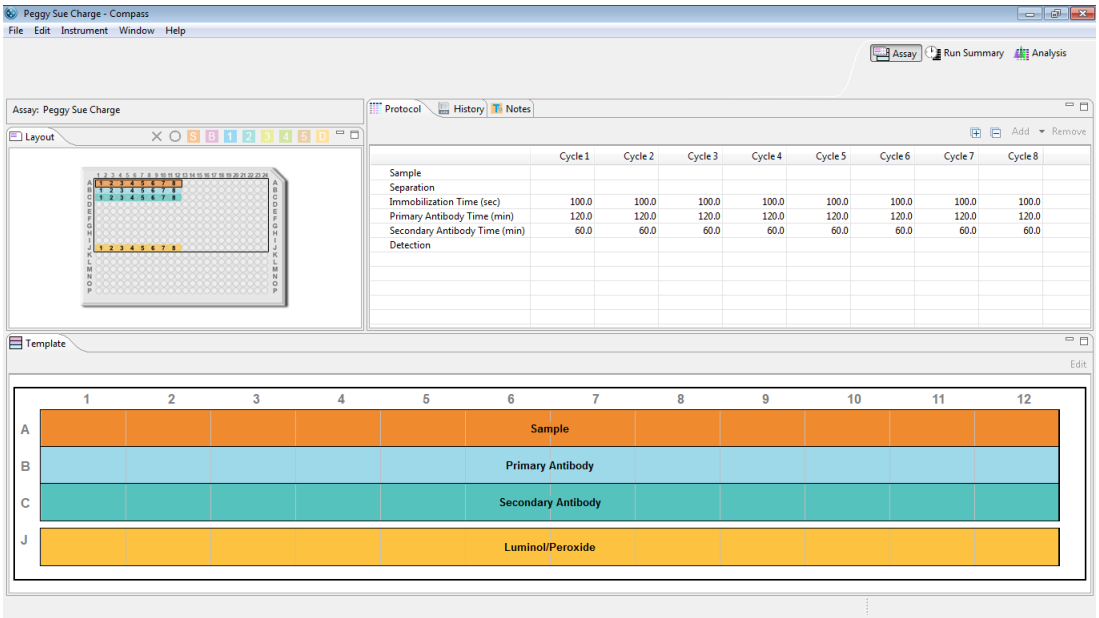
Step 1 - Open a Template Assay

1. Select **File** in the main menu and click **New Assay**:

T



2. A list of template assays that can be used as a starting point for new assays will display. Click **Peggy Sue Charge**. The Peggy Sue template assay and default settings will display in the Assay screen:

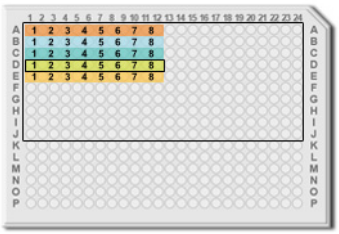


Step 2 - Assign Assay Plate Reagents (Optional)

1. Click on the Layout tab. This pane displays the default row locations of where each reagent should be placed on the assay plate.

NOTE: Up to 96 different samples or conditions (lysates, proteins, antibodies or incubation times) can be assayed using the full eight cycles to accommodate experimental needs.

- **Row A** - Samples

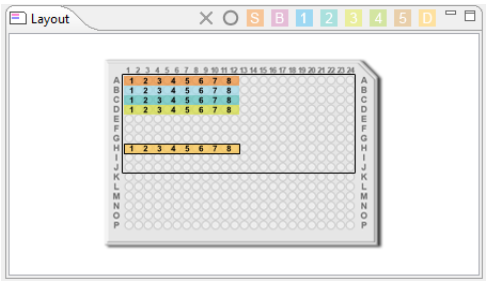


- **Row B** - Primary Antibody
- **Row C**- Secondary Antibody
- **Row D** - Tertiary Antibody (optional)
- **Row E** - Luminol/Peroxide mix

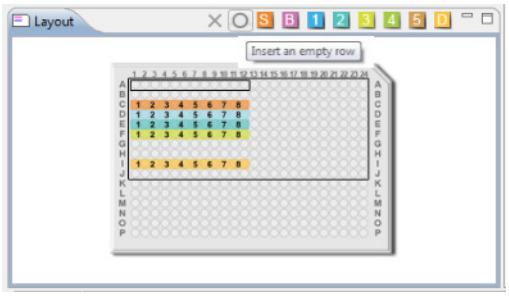
NOTE: For details on sample, reagent and assay plate preparation, please refer to the product insert provided with the Simple Western kits.

---

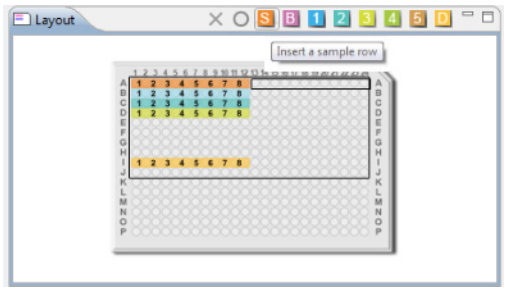
2. If needed, well assignments can be modified as described below. Any row assignments changed in the **Layout** pane are updated in the Protocol pane automatically.
   - **To move a reagent row to another location** - Click the row in the **Layout** pane, then drag and drop it on the new location. The row from which it was moved will be reassigned as empty.



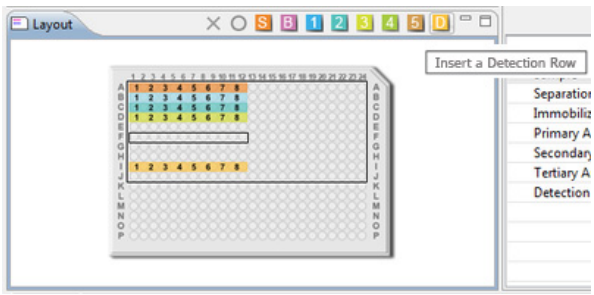
- **To insert a new row** - Click the row below where the new one should be inserted, then click **Insert an empty row** (circle icon) in the **Layout** pane toolbar. A new row will be inserted above the selected row.



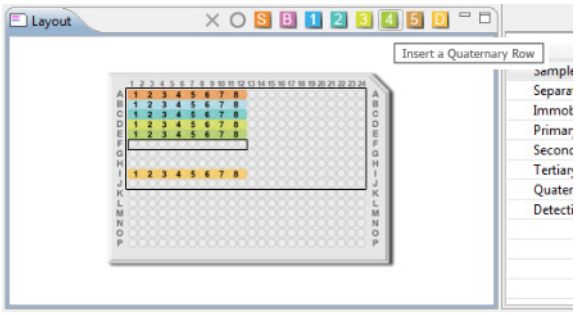
- **To insert a sample row -** Click an empty row or the row below where the new sample row should be inserted, then click **Insert a sample row** (S icon) in the **Layout** pane toolbar. A new sample row will be added in the empty row or inserted above the selected row.



- **To insert a detection row -** Click an empty row or the row below where the new detection row should be inserted, then click **Insert a detection row** (D icon) in the **Layout** pane toolbar. A new detection row will be added in the empty row or inserted above the selected row.



- **To insert a fourth or fifth incubation reagent** - Click an empty row or the row below where the new incubation reagent should be inserted, then click **Insert a Quaternary Row** (4 icon) in the **Layout** pane toolbar. A new incubation reagent row will be added in the empty row or inserted above the selected row.

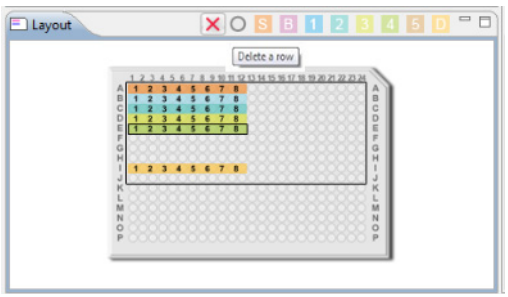


A fifth incubation reagent can now be added by repeating the above and clicking **Insert a Quinary Row** (5 icon) instead.

NOTES:
When using the fourth and fifth incubation steps, only five cycles can typically be performed per run as more water is needed per cycle for the additional wash steps.

Row J is the last row assignment that can be used on the assay plate. New, sample, incubation or luminol rows cannot be inserted if row J already has an assigned reagent. To insert a row in this case, you must first move the contents of row J to another area on the plate.

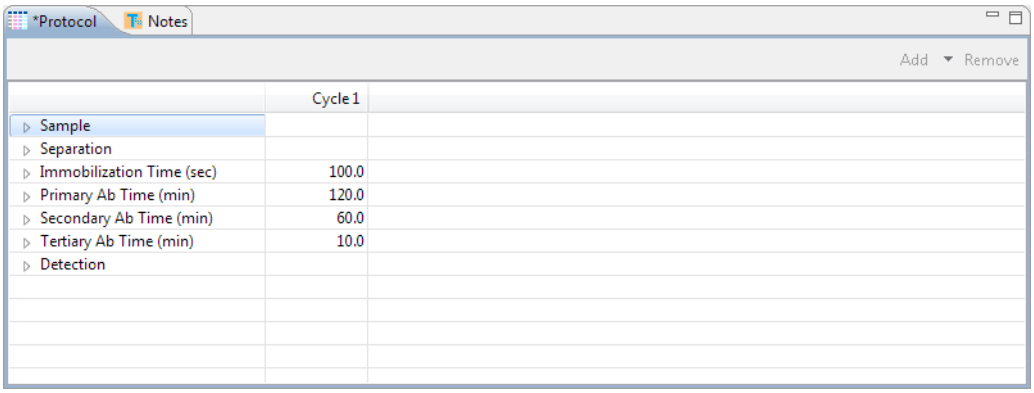
- **To delete a row** - Click the row to be deleted, then click **Delete** (red x icon) in the toolbar. Only empty rows and fourth incubation rows can be deleted. Rows for required assay reagents cannot be deleted.



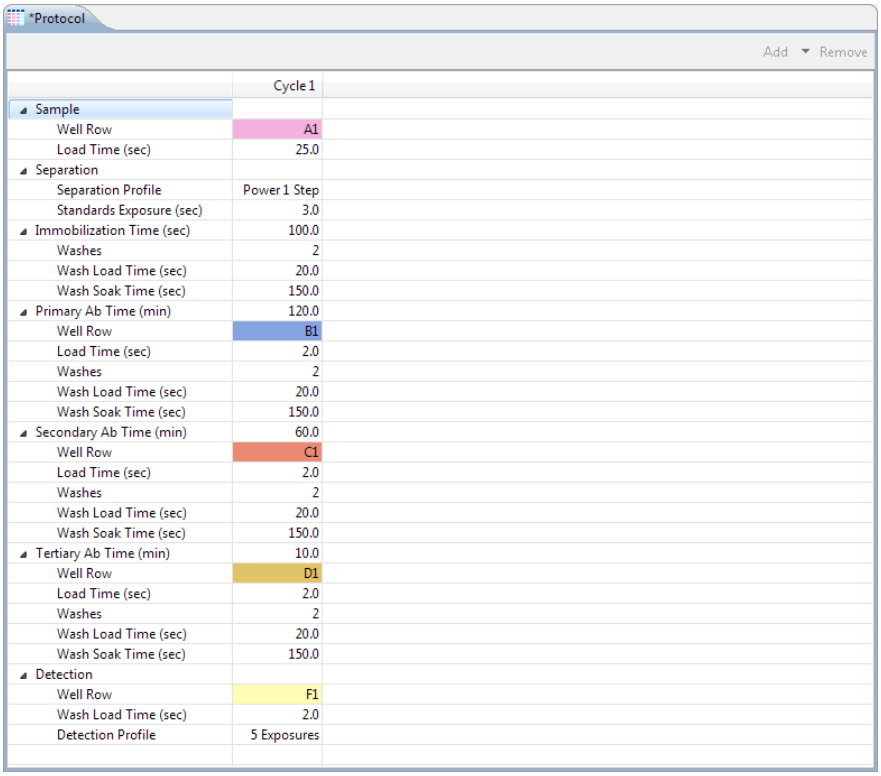
NOTE: Samples, antibodies and blocking buffer can be dispensed in Rows A-J and in columns 1-12 or 13-24. Rows K-P cannot be used for assay reagents.

Step 3 - Modifying the Assay Protocol (Optional)

1. Click on the Protocol tab. This pane displays the individual steps of the assay protocol and allows you to change parameters as needed. When creating a new assay, a default protocol will display which automatically assigns all reagent locations for Cycle 1:



Each row contains an assay protocol step. Each step can contain a reagent assay plate row assignment and one or more parameter settings. To view the details for a step, click on the white arrow next to the step name. We recommend using the default protocol settings for Peggy Sue assays. An expanded list of the default protocol step parameters is shown:

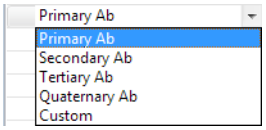


	Cycle 1
Sample	
Well Row	A1
Load Time (sec)	25.0
Separation	
Separation Profile	Power 1 Step
Standards Exposure (sec)	3.0
Immobilization Time (sec)	100.0
Washes	2
Wash Load Time (sec)	20.0
Wash Soak Time (sec)	150.0
Primary Ab Time (min)	120.0
Well Row	B1
Load Time (sec)	2.0
Washes	2
Wash Load Time (sec)	20.0
Wash Soak Time (sec)	150.0
Secondary Ab Time (min)	60.0
Well Row	C1
Load Time (sec)	2.0
Washes	2
Wash Load Time (sec)	20.0
Wash Soak Time (sec)	150.0
Tertiary Ab Time (min)	10.0
Well Row	D1
Load Time (sec)	2.0
Washes	2
Wash Load Time (sec)	20.0
Wash Soak Time (sec)	150.0
Detection	
Well Row	F1
Wash Load Time (sec)	2.0
Detection Profile	5 Exposures

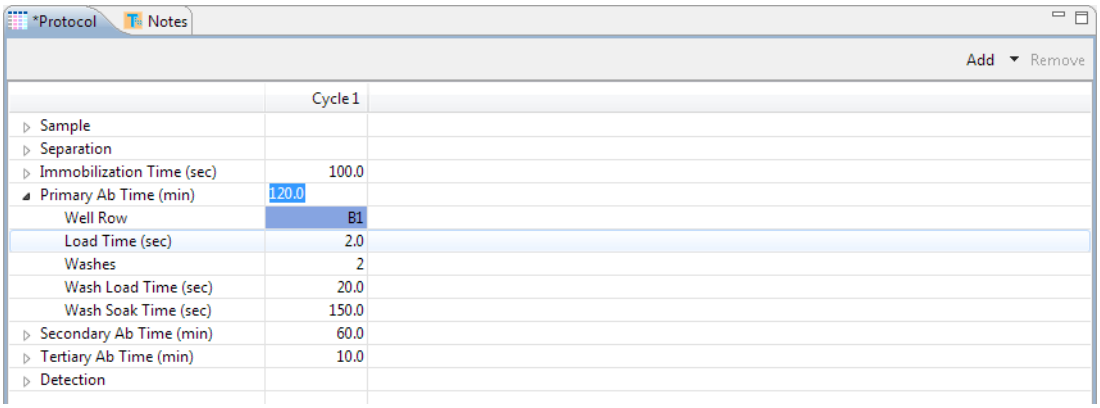
2. Five incubation steps are allowed per protocol. You can select the type of incubation for each step. The available incubation types and their default Simple Western use is as follows:

   - **First incubation** - Primary antibody
   - **Second incubation** - Secondary antibody
   - **Third incubation** - User defined (tertiary antibody)
   - **Fourth incubation** - User defined (quaternary antibody)
   - **Fifth incubation** - User defined (custom)

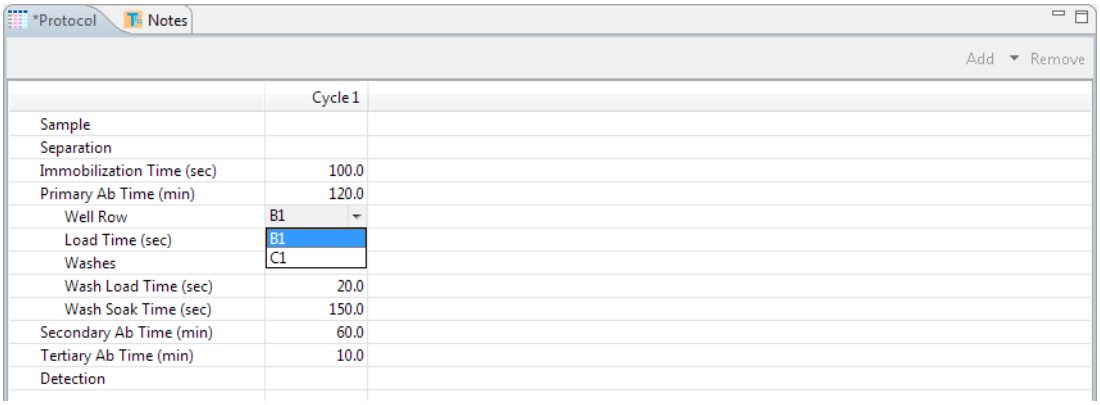
   To change the type, click the incubation step name and select an option from the drop down list.



3. If needed, change the primary incubation time. To do this, click the cell in the value column next to Primary Ab Time (min) and enter a new value in minutes:



4. If needed, change the primary incubation reagent row location. To do this, click the cell in the value column next to Well Row and select a different row on the assay plate:



NOTE: Only rows designated as primary antibody in the Layout tab can be selected in the Well Row drop-down menu.

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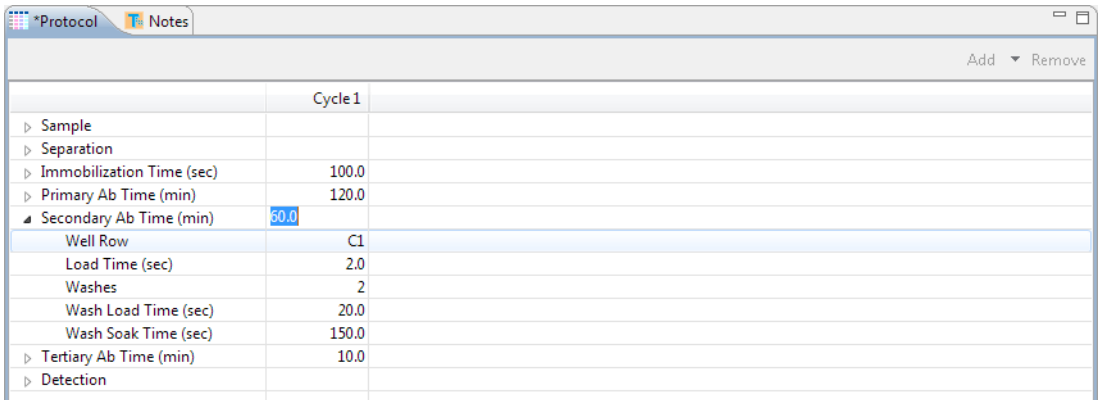
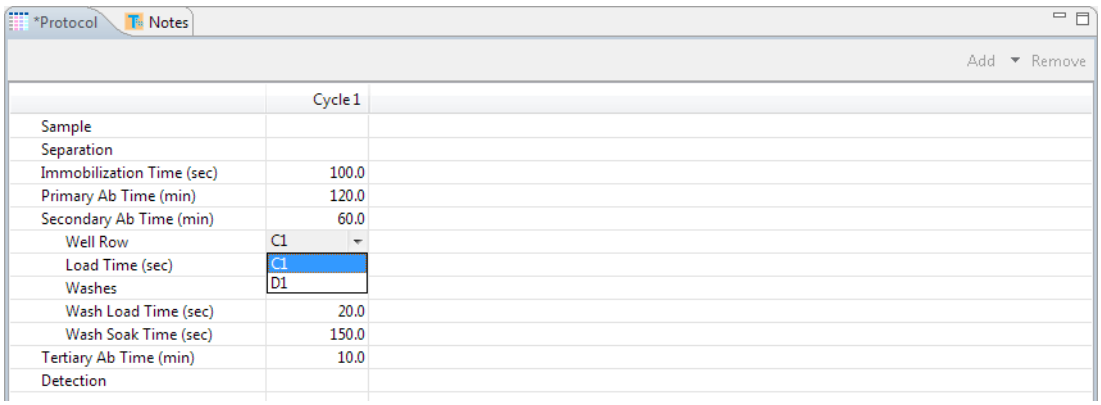
5. If needed, change the secondary incubation time. To do this, click the cell in the value column next to Secondary Ab Time (min) and enter a new value in minutes:



6. If needed, change the secondary incubation reagent row location. To do this, click the cell in the value column next to Well Row and select a different row on the assay plate:



NOTE: Only rows designated as secondary antibody in the Layout tab can be selected in the Well Row drop-down menu.

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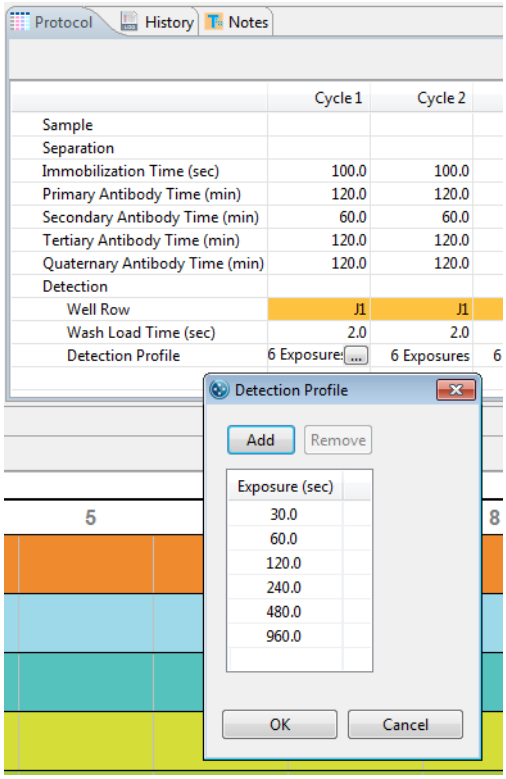
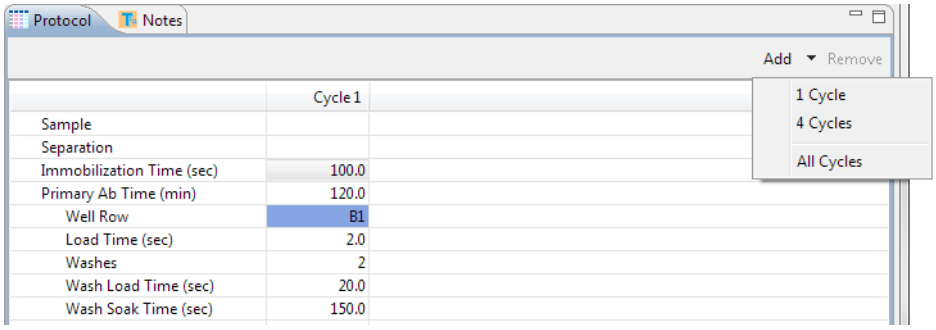
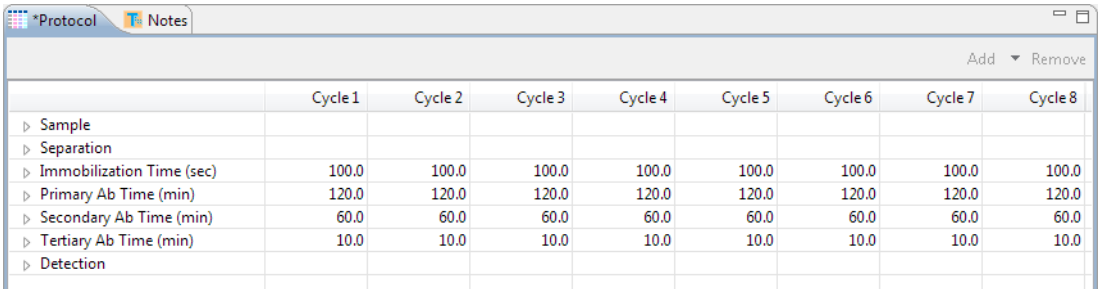
7. Additional exposures can be collected in the assay if desired. To do this, click the white arrow next to Detection to expand the row. Click the cell in the exposure column next to Detection Profile to open the Detection Profile window. Additional times can be added to the protocol by clicking the **Add** button, entering the values and selecting **OK.**



8. Modify any other protocol parameters as needed.
9. To add additional cycles to the assay protocol, click in any cell with a value in a cycle column.
   - To add one cycle, either click **Add** or click the down arrow next to Add and select **1 Cycle**
   - Select **4 Cycles** from the drop down menu to add four additional cycles
   - Select **All Cycles** from the drop down menu to add the number of cycles needed to reach the maximum of eight

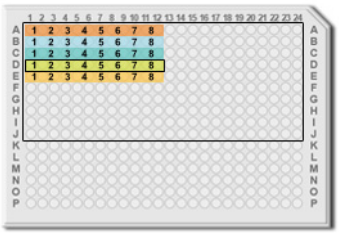


New cycle columns will display using the same parameters used for cycle 1.



	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Sample								
Separation								
Immobilization Time (sec)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Primary Ab Time (min)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Secondary Ab Time (min)	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Tertiary Ab Time (min)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Detection								

Repeat steps 1-8 to change parameters for the added cycles. As cycles are added and reagent locations are selected, cycle number assignments will update in the Layout pane assay plate map:

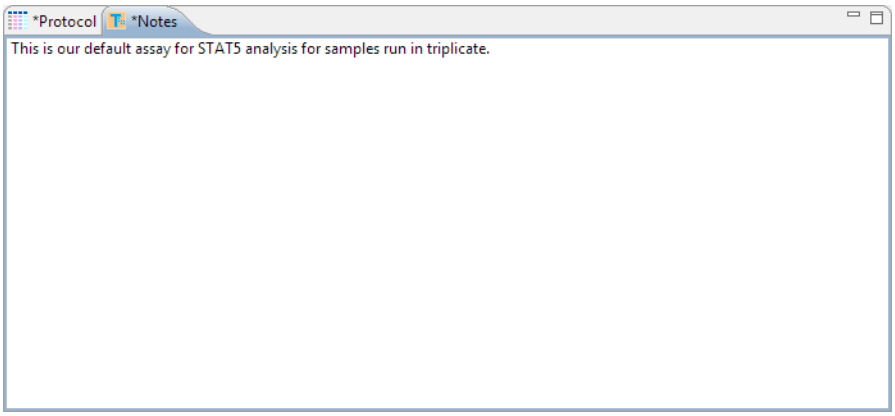


NOTES:
For more information on changing protocol step parameters other than incubation times, contact ProteinSimple Technical Support.

When a protocol is being edited, an asterisk will appear in the Protocol tab to indicate changes have been made and should be saved.

Step 4 - Add Assay Notes (Optional)

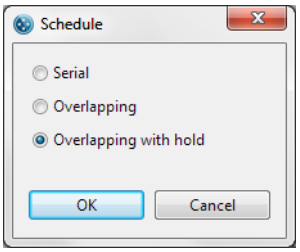
1. Click on the Notes tab.
2. Click in the pane and type any assay or protocol notes. This information will be stored with the assay and run data.



NOTE: When notes are being edited, an asterisk will appear in the Notes tab to indicate changes have been made and should be saved.

Step 5 - Select a Schedule (Optional)

Peggy Sue can execute cycles serially or in parallel. To choose an option, select **Edit** and click **Schedule**.



- **Serial** - Each cycle is executed in sequential order, and each cycle completes before the next one starts. Selecting this option result in the longest run times.
- **Overlapping** - Cycle steps are executed in parallel if timing permits shared use of the separation tray and robot. Each cycle will run through to completion once started. This option offers a reduction in overall run time without affecting the individual run time for each cycle.
- **Overlapping with Hold (default)** - Each cycle executes through the separate step in sequence and then enters a variable length hold step where capillaries remain in the incubation tray. More sample stability is provided with this option as proteins are 'locked' to the capillary wall and therefore more stable than samples on the assay plate. This option also results in the most significant reduction in overall run time.

NOTE: We recommend using the overlapping with hold option. For more information on when to use other schedule options, please contact ProteinSimple Technical Support.

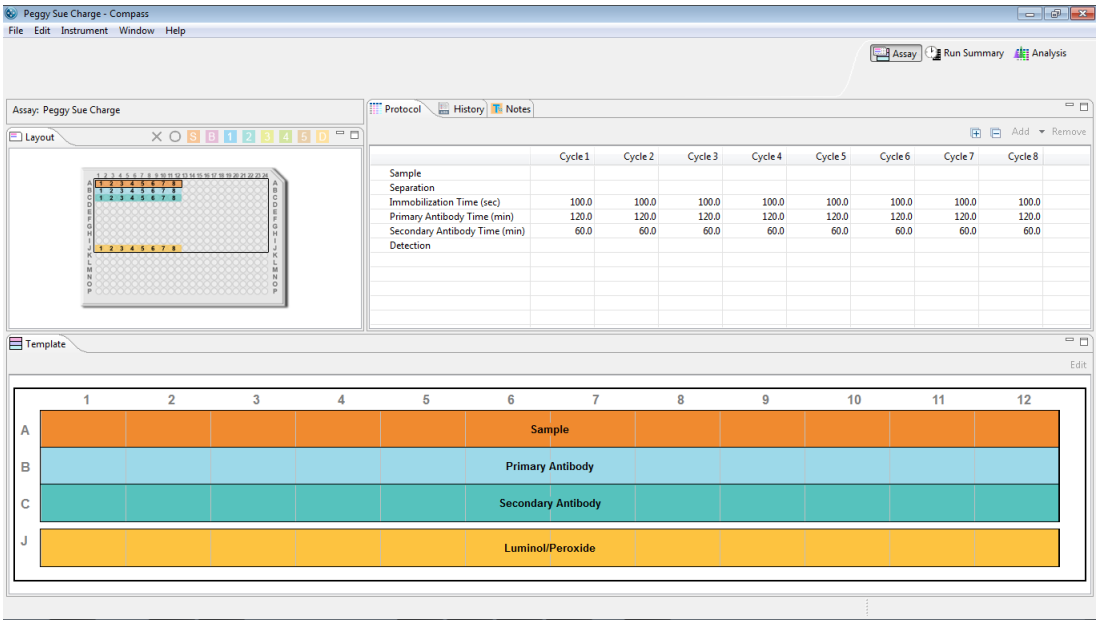
Step 6 - Add Assay Plate Annotations (Optional)

Custom reagent names, notes and annotations can be entered for individual rows and wells on the assay plate. This information will be stored with assay and run data. During post-run analysis, this information will be used to apply custom analysis settings to specific sample names, primary antibody names or sample and primary antibody attributes. This will be explained in more detail in "Analysis Settings Overview" on page 422.

NOTE: Template pane information can also be added or updated after a run is complete.

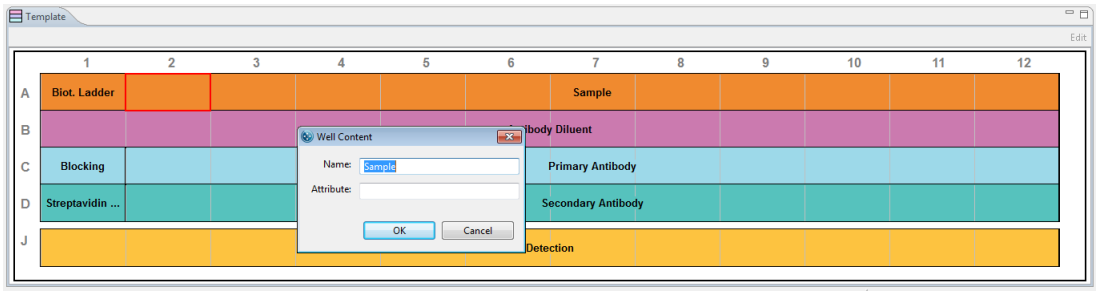
To enter annotations:

1. Click on the Template tab. The default annotations for reagent rows and individual wells on the assay plate will display:

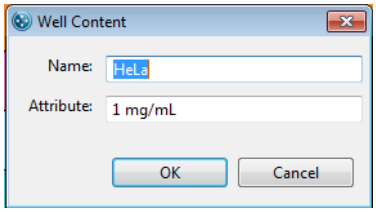


2. Change or add row and well annotations as needed. To do this:

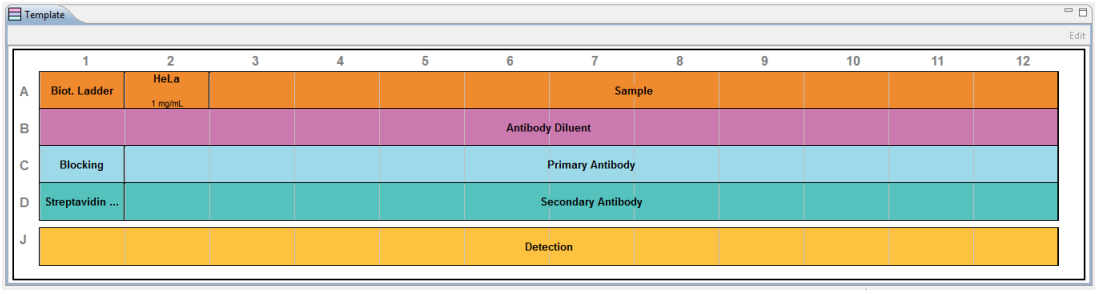
   a. **To enter annotations for a specific well** - Right click the well and select **Edit** or click **Edit** in the upper right corner of the pane or double click the selected well. The following box will display:



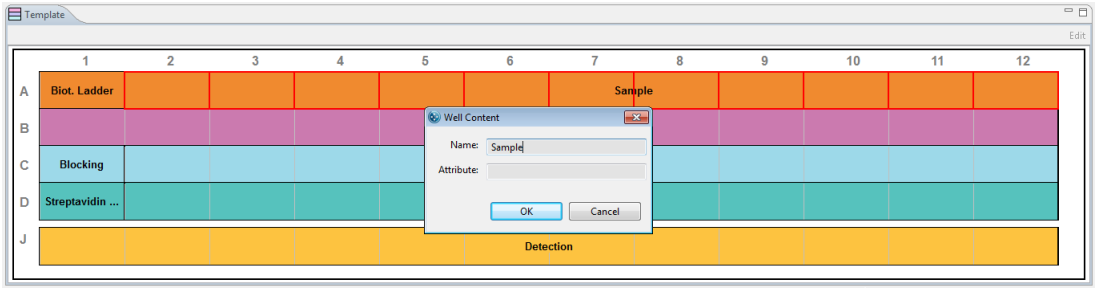
Enter the reagent name, notes or specific attributes (for example, sample concentration or dilution ratio):



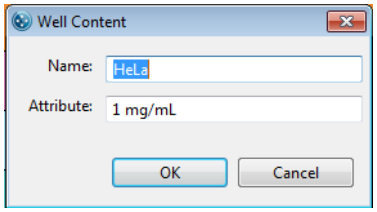
Click **OK**. The new information will display in the selected well:



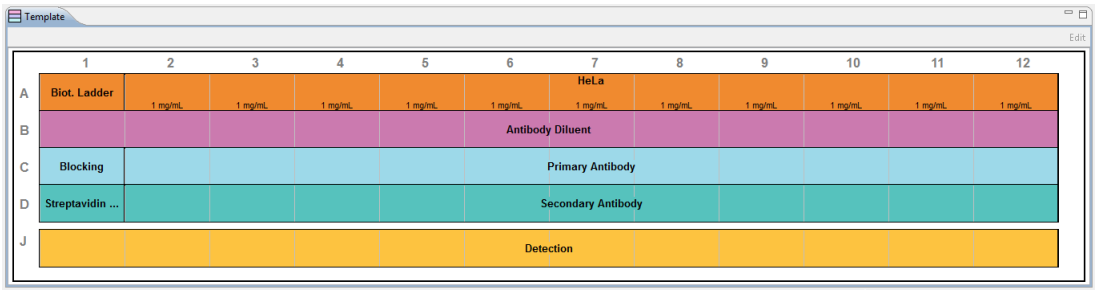
b. **To enter annotations for multiple wells or a row** - To select individual wells, press and hold the Control key and then select individual wells. To select a sequential set of wells or a full row, press and hold the Shift key, then select the first well and last well. Next, right click and select **Edit** or click **Edit** in the upper right corner of the pane. The following box will display:



Enter the reagent name, notes and specific attributes (for example, sample concentration or dilution ratio):



Click **OK**. The new information will display in the selected wells:

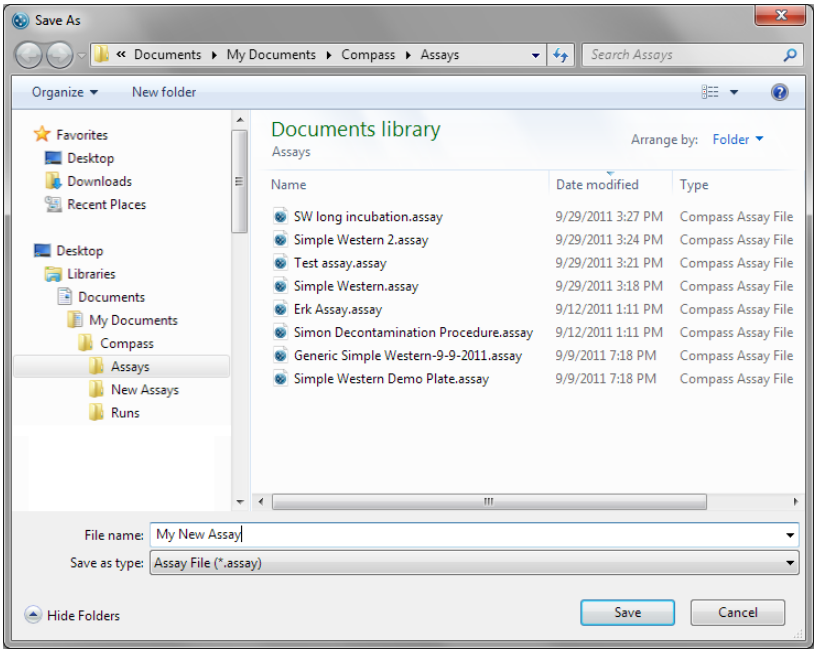


---

*NOTE: When annotations are being edited, an asterisk will appear in the **Template** tab to indicate changes have been made and should be saved. Only Name and Attribute will be used by Compass for Simple Western to annotate the data.*

Step 7 - Save the Assay

1. Select **File** from the main menu and click **Save As**. Enter the assay name and click **Save**.

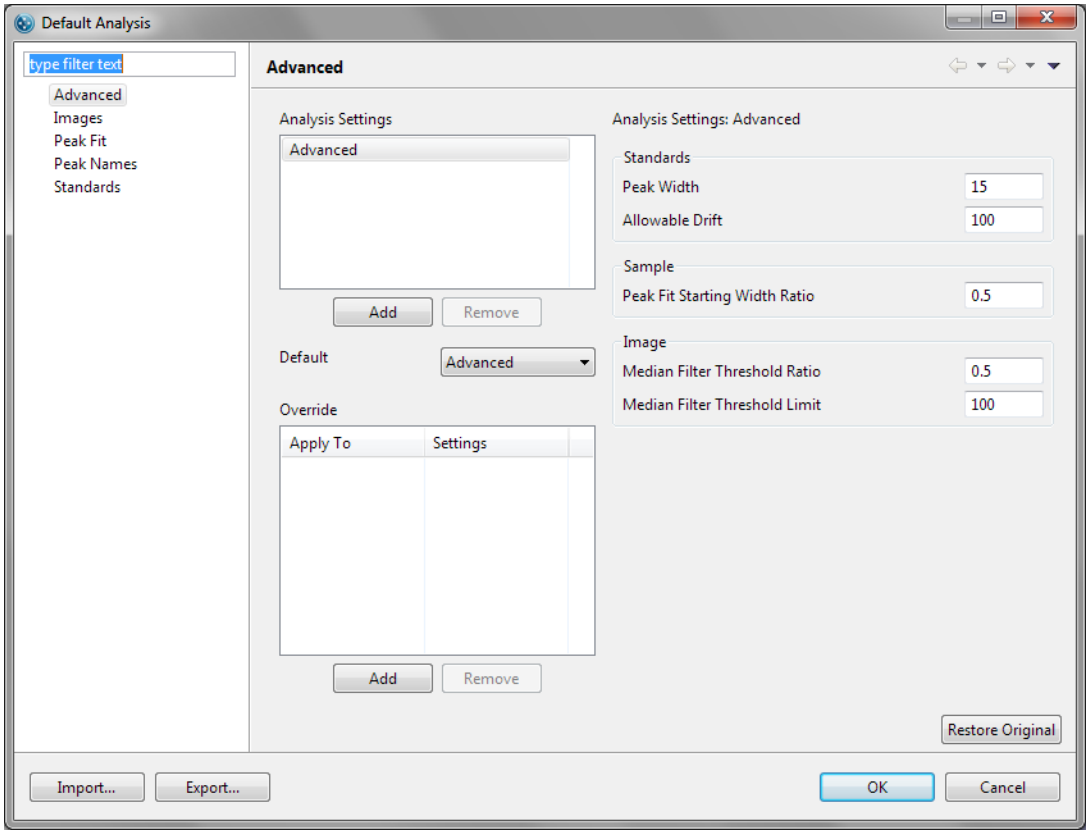


NOTE: New assays are saved in the Compass Assays directory.

Step 8 - Modify Default Analysis Parameters (Optional)

You can preset the parameters used to analyze run data generated with the assay.

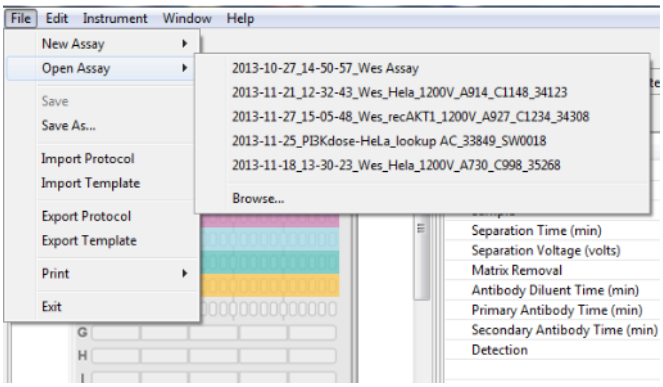
1. Select **Edit** from the main menu and click **Default Analysis**. The following screen will display:



2. We recommend using the default parameters for Simple Western assays. However, you can modify any parameters as needed, then click **OK**. For detailed information on analysis parameters, please refer to "Analysis Settings Overview" on page 422.

Making Changes to an Existing Assay

1. Select **File** in the main menu and click **Open Assay**.

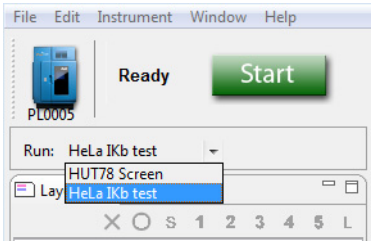


2. A list of the last assays opened will display. Select one of these assays or click **Browse** to open the assay folder and select a different assay.
3. Follow the steps in "Creating a New Assay" on page 111 to make changes and save the assay.

Switching Between Open Assays

If more than one run file is open, you can switch between viewing the assays for each run. To do this:

1. Click the down arrow in the run box.

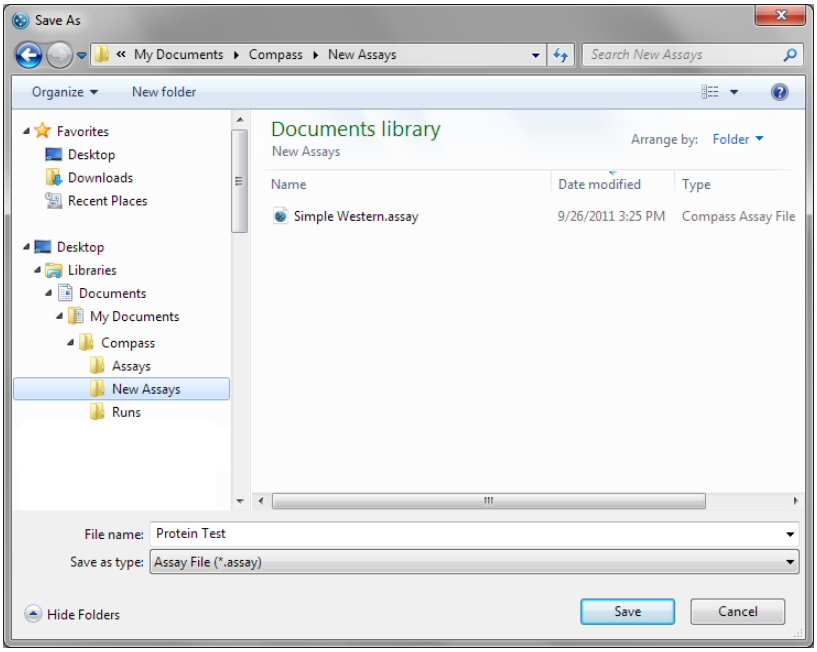


2. Select the run for the assay you want to view from the drop down list.

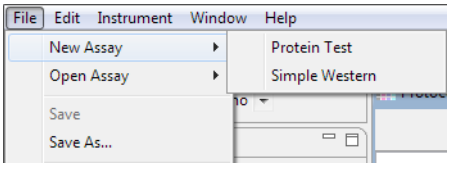
Creating a Template Assay

You can create and save template assays that can be used as a starting point for creating new assays. To do this:

1. Select **File** in the main menu. Click **Open Assay** to open an existing assay or click **New Assay** to open an existing template assay.
2. Follow the steps in "Creating a New Assay" on page 111 to make changes to the assay.
3. When changes are complete, select **File** in the main menu and click **Save As**. Select the New Assays folder:

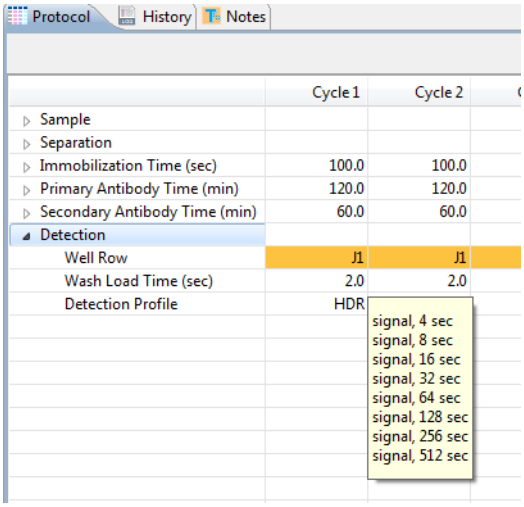


4. Type the name for the new template assay and click **Save**.
5. Select **File** in the main menu and click **New Assay**. The new template assay will now be available in the drop down list:



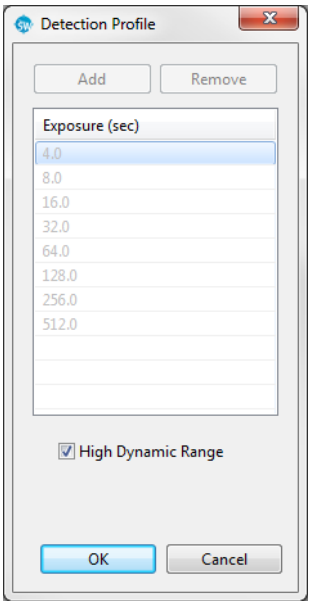
Viewing and Changing the Detection Exposures

To view the current detection profile, roll the cursor over the exposures cell in Protocol pane:



High Dynamic Range Detection Profile

Starting with Compass for Simple Western 3.1, HDR (high dynamic range) is the default detection profile. Exposure times for HDR have been optimized for best performance and can't be edited:

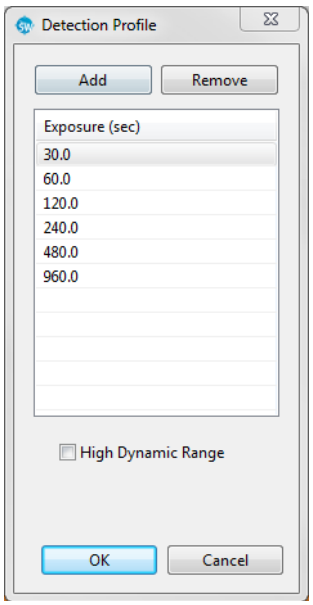


Each row represents an individual exposure that will be taken during the run. To achieve a broader detection dynamic range for a charge assay, you need Compass for Simple Western v3.1 or higher. To download the latest version of the software go to **Help** > **Check for Updates**.

For Peggy Sue and NanoPro 1000, the higher dynamic range is achieved by using a more effective algorithm to create the High Dynamic Range multi-image analysis (now called High Dynamic Range in the Analysis window). Note that for these instruments, you can select HDR for some cycles, and uncheck it for others, giving you great flexibility for data analysis later.

NOTE: See "Images Analysis Settings" on page 430 to find out more about how data sets are analyzed under HDR and standard detection profiles.

If you'd rather not use HDR detection profile, uncheck the box next to HDR. Doing so reverts all instrument functions to a pre-Compass 3.1 state. Under this detection profile, the exposure times can be edited:

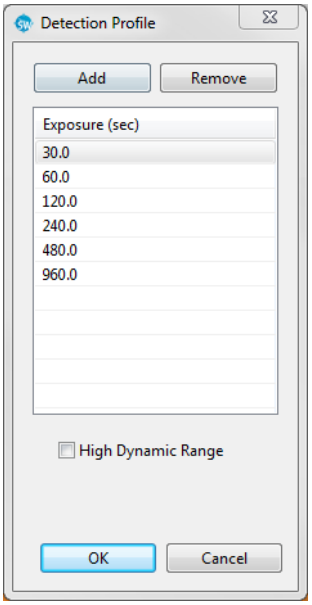


NOTE: Assays created and saved with Compass v2.7 or Compass for Simple Western v3.0 will have HDR unchecked by default. If you want HDR to be the default detection profile for that assay, check the box next to HDR in the Detection Profile, then re-save the assay.

Changing the Detection Profile

While we recommend using the default detection profile, you can change the profile, exposure times and exposure sequences if needed. To do this:

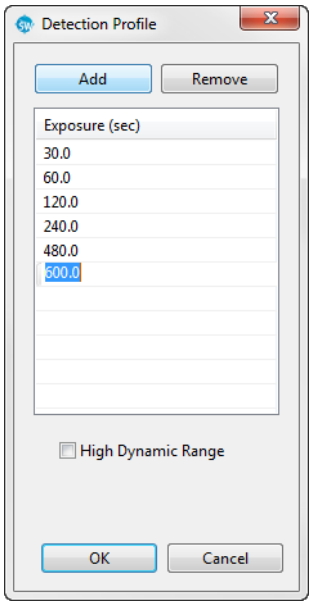
1. Select the exposures cell in the Protocol pane and click the button, or double-click in the cell. The following screen displays:



NOTE: Assays created and saved with Compass v2.7 or Compass for Simple Western v3.0 will have HDR unchecked by default. If you want HDR to be the default detection profile for that assay, check the box next to HDR in the Detection Profile, then re-save the assay.

---

a. **To change an existing exposure time (applies when HDR is unchecked)** - Click in the exposure cell and enter a new time in seconds:



b. **To delete an existing exposure** - Select a type or exposure cell and click **Remove**.

c. **To add a new exposure** - Select **Add**. A new exposure will be added to the end of the list. Click in the exposure cell and enter an exposure time in seconds.

2. Click **OK** to save and exit.

Copying Protocols and Templates

The steps and parameters in the Protocol pane can be copied and d into other documents, as can the graphic image of the annotations in the Template pane.

Copying an Assay Protocol

1. Click on the Protocol tab.
2. Select **Edit** in the main menu and click **Copy**.
3. Open a document (Microsoft® Word®, Excel®, etc.). Right click in the document and select. All assay protocol steps and parameters will be copied into the document as a list in the same format shown in the Protocol pane.

Copying an Assay Template

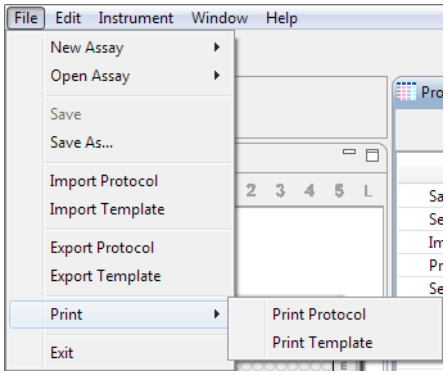
1. Click on the Template tab.
2. Select **Edit** in the main menu and click **Copy**.
3. Open a document (Microsoft® Word®, Excel®, etc.). Right click in the document and select. The assay template will be copied into the document as an image, exactly as it is shown in the Template pane.

Printing Protocols and Templates

The information in the Protocol pane can be printed, as can a graphic image of the information in the Template pane.

Printing an Assay Protocol

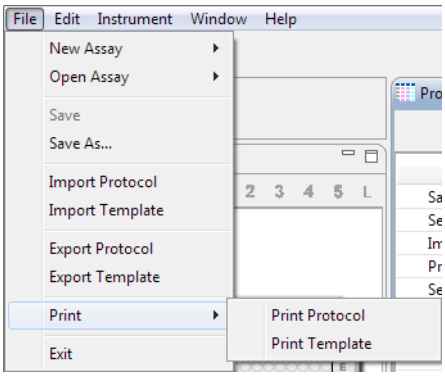
1. Click on the Protocol tab.
2. Select **File** in the main menu, click **Print**, and then click **Print Protocol**.



All assay protocol steps and parameters will be printed as a list in the same format shown in the Protocol pane.

Printing an Assay Template

1. Click on the Template tab.
2. Select **File** in the main menu, click **Print**, and then click **Print Template**.



The assay template will print as an image, exactly as it is shown in the Template pane.

Importing and Exporting Protocols and Templates

The assay protocol in an open assay or run file can be exported as a separate file, as can the assay template annotation information. This allows the same assay protocol and template information to be imported into another assay at a later time, rather than having to re-enter assay protocol and template information manually.

Importing an Assay Protocol

NOTE: Importing an assay protocol imports information into the Protocol pane only.

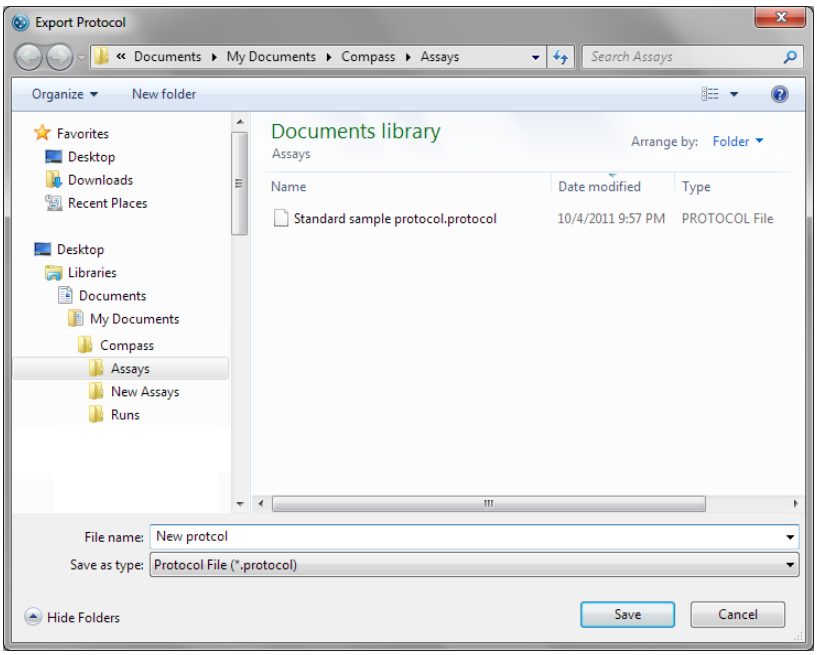
---

1. Open the assay you want to import the assay protocol in to.
2. Select **File** in the main menu and click **Import Protocol**.
3. Select a protocol file (*.protocol) and click **OK**. The imported information will display in the Protocol pane.

Exporting an Assay Protocol

NOTE: Exporting an assay protocol exports information in the Protocol pane only.

1. Open the assay you want to export the assay protocol from.
2. Select **File** in the main menu and click **Export Protocol**. The following window displays:



3. The default directory is Compass/Assays. Change the directory if needed.
4. Enter a protocol name and click **Save**. The protocol will be saved as a *.protocol file.

Importing an Assay Template

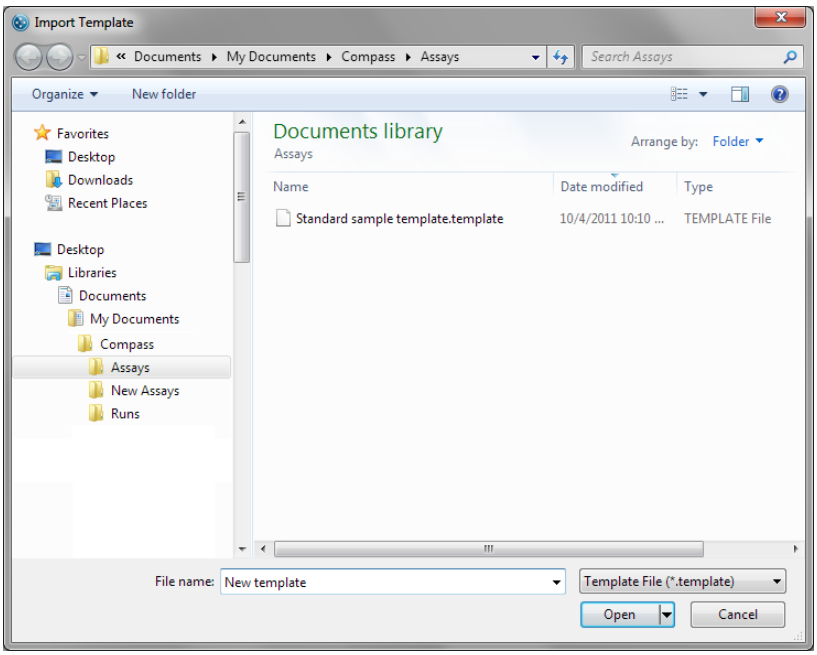
NOTE: Importing an assay template imports information into the Template pane only.

1. Open the assay you want to import the assay template in to.
2. Select **File** in the main menu and click **Import Template**.
3. Select a template file (*.template) and click **OK**. The imported information will display in the Template pane.

Exporting an Assay Template

NOTE: Exporting an assay template exports information in the Template pane only.

1. Open the assay you want to export the assay template from.
2. Select **File** in the main menu and click **Export Template**. The following window displays:



3. The default directory will be Compass/Assays. Change the directory if needed.
4. Enter a template name and click **Save**. The template will be saved as a *.template file.

Chapter 6:

Running a Charge Assay on Peggy Sue

Chapter Overview

- Starting a Run
- Stopping a Run

Starting a Run

Step 1 - Get Ready

1. Open Compass for Simple Western software.
2. Prepare instrument: empty waste, refill water and add a new manifold sponge.
3. Create or open desired assay file.
4. Prepare assay plate following the procedure described in the product insert.

IMPORTANT

To prevent well evaporation and ensure best results, keep a lid on the assay plate until ready to use.

---

5. While plate is spinning, add Wash Buffer, Anolyte and Catholyte to resource tray cups. Place capillary box in the designated resource tray position.

IMPORTANT

Capillaries are light sensitive. Keep the cover on the box until you are ready to transfer the capillary box to the resource tray.

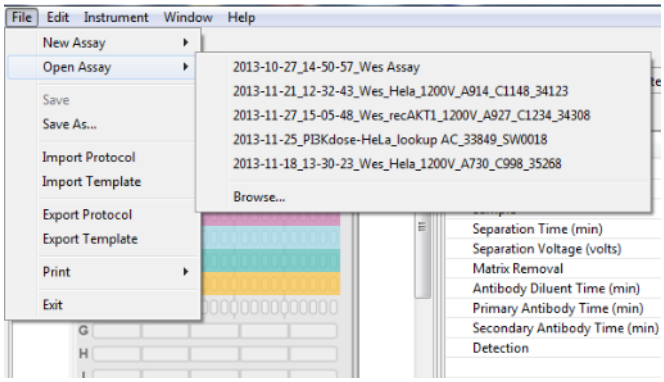
---

6. Place assay plate into the sample tray of the instrument and press **Start**.

Step 2 - Start the Run

1. New run of an existing assay:

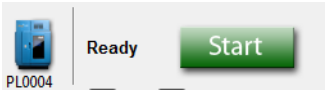
   a.Select **File** in the main menu and click **Open Assay**.



b. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different assay.

2. Alternatively, choose **New Assay** and select **Peggy Sue Charge** to get the default Peggy Sue assay conditions.

c. The **Start** button will display. This indicates than an assay has been loaded.



a. Go to the Assay screen and verify this is the assay you want to use. If not, select **File** in the main menu, click **Open Assay**, and select another assay.

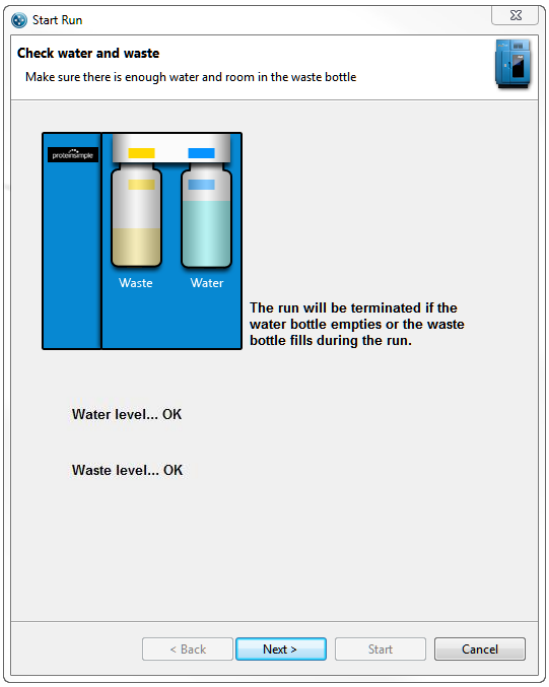
3. Click **Start**. This will launch the **Start Run Wizard**.

---

*NOTE: If the manifold was not cleaned prior to starting the run, a message indicating this will display. If this occurs, click **Yes** to cancel the run and perform the manifold cleaning.*

---

4. **Check Water and Waste.** The fluid levels in the accessory module bottles will be checked by the software. If the level in both bottles will allow Peggy Sue to complete the run, the wizard screen will display **Water Level OK** and **Waste Level OK** messages. Click **Next** to proceed.

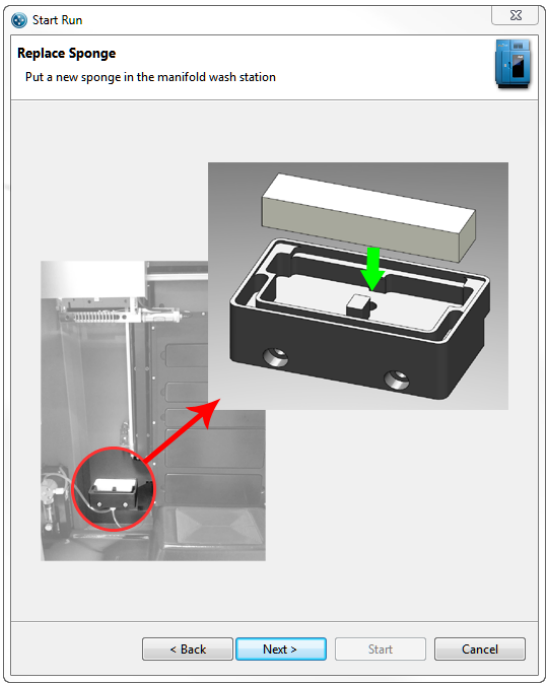


---

*NOTE: If the waste level is too high or the water level is too low to complete the run, messages to indicate one or both will be presented in this screen. If this occurs, fill or empty each bottle as indicated using the procedures outlined in the Peggy Sue User Guide. When this is complete, the error status will be automatically updated and allow the **Start Run Wizard** to proceed.*

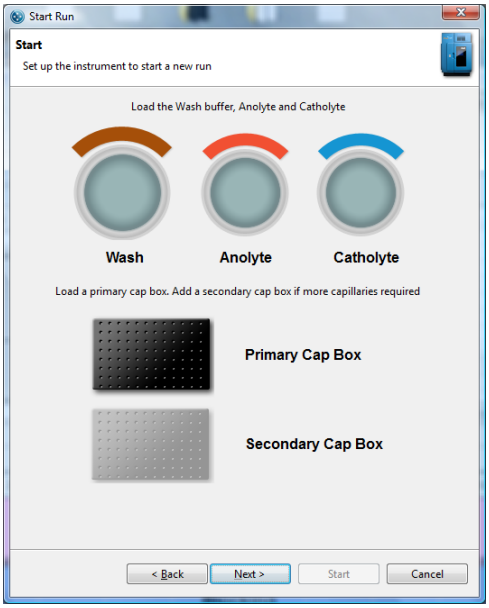
---

5. **Replace Sponge.** A new sponge should be used each time a new experimental run is started. Discard the old sponge and put a new sponge in the manifold wash station.



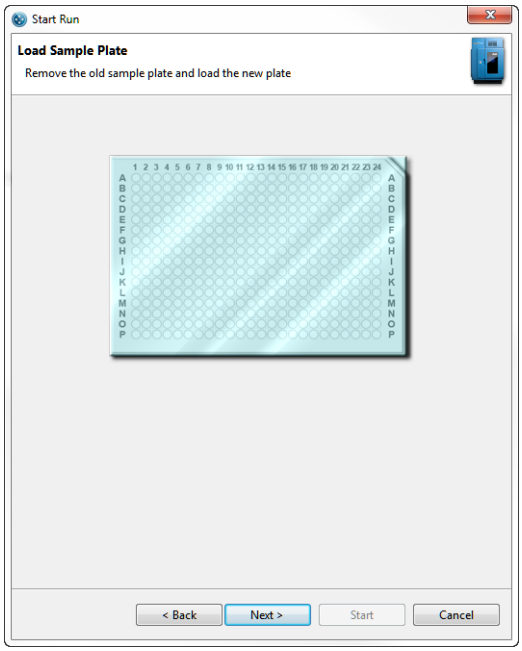
6. **Start.** The resource tray will automatically open. Fill the Wash Buffer, Anolyte and Catholyte cups and insert the capillary box in the primary capillary box position. Add a secondary capillary box if more capillaries are required. Click **Next** when finished. The resource tray will close.

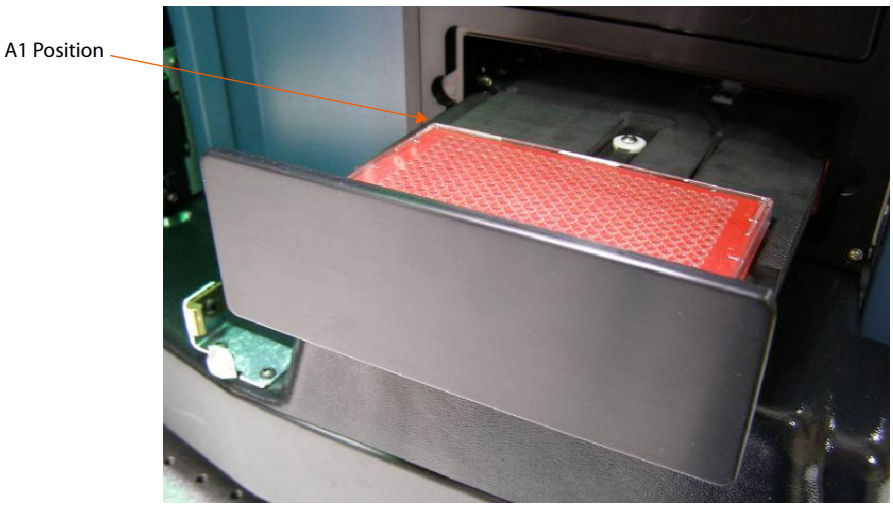
NOTE: Make sure enough capillaries are loaded to complete the run. A capillary box should be inserted in the primary position first and if additional capillaries are needed, use the secondary position. When the primary box is depleted, Peggy Sue will automatically move to the secondary box. Discard leftover Running Buffer, Wash Buffer and Matrix Removal Buffer prior to refilling bottles for new run.



NOTE: You can also refer to the labels on the resource tray for proper insertion of reagents.

7. **Load Sample Plate.** The sample tray will automatically open. Place the lidded 384-well plate on the cooling block in the tray, ensuring that the A1 well position is aligned with the upper left corner of the cold block. When this is complete, click **Next**. The sample tray will close.





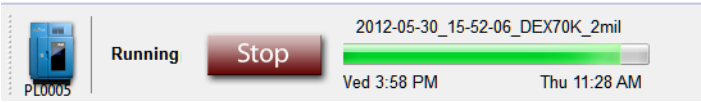
NOTES:
Peggy Sue requires that plate lids be used on sample plates. If a lid is not detected, a message will be displayed in the Start Run Wizard. Compass for Simple Western will reopen the sample tray to allow you to place the lid on the sample plate.

When inserting the sample plate, ensure that the plate is firmly seated and level on the cold block. Plates that are not level can interfere with the movement of the sample tray.

---

8. **Begin the Automated Run.** The data file name will automatically default to the assay name appended with the current date and time. To change the file name, begin typing in the text box. To change the directory where the data file will be stored, click **Browse**:

Click **Start** to begin the run. Instrument status will change to running, and the stop button and progress bar will display:



The run will continue until complete (~14-19 hours depending on the assay).

Step 3 - Post-Run Procedures

1. Empty the capillary discard tray.
2. Remove the assay plate.
3. Dispose of the assay plate and capillaries. Disposal will depend on the samples that have been assayed. If sample origins are unknown, we recommend that used capillaries and plates be disposed of as biohazard waste.

!WARNING! SHARPS HAZARD

The capillaries may present a potential sharps hazard. Dispose of used capillaries in biomedical waste sharps containers.

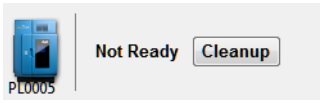
!WARNING! BIOHAZARD

Samples and waste bottle contents should be handled by procedures recommended in the CDC/NIH manual: Biosafety in Microbiological and Biomedical Laboratories (BMBL). The manual is available from the U.S. Government Printing Office or online at http://www.cdc.gov/biosafety/publications/bmbl5/.

Depending on the samples used, waste bottle contents may constitute a biohazard. Use precaution when emptying the waste bottle. Dispose of waste bottle contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations. Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste vial before you store, handle, or dispose of chemical waste.

Stopping a Run

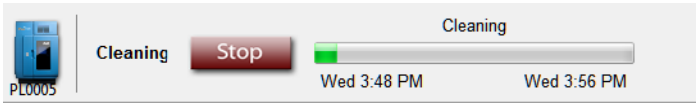
1. To stop a run, click **Stop**. When the run stops, instrument status will go to Not Ready and a Cleanup button displays:



NOTE: If a run is stopped prior to completion, Peggy Sue must perform a cleaning protocol to remove used capillaries from system trays. This is required to prepare the system for the next run.

---

2. Click **Cleanup**.



Allow Peggy Sue to complete the cleaning protocol which takes about ten minutes. When complete, instrument status will change to Ready and a new run can be started.

Chapter 7:

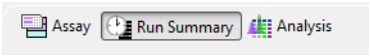
Run Status

Chapter Overview

- Run Summary Screen Overview
- Opening Run Files
- Viewing File and Run Status Information
- Watching Standards Separation Movies
- Viewing Current and Voltage Plots
- Switching Between Open Run Files
- Closing Run Files

Run Summary Screen Overview

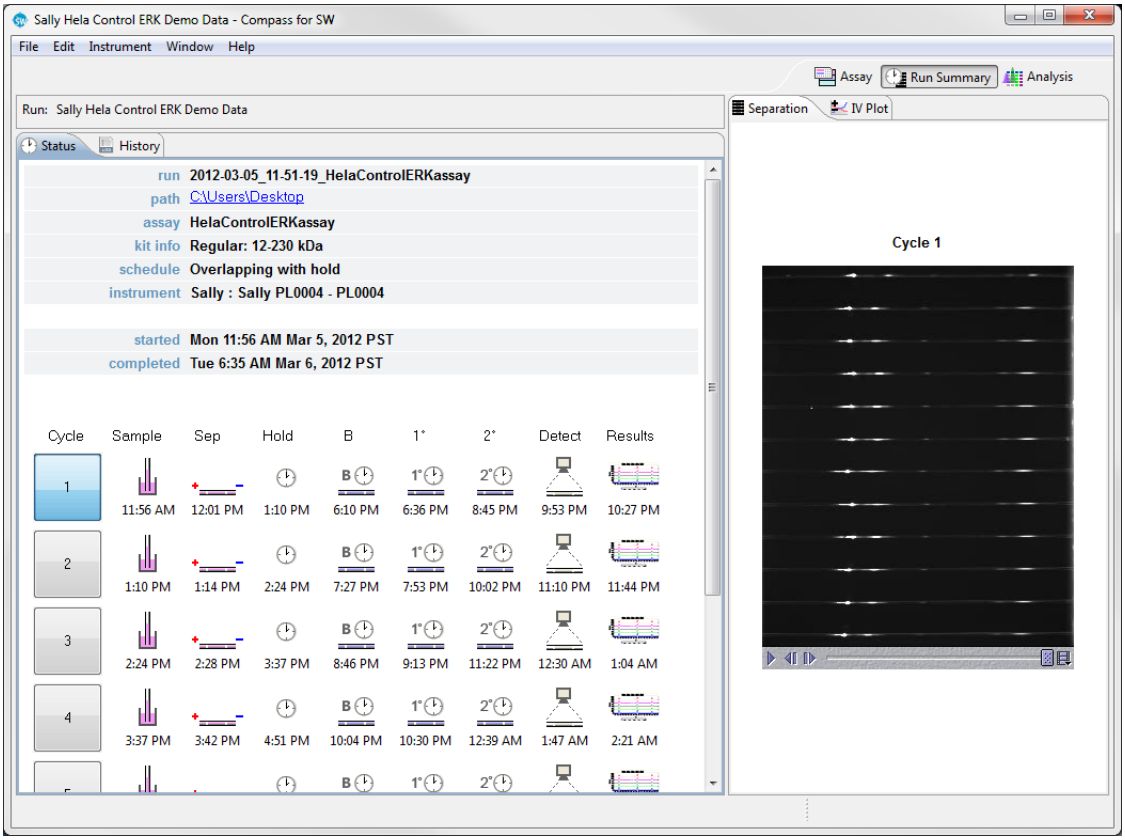
The Run Summary screen is used to monitor run progress, watch movies of the fluorescent standards separation, and view current and voltage plots for a run. To access this screen, click Run Summary in the screen tab:



Run Summary Screen Panes

The Run Summary screen has three panes:

- **Status** - Displays run file information and current status of a run in progress.
- **Separation** - Lets you view a movie of the fluorescent standards separation for each cycle of the experimental run.
- **IV Plot** - Lets you view plots of the total current and voltage measured during separation for all capillaries for each cycle of the experimental run.



Software Menus Active in the Run Summary Screen

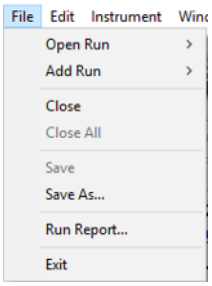
The following software menus are available:

- File
- Edit
- Instrument (when Compass for Simple Western is connected to an instrument)
- Window
- Help

The File and Edit menu options specific to the Run Summary screen are described next.

File Menu

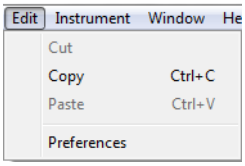
The following **File** menu options are active:



- **Open Run** - Opens a run file.
- **Add Run** - Open and view other run files in addition to the one that is already open.
- **Close** - Closes the run file currently being viewed.
- **Close All** - Closes all open run files.
- **Save** - Saves the open run file.
- **Save As** - Saves the open run file under a different file name.
- **Run Report** - Creates a report of the run in .pdf format.
- **Exit** - Closes Compass for Simple Western.

Edit Menu

The following Edit menu options are active:



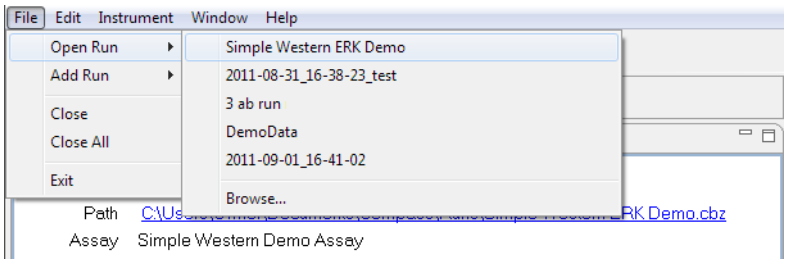
- **Preferences** - Set and save custom preferences for data export, plot colors in the graph and Twitter settings. See Chapter 11, *"Setting Your Preferences"* for more information.

Opening Run Files

You can open one run file or multiple run files at a time to compare information between runs.

Opening One Run File

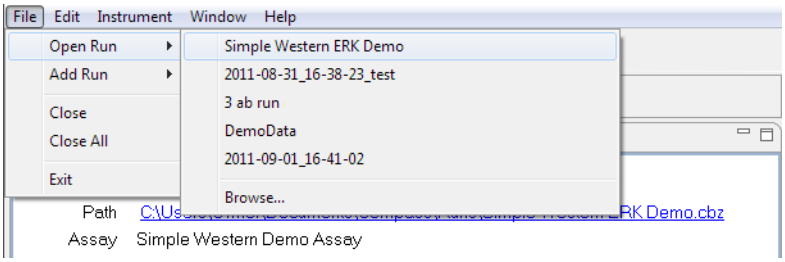
1. Select **File** in the main menu and click **Open Run**.



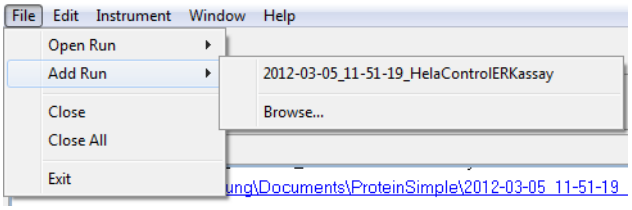
2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.

Opening Multiple Run Files

1. To open the first run file, select **File** in the main menu and click **Open Run**.



2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.
3. To open another run file, select **File** in the main menu and click **Add Run**.



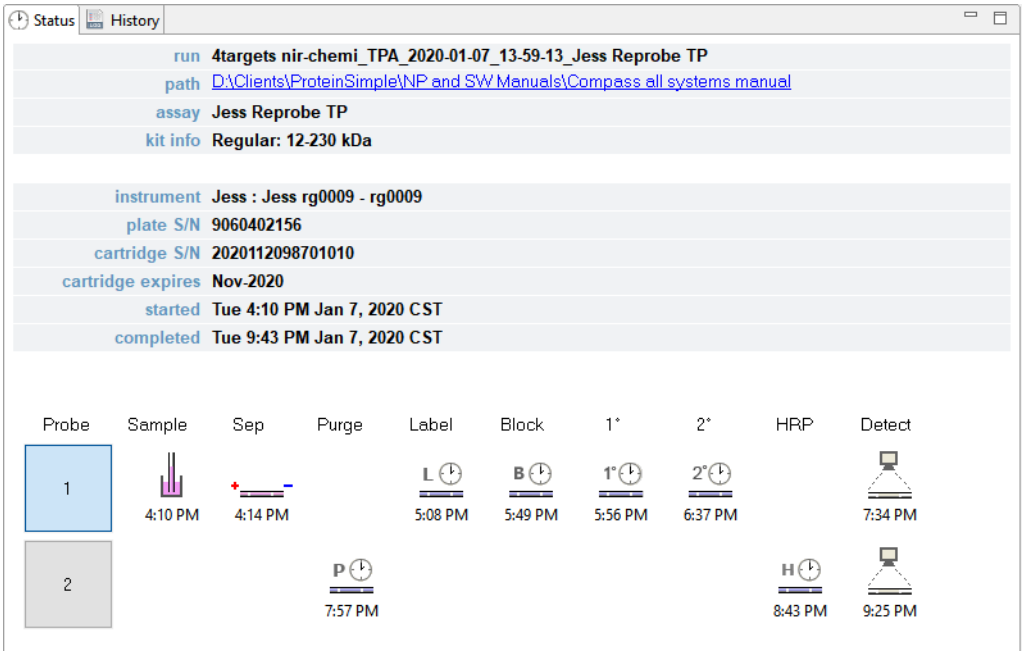
4. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.
5. Repeat the last two steps to open additional runs.

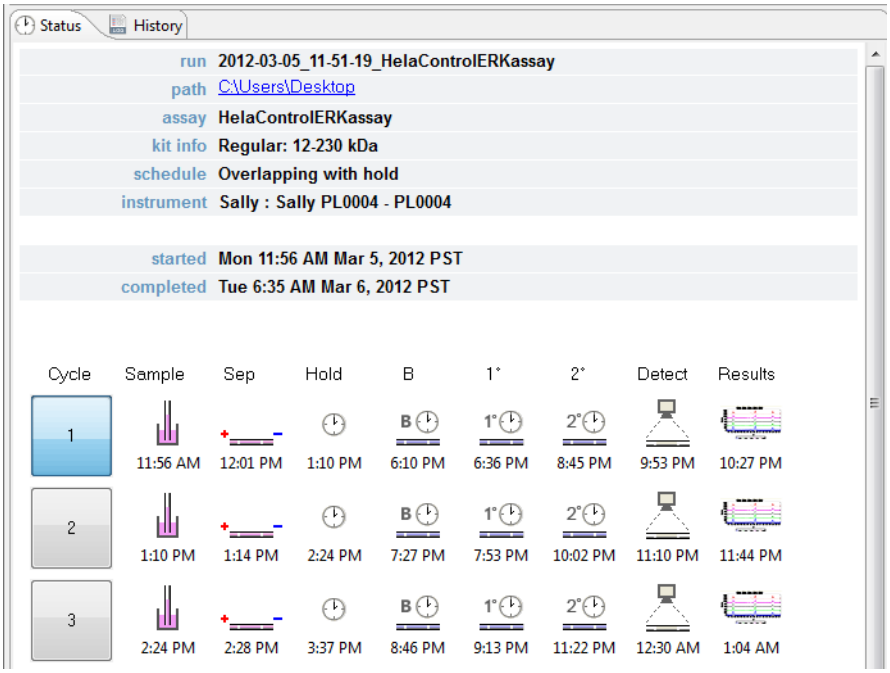
NOTES:
When adding multiple data files for analysis, they must contain matching detection channels (for example chemi, IR/NIR fluorescence, or IR/NIR fluorescence with Protein Normalization).

When adding multiple RePlex data files (Jess only), the channels for both probes must contain matching detection files. For example, a chemiluminescence with Total Protein data file can't be added to a chemiluminescence/chemiluminescence data file.

Viewing File and Run Status Information

Information specific to each run file is shown in the Status pane:





The run file name, path (directory location) and assay used is displayed along with instrument serial number and the run start/complete date and time.

- **To go to the run file directory location** - Double click the path hyperlink, or right-click and select **Open Directory**.
- **To copy the path** - Right-click on the path hyperlink and click **Copy**. The path can then be copied into documents. The path can also be copied into the Windows Explorer address bar to launch Compass for Simple Western and open the run file automatically.
- **Kit info** - Compass for Simple Western v2.7 and higher displays the type of kit used to run the assay (regular for immunoassays, total protein or charge), the molecular range and whether or not the split Running Buffer was used.
- **Plate S/N** - For Jess and Wes, the plate serial number (S/N) information is captured and displayed for all assays.

Assay Steps: Size-based Assays

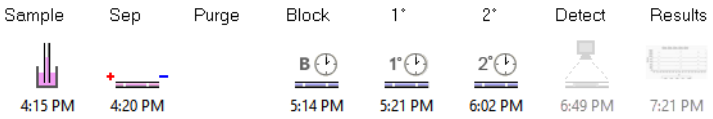
Each assay step that is executed during the run is represented by an icon. The time the individual step is scheduled to start is shown under each icon. Details on what happens during each assay step is as follows:

RePlex Assays (Jess Only)

Step	Description
Sample  12:54 PM	**Sample Loading Step** - Capillaries are moved to the assay plate in the sample tray where Separation Matrix, Stacking Matrix, biotinylated ladder and samples are aspirated. Capillaries are then transferred to the running buffer trough.
Sep  12:56 PM	**Separation Step** - Samples and fluorescent standards are separated in the capillaries. Capillaries are then exposed to UV light, which immobilizes the separated proteins to the walls of each capillary. During the separation, voltage and current are monitored and displayed in the IV Plot. Separation of the fluorescent standards is recorded and a movie of the separation is compiled for viewing when separation is complete in the Separation pane. After separation, Matrix Removal Buffer is aspirated.
Label  5:08 PM	**Label Step (Total Protein only)**- Capillaries are moved to the assay plate in the sample tray and Biotin labeling reagent is aspirated. When incubation is complete, Wash Buffer is aspirated.
Block  2:00 PM	**Blocking Step** - Capillaries are moved to the assay plate in the sample tray and blocking reagent (Milk-Free Antibody Diluent) is aspirated. When incubation is complete, Wash Buffer is aspirated.
1°  2:16 PM	**Primary Antibody (1°) Step** - Capillaries are moved to the assay plate in the sample tray and primary antibody is aspirated. When incubation is complete, Wash Buffer is aspirated.
2°  4:22 PM	**Secondary Antibody (2°) Step -** Capillaries are moved to the assay plate in the sample tray and secondary HRP/NIR/IR antibody conjugate and Streptavidin-HRP/NIR is aspirated. When incubation is complete, Wash Buffer is aspirated.
HRP  8:43 PM	**HRP Step (Total Protein only)** - Capillaries are moved to the assay plate in the sample tray and Total Protein Streptavidin-HRP is aspirated. When incubation is complete, Wash Buffer is aspirated.

Step	Description
Detect  5:28 PM	**Detect Step -** Capillaries are moved to the assay plate in the sample tray and Luminol-Peroxide solution is aspirated (chemiluminescence only). Emitted chemiluminescent light is detected with the CCD camera. For fluorescence detection on Jess, emitted fluorescent signal is detected.
Purge  7:57 PM	**Purge Step** - Capillaries are moved to the assay plate in the sample tray and Wash Buffer is aspirated. RePlex reagent mix is then aspirated to remove the primary and secondary antibodies from the immobilized sample proteins. This is followed by another round of Wash Buffer.
Results  6:00 PM	**Results Step -** Results are available in the Analysis screen.

When a run is in progress, icons for steps that have not executed will be grey (inactive). In the following example, the Secondary Antibody (2°) step is executing and the Detect and Results steps have not started:

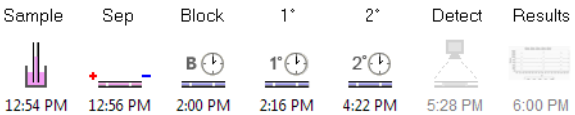


Standard Size Assays

Step	Description
Sample  12:54 PM	**Sample Loading Step** - Capillaries are moved to the assay plate in the sample tray where Separation Matrix, Stacking Matrix, biotinylated ladder and samples are aspirated. Capillaries are then transferred to the running buffer trough (Wes/Jess) or separation tray (Sally Sue/Peggy Sue).
Sep  12:56 PM	**Separation Step** - Samples and fluorescent standards are separated in the capillaries. Capillaries are then exposed to UV light, which immobilizes the separated proteins to the walls of each capillary. During the separation, voltage and current are monitored and displayed in the IV Plot. Separation of the fluorescent standards is recorded and a movie of the separation is compiled for viewing when separation is complete in the Separation pane. After separation, Matrix Removal Buffer (Jess/Wes) or Wash Buffer (Peggy Sue/Sally Sue) is aspirated.
PN  4:38 PM	**Protein Normalization Step (Jess only)** - Capillaries are moved to the assay plate in the sample tray and the protein normalization reagent is aspirated. When incubation is complete, Wash Buffer is aspirated.
Block  2:00 PM	**Blocking Step** - Capillaries are moved to the assay plate in the sample tray and blocking reagent (Milk-Free Antibody Diluent) is aspirated. Capillaries are then transferred to an incubator tray (Sally Sue/Peggy Sue). When incubation is complete, Wash Buffer is aspirated.
1°  2:16 PM	**Primary Antibody or Total Protein Labeling Reagent (1°) Step** - Capillaries are moved to the assay plate in the sample tray and primary antibody or labeling reagent is aspirated. Capillaries are then transferred to an incubator tray (Sally Sue/Peggy Sue). When incubation is complete, Wash Buffer is aspirated.
2°  4:22 PM	**Secondary Antibody or Total Protein Streptavidin-HRP (2°) Step -** Capillaries are moved to the assay plate in the sample tray and secondary HRP/NIR/IR antibody conjugate and Streptavidin-HRP/NIR (Immunoassays), or Total Protein Streptavidin-HRP (Total Protein Assays) is aspirated. Capillaries are then transferred to an incubator tray (Sally Sue/Peggy Sue). When incubation is complete, Wash Buffer is aspirated.
Detect  5:28 PM	**Detect Step -** Capillaries are moved to the assay plate in the sample tray and Luminol-Peroxide solution is aspirated. Capillaries are then transferred to the separation tray (Sally Sue/Peggy Sue) where the emitted chemiluminescent light is detected with the CCD camera. For fluorescence detection on Jess, emitted fluorescent signal is detected.

Step	Description
Results  6:00 PM	**Results Step -** Results are available in the Analysis screen.
Hold  1:10 PM	**Hold Step (Sally Sue/Peggy Sue only) -** This step is used for overlapping or overlapping with hold schedule options. A cycle that has completed a specific step will go into a hold step while the remaining cycles execute the same step in parallel. For example, once cycle1 completes the separate step, it may go into a hold step while cycle 2 executes the separate step. This execute and hold pattern will continue until all cycles have completed the separate step.

When a run is in progress, icons for steps that have not executed will be grey (inactive). In the following example, the Secondary Antibody (2°) step is executing and the Detect and Results steps have not started:



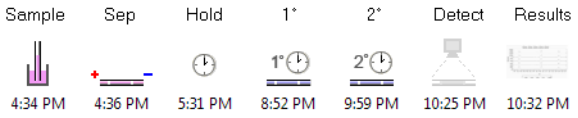
Assay Steps: Charge-based Assays

Each assay step that is executed during the run is represented by an icon. The time the individual step is scheduled to start is shown under each icon. Details on what happens during each assay step is as follows:

Step	Description
Sample  12:54 PM	**Sample Loading Step** - Capillaries are moved to the assay plate in the sample tray and samples are aspirated. Capillaries are then transferred to the separation tray.
Sep  12:56 PM	**Separation Step** - Samples, ampholyte mix and fluorescent pI standards are separated in the capillaries. Capillaries are then exposed to UV light, which immobilizes the separated proteins to the walls of each capillary. During the separation, voltage and current are monitored and displayed in the IV Plot. Separation of the fluorescent standards is recorded and a movie of the separation is compiled for viewing in the Separation pane after separation is complete.

Step	Description
1° 2:16 PM	**Primary Antibody (1°) Step** - Capillaries are moved to the assay plate in the sample tray and primary antibody is aspirated. Capillaries are then transferred to an incubator tray. When incubation is complete, Wash Buffer is aspirated.
2° 4:22 PM	**Secondary Antibody (2°) Step -** Capillaries are moved to the assay plate in the sample tray and secondary HRP-conjugated antibody is aspirated. Capillaries are then transferred to an incubator tray. When incubation is complete, Wash Buffer is aspirated.
Detect 5:28 PM	**Detect Step -** Capillaries are moved to the assay plate in the sample tray and Luminol-Peroxide solution is aspirated. Capillaries are then transferred to the separation tray where the emitted chemiluminescent light is detected with the CCD camera.
Results 6:00 PM	**Results Step -** Results are available in the Analysis screen.
Hold 1:10 PM	**Hold Step -** This step is used for overlapping or overlapping with hold schedule options. A cycle that has completed a specific step will go into a hold step while the remaining cycles execute the same step in parallel. For example, once cycle1 completes the separate step, it may go into a hold step while cycle 2 executes the separate step. This execute and hold pattern will continue until all cycles have completed the separate step.

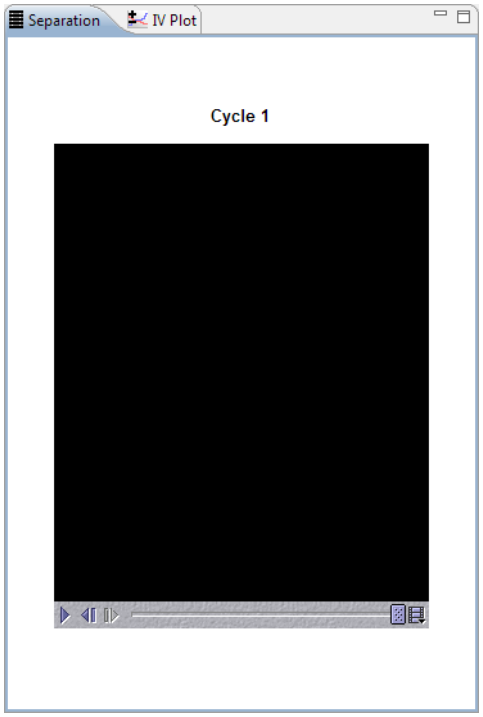
When a run is in progress, icons for steps that have not executed will be grey (inactive). In the following example, the Secondary Antibody (2°) step is executing and the Detect and Results steps have not started:



Watching Standards Separation Movies

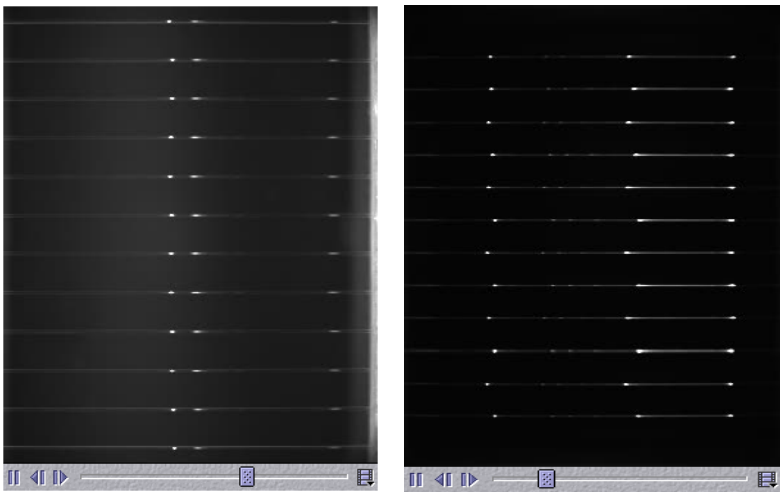
You can view a movie of the fluorescent standards separation in all 12 capillaries. To do this:

1. Click the **Separation** tab.

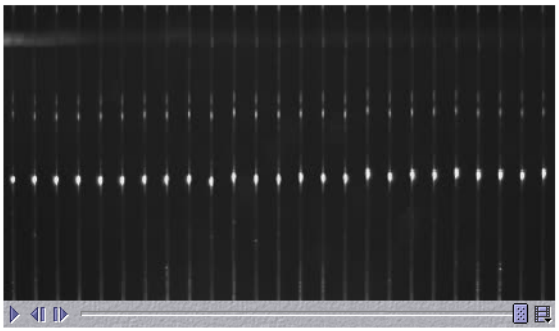


2. The player control panel has play/pause, rewind and fast forward buttons, and a slider bar that allows you to scroll through the movie manually:

Click **Play** (button on far left) to view the movie. In the examples below, standards for a Peggy Sue/Sally Sue size assay (top left) and a charge assay (top right) are shown, as well as a Jess RePlex size assay (bottom):



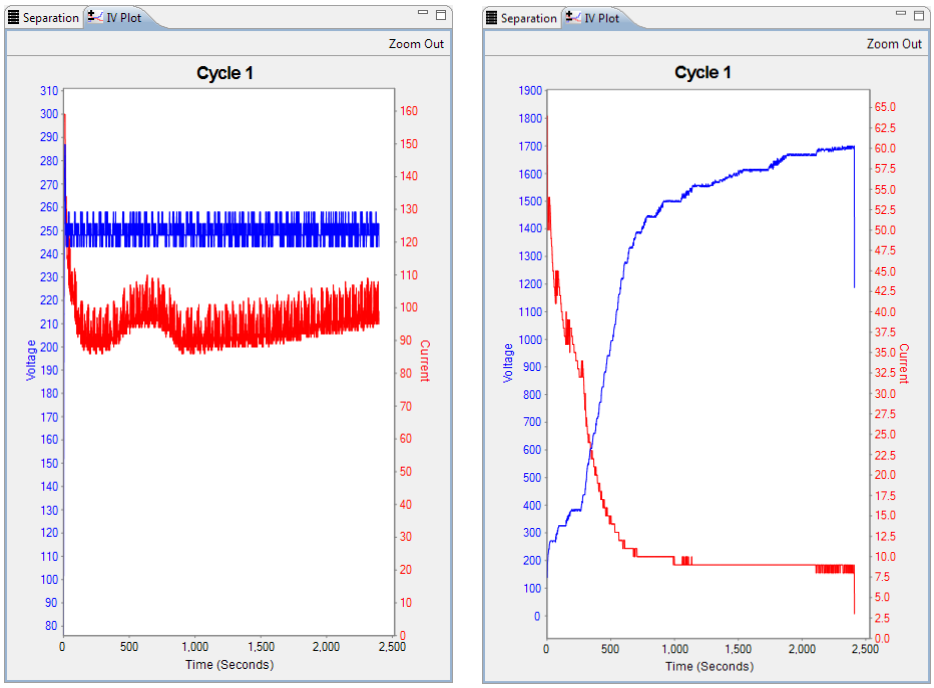
Probe 1



NOTE: Complete separation movies of the fluorescent standards are not available until the separation step has finished executing. If the movie is played while the separation step is executing, the movie will only show separation progress up to the current point in time.

Viewing Current and Voltage Plots

You can view plots of the total current and voltage measured during separation for all 12 capillaries. To do this, click the **IV Plot** tab. In the examples below, the IV plot for a size assay is on the left, and the IV plot for a charge assay is on the right:



The blue Y-axis and plot shows the run voltage in volts (V), and the red Y-axis and plot shows the run current in micro amps (µA). The X-axis displays time in seconds.

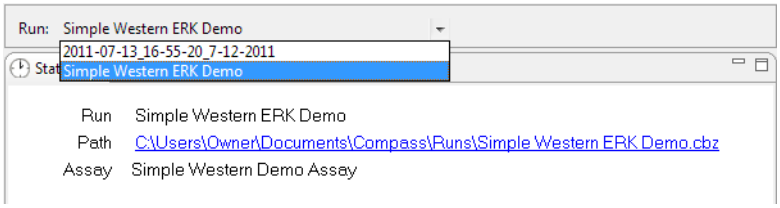
- **To zoom in on an area of the plot** - Hold the mouse button down and draw a box around the area with the mouse.
- **To zoom out** - Click **Zoom Out** in the upper right corner of the pane.

NOTE: The IV plot for a run in progress will not be available until the separation step starts executing. The plot is then displayed in real-time.

Switching Between Open Run Files

If more than one run file is open, you can switch between viewing the run information in each. To do this:

1. Click the down arrow in the run box.



2. Select the run you want to view from the drop down list.

Closing Run Files

If more than one run file is open, you can close just one file or all the open files at the same time.

- **To close the run file being viewed** - Select **File** from the main menu and click **Close**.
- **To close all open run files** - Select **File** from the main menu and click **Close All**.

Chapter 8:

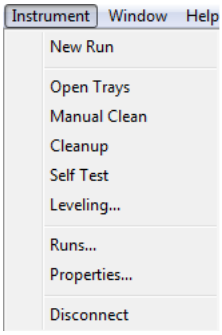
Controlling Jess, Wes, Sally Sue and Peggy Sue

Chapter Overview

- Instrument Control
- Self Test
- Viewing and Changing System Properties
- Viewing Log Files
- Status Modes

Instrument Control

The Instrument menu allows you to control Jess, Wes, Sally Sue and Peggy Sue.



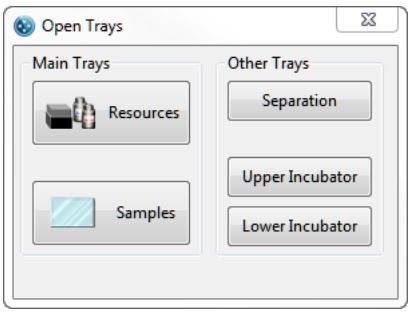
NOTE: Instrument menu options are active only when a computer with Compass for Simple Western is connected directly to Jess, Wes, Sally Sue or Peggy Sue. Available functions will vary depending on the instrument.

Starting a New Run

To start a new run, select **Instrument** in the main menu and click **New Run**. Then follow the steps described in "Step 2 - Start the Run" on page 86 for size assays or "Step 2 - Start the Run" on page 140 for charge assays.

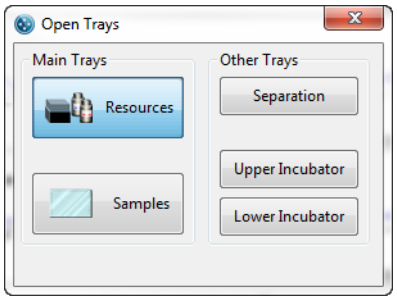
Opening Trays (Sally Sue and Peggy Sue)

To open any of the five trays, select **Instrument** and click **Open Trays**. The tray control window will appear:



Open a tray by clicking on its button. The button will become highlighted indicating the tray is open.

NOTE: Only one tray can be open at a time.



To close a tray, click the corresponding tray button again.

NOTE: If the tray control window is closed when a tray is open, the tray will close automatically.

Cleaning

Two cleaning options are available for Sally Sue and Peggy Sue.

Manual Clean

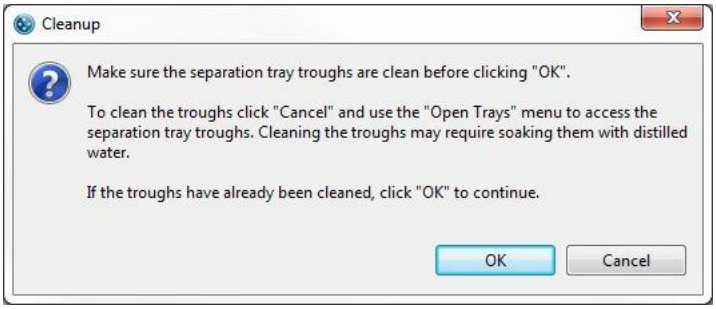
This option is used for general manual cleaning and cleaning the manifold head. To do a manual cleaning, select **Instrument** and click **Manual Clean**. The manifold head will move to a safe position for easy access and the vacuum will turn on.

NOTE: Please contact Protein Simple Technical Support toll-free in the US and Canada at 1-888-607-9692 (option 3) if you have any questions regarding the manifold cleaning procedure.

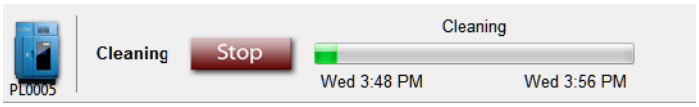
Cleanup

This option is a fully automated cleaning step. The manifold head is flushed, the separation tray troughs are aspirated and washed, and any capillaries left in the trays or gripper are picked up and discarded. This option should be selected when the instrument has not been used for more than a week or if a run error occurs. Cleaning takes about 8 minutes to complete.

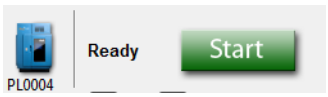
To start the protocol, select **Instrument** and click **Cleanup**. A window will appear with instructions:



Sally Sue's or Peggy Sue's status will change to cleaning, and the stop button and the cleaning progress bar display. The **Assay** screen provides cleaning status details:



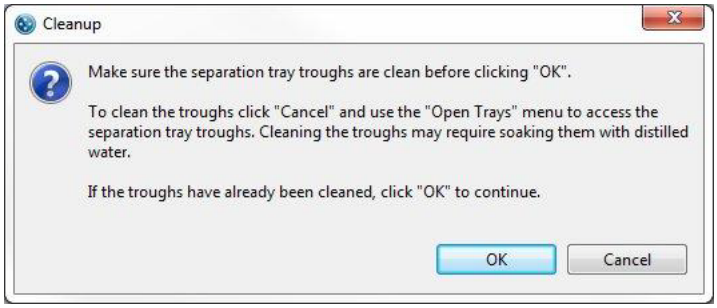
When cleaning is complete, instrument status will change to **Ready**.



Cleaning After a Run Error

Additional cleaning steps are required if an error occurs that stops the run. When this happens, the red Error status light on Sally Sue's or Peggy Sue's front panel will come on.

Click on the **Reset** button displayed in Compass for Simple Western software. The following instructions will appear:

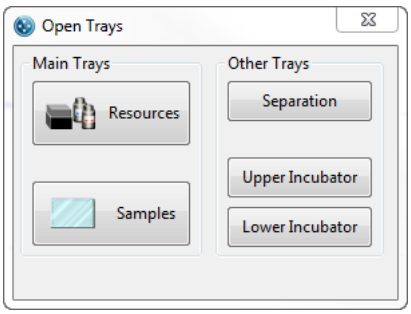


If the troughs in the separation tray are empty, click on **OK** and proceed with “Cleanup” on page 167.

If Running Buffer is present in the separation tray, click on **Cancel** and manually remove the buffer. Evaporation of the Running Buffer will result in a highly viscous residue which the automatic cleaning feature cannot remove.

To remove the Running Buffer:

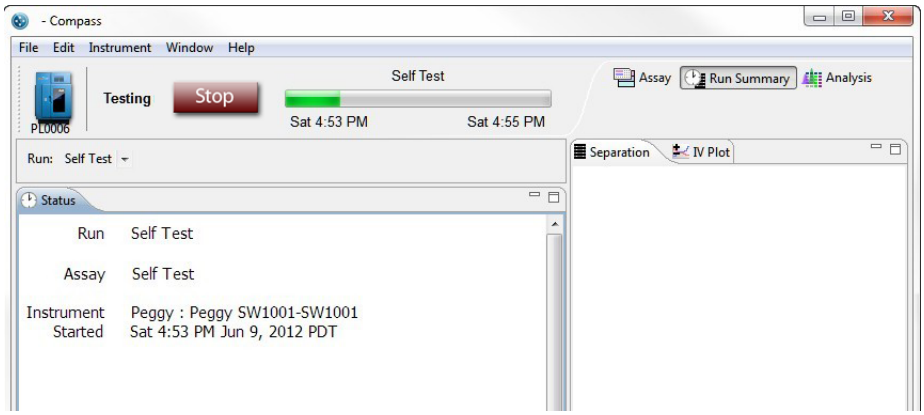
1. Select **Instrument** and click **Open Trays**.



2. Click **Separation** to open the separation tray.
3. Add 800 µL of deionized water to the troughs in the separation tray and soak for 20 minutes.
4. Remove the water by either aspirating with a pipette or with the vacuum wand located on the inside of Sally Sue’s or Peggy Sue's left door.
5. Repeat the steps above until the Running Buffer or residues are completely removed.
6. To complete the cleaning process, select **Instrument** and click **Cleanup**.

Self Test

Jess, Wes, Sally Sue and Peggy Sue can perform a series of self tests to check for proper instrument performance. To start the test, select **Instrument** and click **Self Test**. The test takes approximately 15 to 30 minutes depending on the instrument.



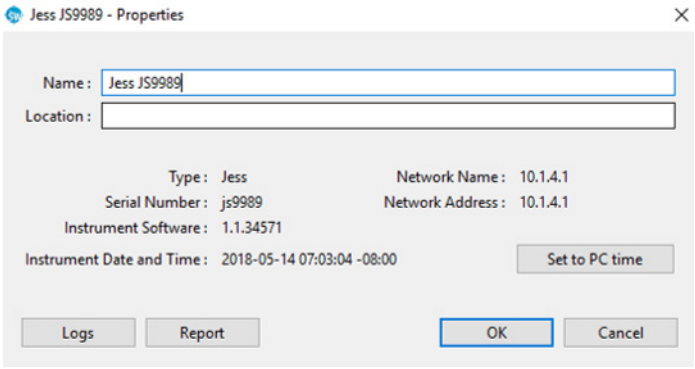
NOTE: We recommend performing the self test prior to starting a run.

To view the test log at completion of the test, select **Instrument**, click **Properties** and click **View Logs**. See "Self Test Logs" on page 174 for more information.

Viewing and Changing System Properties

Select **Instrument** and click **Properties** to display system properties which include:

- Name
- Location
- Type
- Serial number
- Instrument software version (firmware)
- Network name and address
- Date and time of the instrument clock

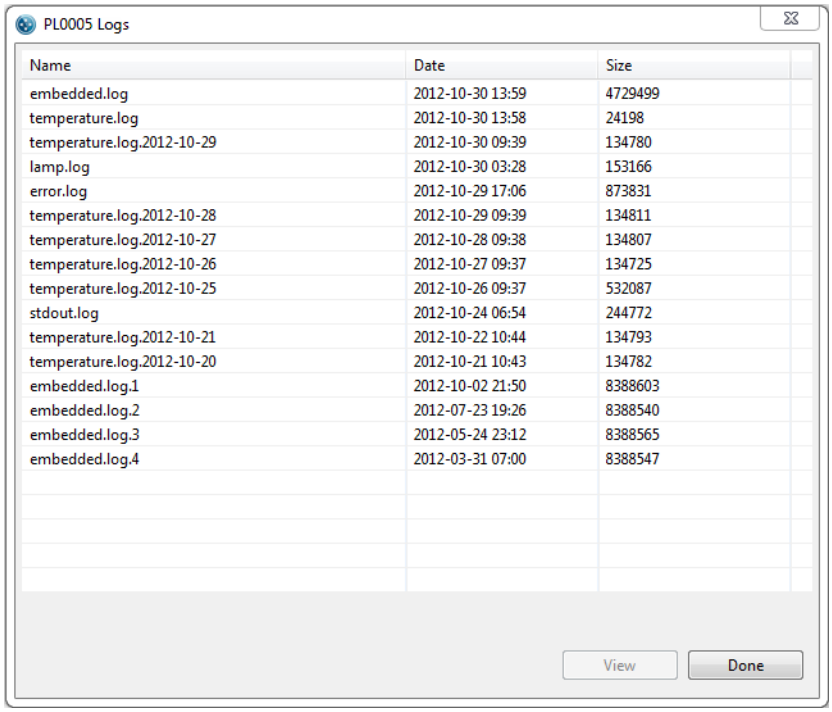


- **To change system name or location** - click in the name or location boxes and enter the new information.
- **To sync the instrument clock with the computer** - click **Set to PC time**.

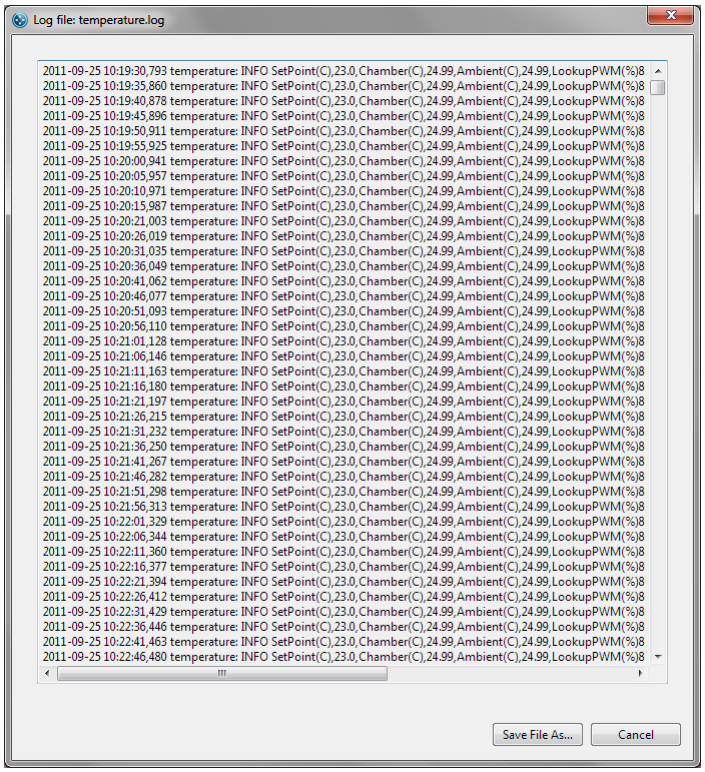
Viewing Log Files

Error Logs

1. Select **Instrument** in the main menu and click **Properties** to display system properties.
2. Click **View Error Log**. A list of system logs will display:



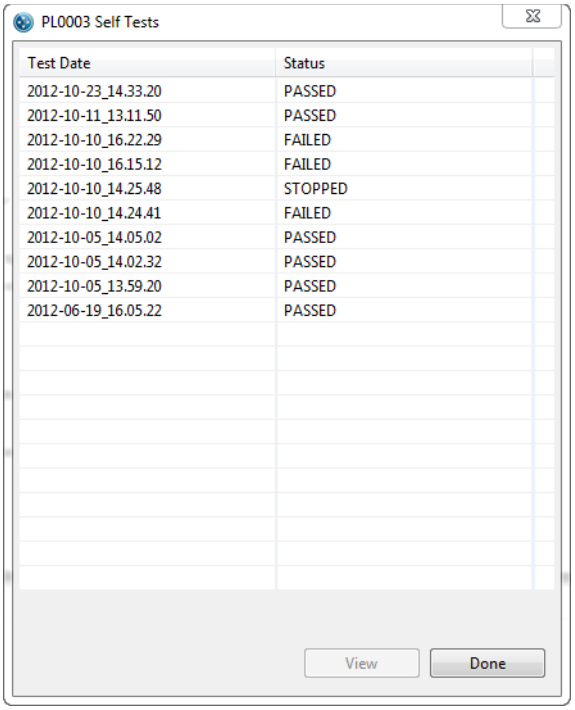
3. Select a log file and click **View**. The log details will display:



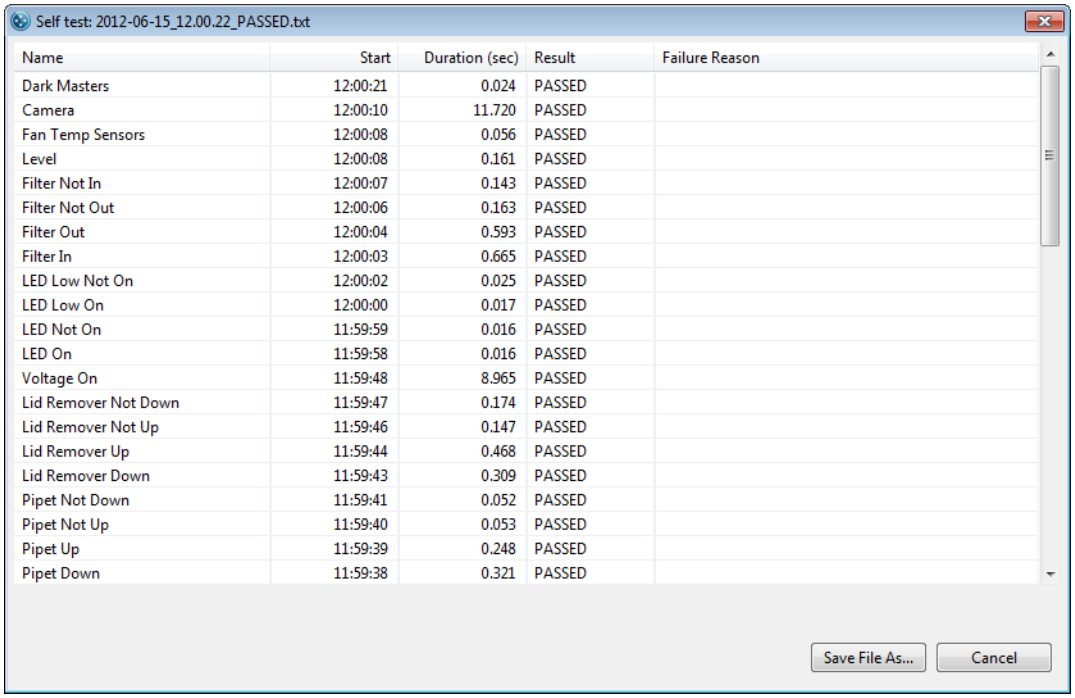
4. Click **Save File As** to save a copy of the log file.

Self Test Logs

1. Select **Instrument** in the main menu and click **Properties** to display system properties.
2. Click **View Test Log**. A list of self test logs will display:



3. Select a log file and click **View**. The individual test details will display:



4. Click **Save File As** to save a copy of the log file.

Status Modes

The instrument status bar displays status, buttons and progress bars depending on what Jess, Wes, Sally Sue or Peggy Sue is doing.

- **Ready/Start button** - The instrument is ready and an assay is loaded. Click **Start** to begin a run.
- **Not Ready/Clean button** - The instrument is not ready and must perform system cleaning. Click **Clean** to start the cleaning protocol.
- **Not Ready/Reset button** - The instrument is not ready and must reinitialize. Click **Reset** to start the initialization protocol.
- **Running/Stop button** - The instrument is running an assay. The run name, time the run started and when it will complete display in the run progress bar. Click **Stop** to stop the run.
- **Cleaning/button not active** - The instrument is running a cleaning protocol. The time the cleaning protocol started and when it will complete display in the run progress bar.
- **Error/Reset button** - An error has occurred. Go to the **Status** window in the **Run Summary** screen to view details. When the source of the error is corrected, click **Reset**.

Chapter 9:

Size Assay Data Analysis

Chapter Overview

- Analysis Screen Overview
- Opening Run Files
- How Run Data is Displayed in the Analysis Screen
- Viewing Run Data
- Run Data Notifications and Warnings
- Checking Your Results
- Group Statistics
- Copying Data Views and Results Tables
- Exporting Run Files
- Running Reports
- Changing Sample Protein Identification
- Changing the Virtual Blot View
- Changing the Electropherogram View
- Closing Run Files
- Analysis Settings Overview
- Images Analysis Settings
- Normalization (Jess only)
- Peak Names Settings
- Standard Curve Settings
- System or Loading Control Settings

- Standard Curve Settings
- Importing and Exporting Analysis Settings

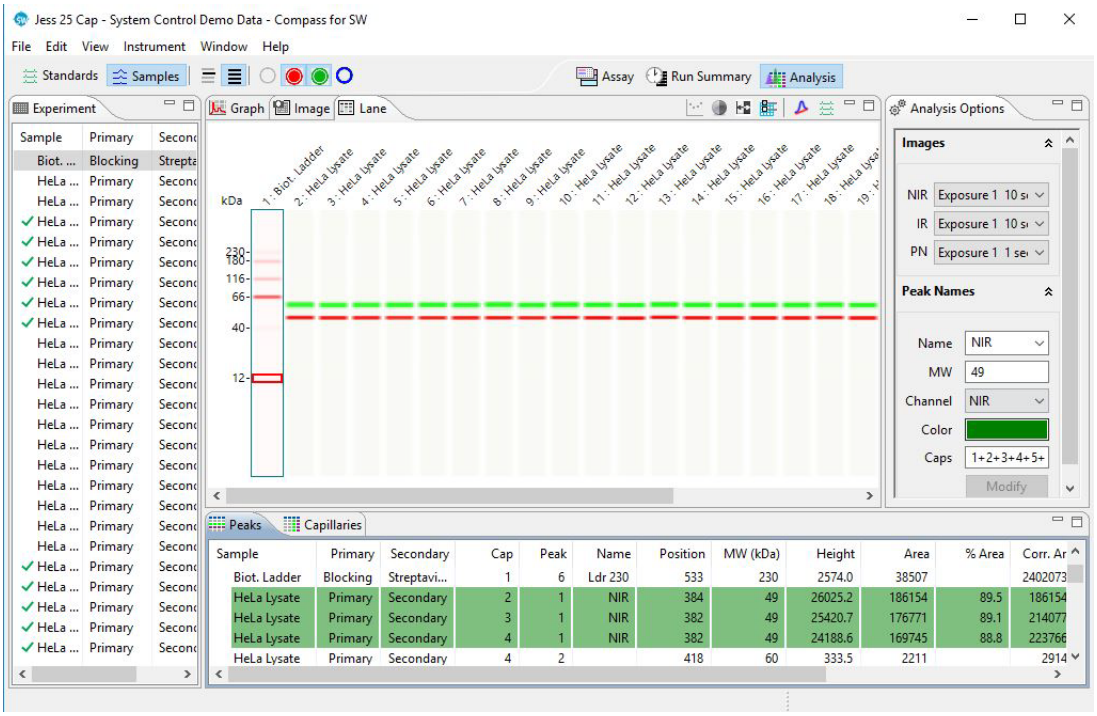
Analysis Screen Overview

The Analysis screen is used to view run data including electropherograms, capillary images, lane view data and tabulated results. Any post-run analysis is also done in this screen. To access this screen, click **Analysis** in the screen tab:

Analysis Screen Panes

The Analysis screen has seven panes, each displays the following data for up to 96 capillaries per experimental run:

- **Experiment** - Lists the assay protocol steps and assay template information.
- **Graph** - Displays electropherogram data for sample proteins, fluorescent standards or capillary registrations.
- **Image** - Displays a 12-capillary image of the separated sample proteins, fluorescent standards or capillary registrations.
- **Lane** - Displays data for sample proteins, fluorescent standards or capillary registrations as bands in individual lanes. This virtual blot-like image is similar to traditional blot results.
- **Peaks** - Lists the tabulated results for sample proteins and information for identified fluorescent standards and capillary registrations.
- **Capillaries** - Displays a list of the sample proteins Compass for Simple Western named automatically using the user-defined peak name analysis parameters.
- **Analysis Options** - Displays options to change various exposure settings and edit/label peak attributes.



NOTE: The reported molecular weight for sample proteins in Compass for Simple Western may vary from apparent molecular weights using other techniques and based on sample and assay conditions.

Software Menus Active in the Analysis Screen

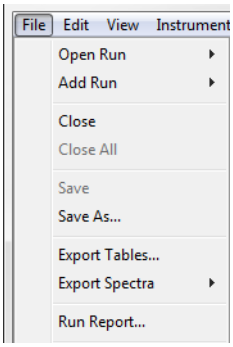
The following software menus are available:

- File
- Edit
- View
- Instrument (when Compass for Simple Western is connected to Jess, Wes, Sally Sue or Peggy Sue)
- Window
- Help

The File, Edit and View menu options specific to the Analysis screen are described next.

File Menu

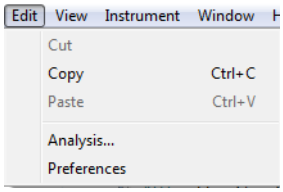
The following File menu options are active:



- **Open Run** - Opens a run file.
- **Add Run** - Opens and views other run files in addition to those that are already open.
- **Close** - Closes the run file currently being viewed.
- **Close All** - Closes all open run files.
- **Save** - Saves changes to the open run file.
- **Save As** - Saves changes to the open run file under a different file name.
- **Export Tables** - Exports the results for all capillaries in the run in .txt format.
- **Export Spectra** - Exports the raw data traces for each capillary in the run in .txt or .cdf format.
- **Run Report** - Creates a report of the run in .pdf format.
- **Exit** - Closes Compass for Simple Western.

Edit Menu

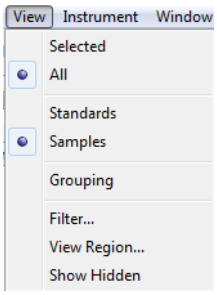
The following Edit menu options are active:



- **Copy** - Lets you copy data shown in the graph, lane, peaks or capillaries panes. See "Copying Data Views and Results Tables" on page 244 for more information.
- **Analysis** - Displays the analysis settings used to analyze the run data and lets you change them as needed. See "Analysis Settings Overview" on page 292 for more information.
- **Preferences** - Lets you set and save custom preferences for data export, plot colors in the graph and Twitter settings. See Chapter 11, *"Setting Your Preferences"* for more information.

View Menu

The following View menu options are active:



- **Selected View** - Displays data in a per capillary (single) view format.
- **All View** - Displays data in a per 12- or 25-capillary (multiple) view format.
- **Standards** - Lets you change the data view to show only the fluorescent standards.
- **Registration** - Lets you change the data view to show only the capillary registrations (Sally Sue and Peggy Sue only).
- **Samples** - Lets you change the data view to show sample proteins.
- **Filter** - Lets you display data only for specific capillaries or named proteins.
- **View Region** - Lets you change the molecular weight (x-axis) range of the data displayed.

- **Show Hidden**- Shows capillaries that are hidden from the data view.

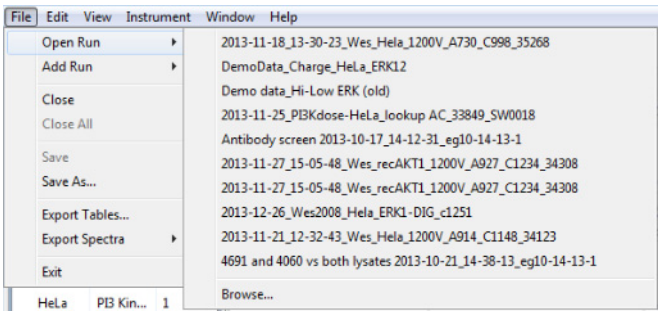
Opening Run Files

You can open one or multiple run files at a time to compare data between runs.

Opening One Run File

NOTE: If you need to open a run file when another run is executing, launch another instance of Compass for Simple Western first, then open the file.

1. Select **File** in the main menu and click **Open Run**.

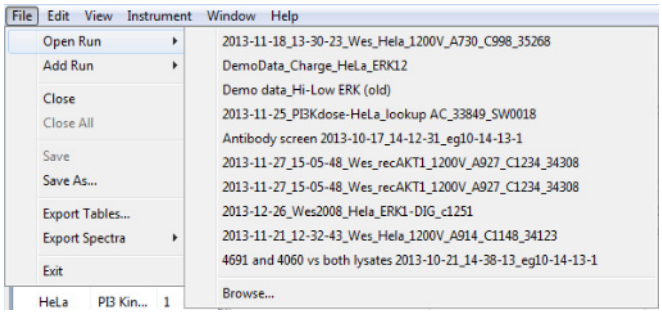


2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.

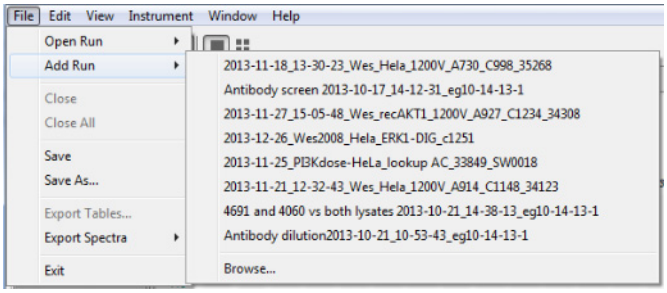
Opening Multiple Run Files

NOTE: If you need to open a run file when another run is executing, launch another instance of Compass for Simple Western first, then open the file.

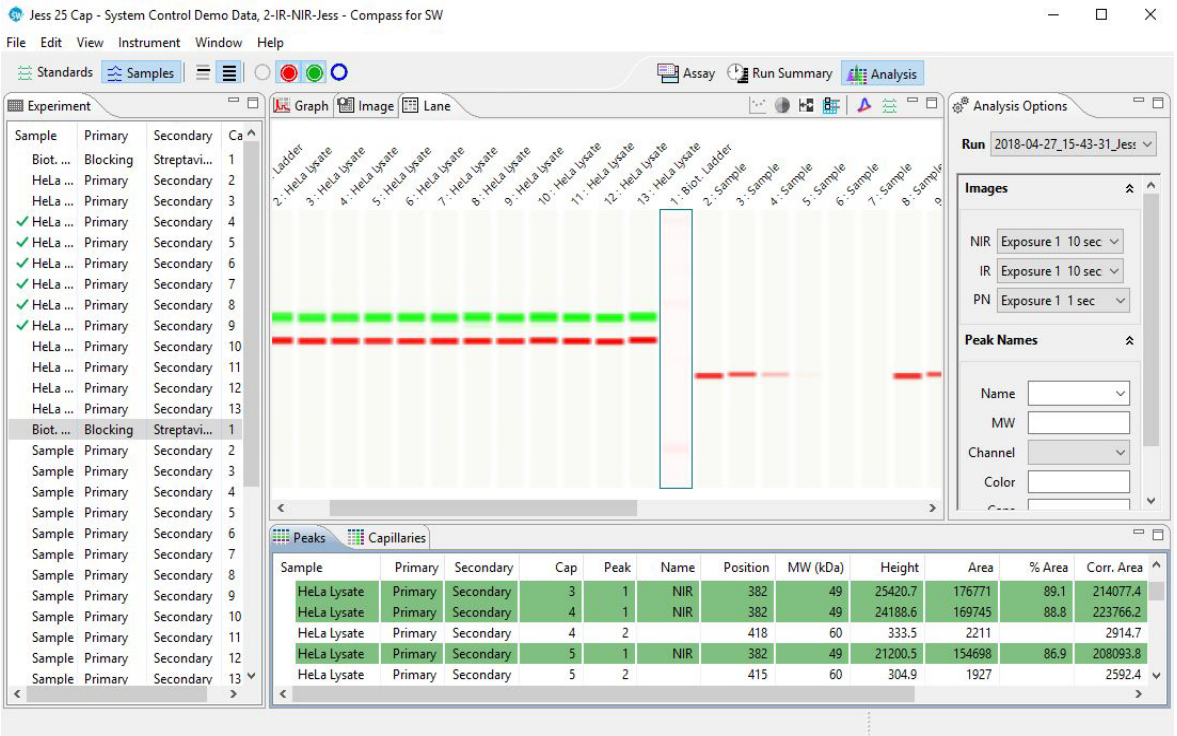
1. To open the first run file, select **File** in the main menu and click **Open Run**.



2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.
3. To open another run file, select **File** in the main menu and click **Add Run**.



4. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file. When a run is added, its data will append to the open run file, and will display as a second set of up to 25 capillaries (Jess/Wes) or 96 capillaries (Sally Sue/Peggy Sue) in all screen panes. The second run file name will also appear in the Compass title bar:



5. Repeat the last two steps to add additional runs.

NOTES:
When adding multiple data files for analysis, they must contain matching detection channels (for example chemi, IR/NIR fluorescence, or IR/NIR fluorescence with Protein Normalization).

When adding multiple RePlex data files (Jess only), the channels for both probes must contain matching detection files. For example, a chemiluminescence with Total Protein data file can't be added to a chemiluminescence/chemiluminescence data file.

How Run Data is Displayed in the Analysis Screen

Data in the run file is compartmentalized for easy review in one of six panes in the Analysis screen.

Experiment Pane: Assay and Capillary Information

The experiment pane displays assay and capillary information for each of the 25 capillaries (Jess/Wes) or 96 capillaries (Sally Sue/Peggy Sue) in the run. A maximized view of the experiment panes for a standard Immunoassay on Sally Sue and a RePlex Assay with two fluorescent NIR Immunoassays on Jess are shown below.

Experiment

Sample	Primary	Cycle	Cap	S	1	2	3
✓ Biotinylated Ladder	Blocking	1	1	C1	D1	E1	F1
✓ K562	anti-ERK1/2	1	2	C2	D2	E2	F2
K562	anti-ERK1/2	1	3	C3	D3	E3	F3
✓ K562	anti-ERK1/2	1	4	C4	D4	E4	F4
✓ K562	anti-ERK1/2	1	5	C5	D5	E5	F5
✓ K562	anti-ERK1/2	1	6	C6	D6	E6	F6
K562	anti-ERK1/2	1	7	C7	D7	E7	F7
K562	anti-ERK1/2	1	8	C8	D8	E8	F8
K562	anti-ERK1/2	1	9	C9	D9	E9	F9
K562	anti-ERK1/2	1	10	C10	D10	E10	F10
K562	anti-ERK1/2	1	11	C11	D11	E11	F11
RTU K562	anti-ERK1/2	1	12	C12	D12	E12	F12

Experiment

Sample	Primary 1	Primary 2	Cap
Biot. ...	Antibod...	Primary II	1
0.2 m...	MFAD	bActin	2
0.2 m...	bActin	bActin	3
0.2 m...	Park7	bActin	4
0.2 m...	MFAD	bActin	5
0.2 m...	bActin	bActin	6
0.2 m...	Park7	bActin	7
0.2 m...	MFAD	bActin	8
0.2 m...	bActin	bActin	9
0.2 m...	Park7	bActin	10
0.2 m...	MFAD	bActin	11
0.2 m...	bActin	bActin	12

- **To view all columns** - Click the **Experiment** tab, then use the scroll bar or click **Maximize** in the upper right corner.
- **To resize columns** - Click the **Experiment** tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Experiment pane column descriptions for the default assay are as follows:

- **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.

NOTE: Data notification icons will display in the sample column if Compass for Simple Western detected a potential analysis issue or data was manually modified by the user. For more information see "Run Data Notifications and Warnings" on page 227.

- **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Primary 1 (Jess RePlex Assays only)** - Primary antibody name for Probe 1. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.

- **Primary 2 (Jess RePlex Assays only)** - Primary antibody name for Probe 2. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Secondary** - Secondary antibody name. If secondary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Secondary (default name) will display.

NOTE: The Secondary column will not display for RePlex Assays on Jess with two immunoassays.

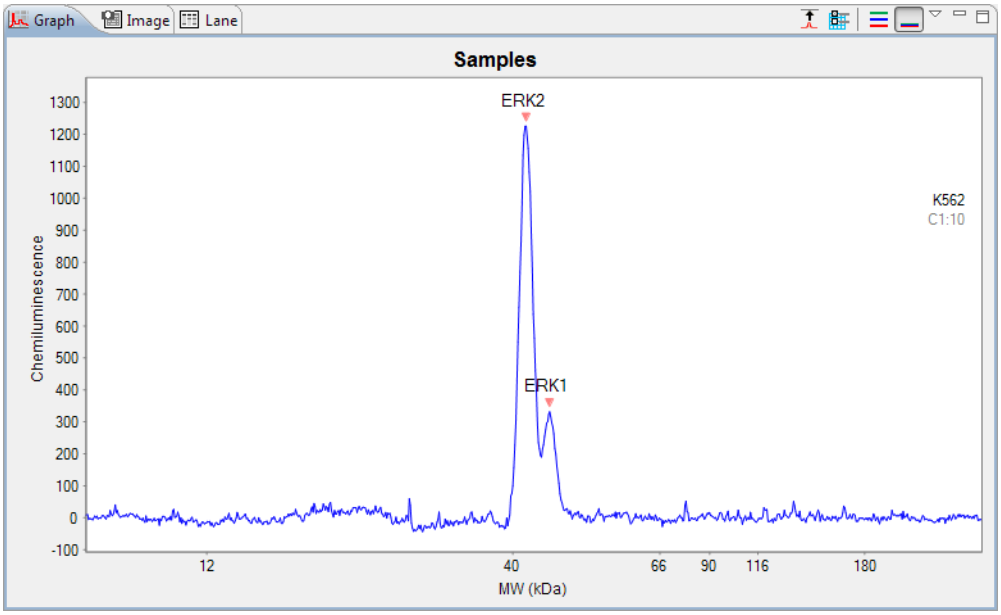
- **Cycle (Sally Sue/Peggy Sue only)** - Run cycle number. There are 12 capillaries in one cycle for Sally Sue/Peggy Sue.

NOTE: Sally Sue and Peggy Sue can run up to eight cycles (12 capillaries per cycle) in an experiment. Each cycle is designated in the Experiment summary.

- **Cap** - Capillary number.
- **S (Peggy Sue/Sally Sue only)** - Well on the assay plate used for sample.
- **1 (Peggy Sue/Sally Sue only)** - Well on the assay plate used for primary antibody or Total Protein labeling reagent.
- **2 (Peggy Sue/Sally Sue only)** - Well on the assay plate used for secondary HRP-conjugate or Total Protein Streptavidin-HRP.
- **3 (Peggy Sue/Sally Sue only)** - Well on the assay plate used for a tertiary antibody.

Graph Pane: Electropherogram Data

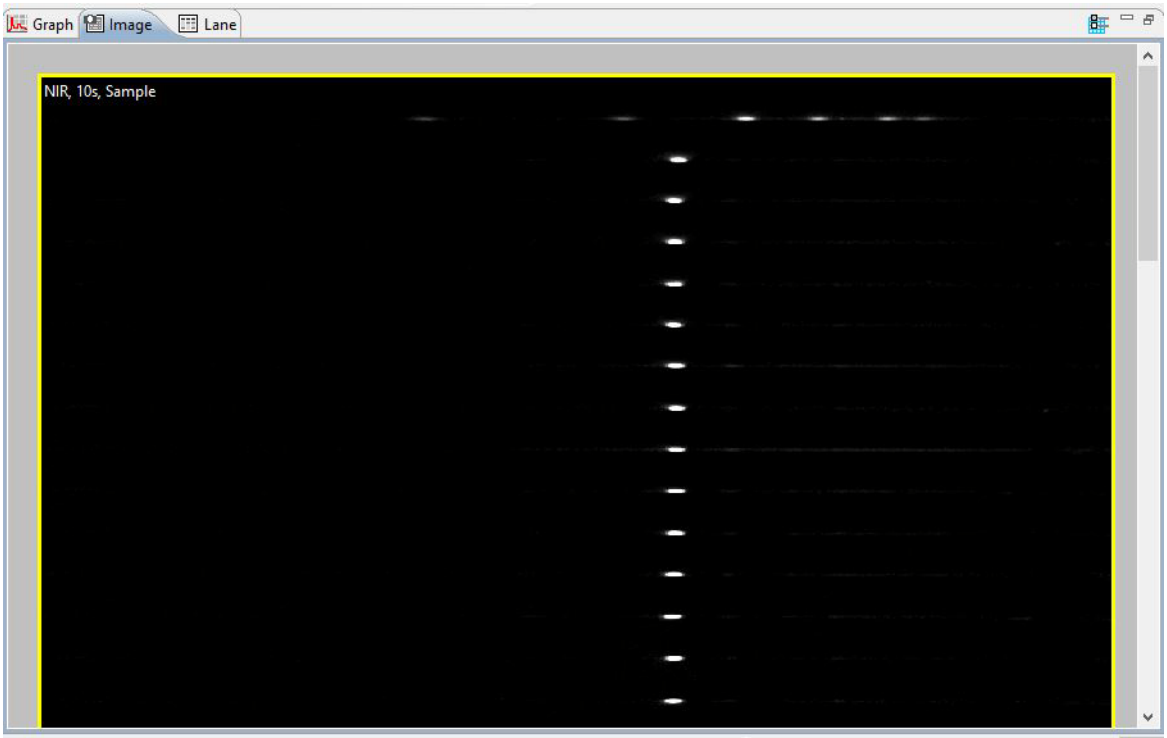
Click the **Graph** tab to view data for sample proteins, fluorescent standards or capillary registrations. Data for samples is shown in the following example, and proteins are displayed as peaks:



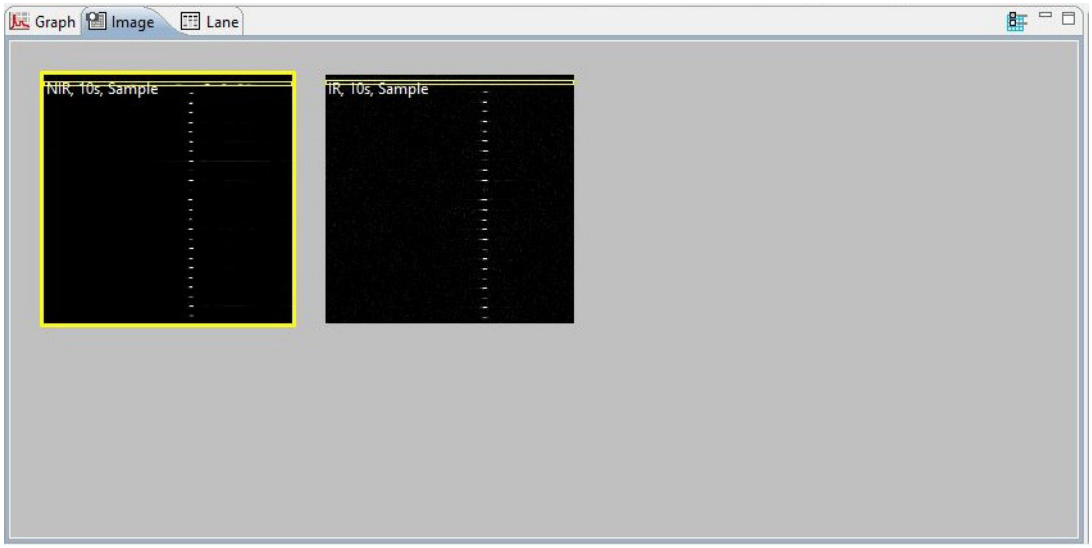
More Graph view options will be described in more detail in “Changing the Electropherogram View” on page 268.

Image Pane: Capillary Separation Image Data

Click the **Image** tab to view final separation images of sample proteins, fluorescent standards or capillary registrations for up to 96 capillaries per experimental run. Image data for samples is shown in the following example:

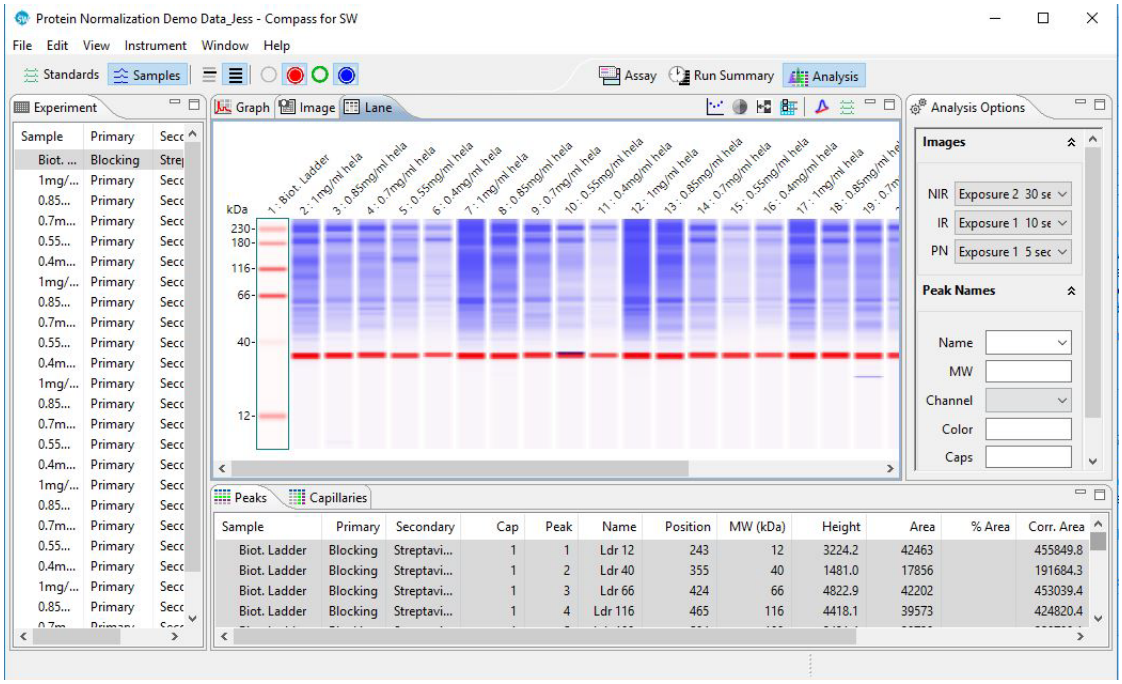


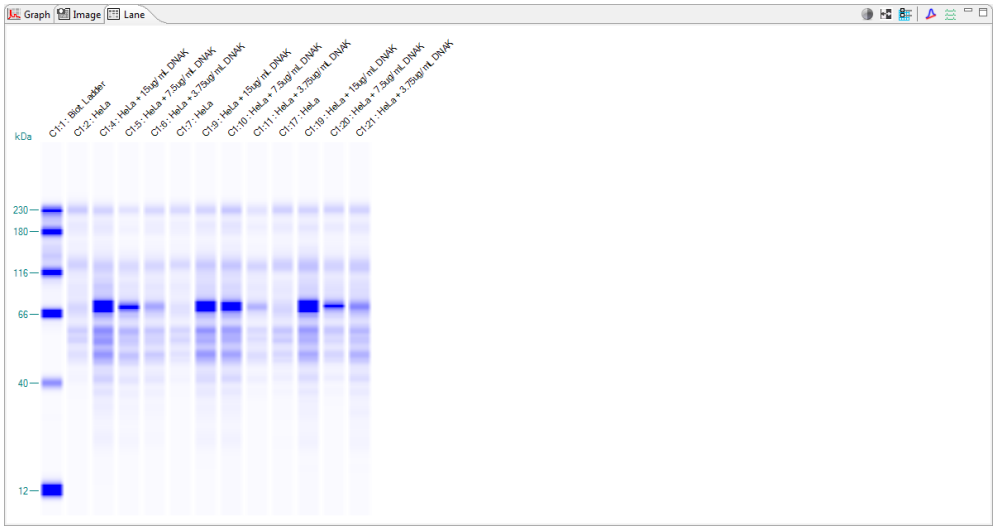
Select the blue **All Images** icon at the top right of the Image view pane to display sample, raw, and background images. Image data for multiple sample types is shown in the following example:



Lane Pane: Virtual Blot-Like Image Data

Click the **Lane** tab to view data for sample proteins, fluorescent standards or capillary registrations as bands in individual lanes. This virtual blot-like image is similar to traditional blot results. Data for samples in the lane view for an Immunoassay (immunodetected proteins), Protein Normalization (for Jess only), and from a Total Protein Assay are displayed as bands in the examples below.





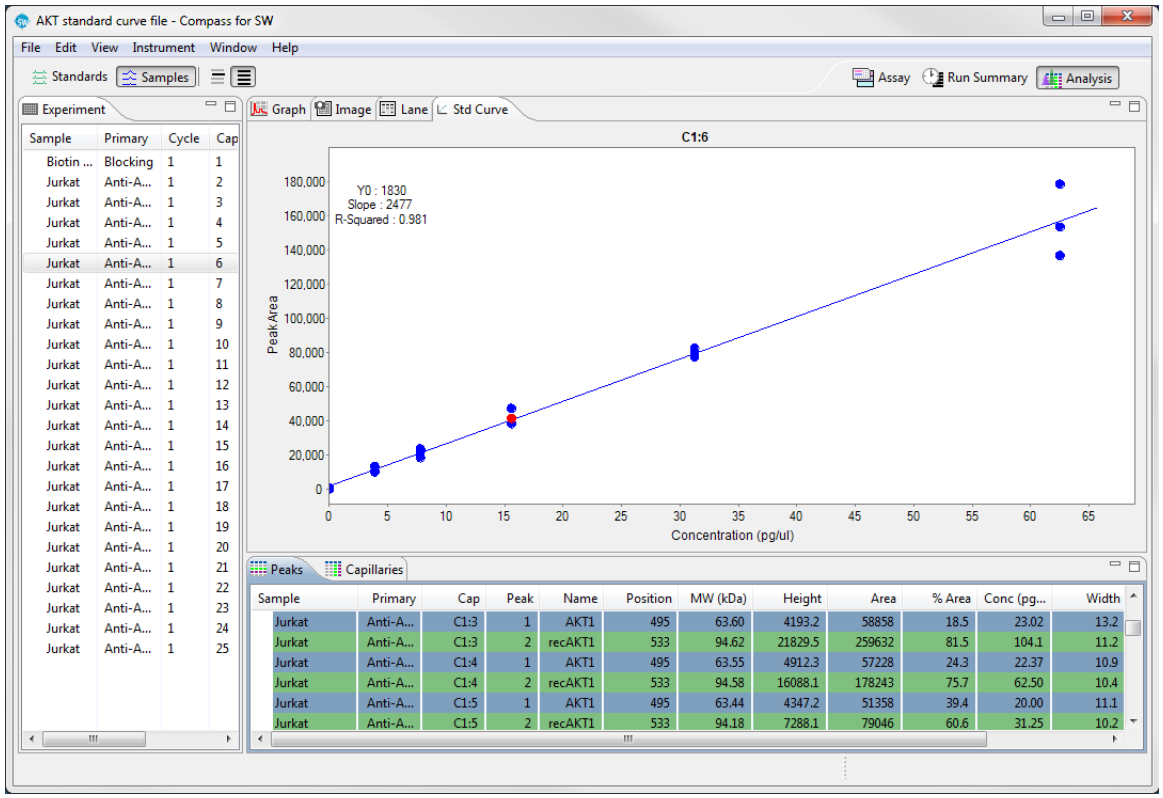
To view information for a band, roll the mouse over a band until the info box appears.

NOTE: The reported molecular weight for sample proteins detected by an Immunoassay or a Total Protein Assay in Compass for Simple Western may vary from apparent molecular weights using other techniques and based on sample and assay conditions.

Lane data displayed in the virtual blot is automatically aligned by Compass for Simple Western. To view raw, unaligned lane data and learn more about virtual blot viewing options, see "Changing the Virtual Blot View" on page 261.

Std Curve Pane: Standard Curve Fit Data

Click the **Std Curve** tab to view a linear or 4-parameter curve fit of your standard curve proteins. Data for the samples in the std curve view is shown in the following example where proteins are displayed as dots.



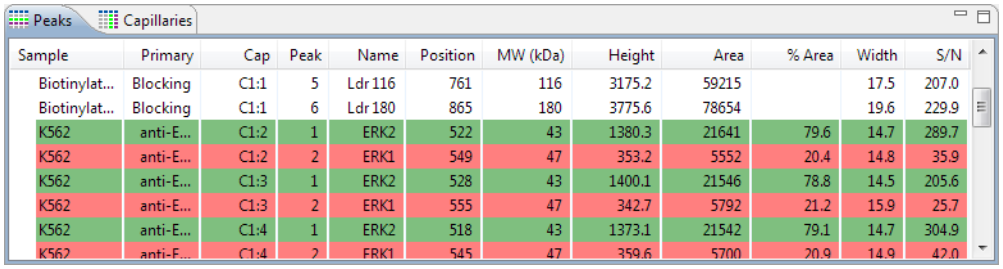
Sample	Primary	Cycle	Cap
Biotin ...	Blocking	1	1
Jurkat	Anti-A...	1	2
Jurkat	Anti-A...	1	3
Jurkat	Anti-A...	1	4
Jurkat	Anti-A...	1	5
Jurkat	Anti-A...	1	6
Jurkat	Anti-A...	1	7
Jurkat	Anti-A...	1	8
Jurkat	Anti-A...	1	9
Jurkat	Anti-A...	1	10
Jurkat	Anti-A...	1	11
Jurkat	Anti-A...	1	12
Jurkat	Anti-A...	1	13
Jurkat	Anti-A...	1	14
Jurkat	Anti-A...	1	15
Jurkat	Anti-A...	1	16
Jurkat	Anti-A...	1	17
Jurkat	Anti-A...	1	18
Jurkat	Anti-A...	1	19
Jurkat	Anti-A...	1	20
Jurkat	Anti-A...	1	21
Jurkat	Anti-A...	1	22
Jurkat	Anti-A...	1	23
Jurkat	Anti-A...	1	24
Jurkat	Anti-A...	1	25

Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Conc (pg...	Width
Jurkat	Anti-A...	C1:3	1	AKT1	495	63.60	4193.2	58858	18.5	23.02	13.2
Jurkat	Anti-A...	C1:3	2	recAKT1	533	94.62	21829.5	259632	81.5	104.1	11.2
Jurkat	Anti-A...	C1:4	1	AKT1	495	63.55	4912.3	57228	24.3	22.37	10.9
Jurkat	Anti-A...	C1:4	2	recAKT1	533	94.58	16088.1	178243	75.7	62.50	10.4
Jurkat	Anti-A...	C1:5	1	AKT1	495	63.44	4347.2	51358	39.4	20.00	11.1
Jurkat	Anti-A...	C1:5	2	recAKT1	533	94.18	7288.1	79046	60.6	31.25	10.2

Concentration of the sample proteins is automatically determined by Compass based on defined values of the standard curve.

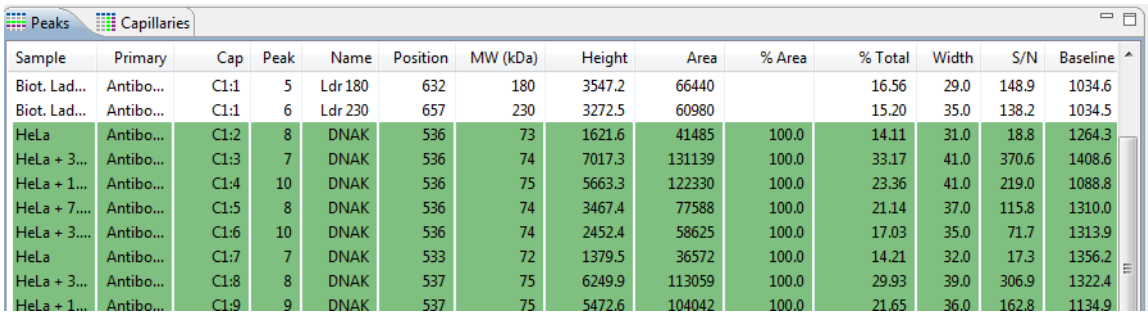
Peaks Pane: Calculated Results

Click the **Peaks** tab to view tabulated results for sample proteins, normalized sample proteins shown as Corr. Area (when Normalization is enabled), fluorescent standards or capillary registrations. Each row in the table shows the individual results for each peak detected in each capillary. Data for samples in the peaks table for an Immunoassay, a Total Protein Assay, Protein Normalization (Jess only) and a RePlex Assay (Jess only) are shown in the following examples:



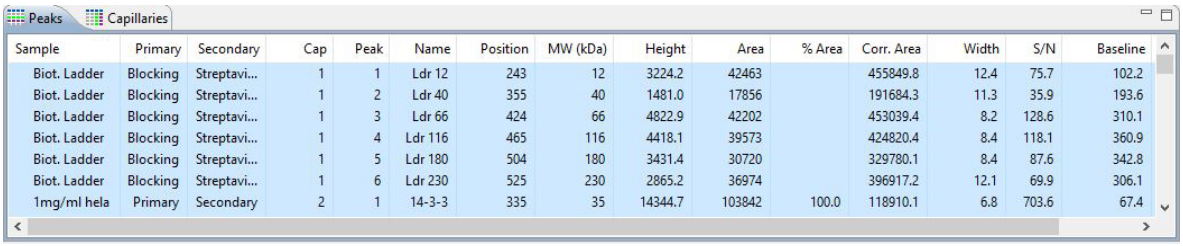
Peaks | Capillaries

Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width	S/N
Biotinylat...	Blocking	C1:1	5	Ldr 116	761	116	3175.2	59215		17.5	207.0
Biotinylat...	Blocking	C1:1	6	Ldr 180	865	180	3775.6	78654		19.6	229.9
K562	anti-E...	C1:2	1	ERK2	522	43	1380.3	21641	79.6	14.7	289.7
K562	anti-E...	C1:2	2	ERK1	549	47	353.2	5552	20.4	14.8	35.9
K562	anti-E...	C1:3	1	ERK2	528	43	1400.1	21546	78.8	14.5	205.6
K562	anti-E...	C1:3	2	ERK1	555	47	342.7	5792	21.2	15.9	25.7
K562	anti-E...	C1:4	1	ERK2	518	43	1373.1	21542	79.1	14.7	304.9
K562	anti-E...	C1:4	2	ERK1	545	47	359.6	5700	20.9	14.9	42.0



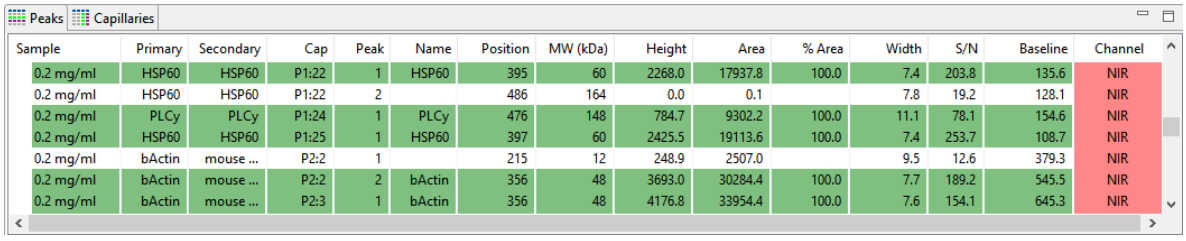
Peaks | Capillaries

Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	% Total	Width	S/N	Baseline
Biot. Lad...	Antibo...	C1:1	5	Ldr 180	632	180	3547.2	66440		16.56	29.0	148.9	1034.6
Biot. Lad...	Antibo...	C1:1	6	Ldr 230	657	230	3272.5	60980		15.20	35.0	138.2	1034.5
HeLa	Antibo...	C1:2	8	DNAK	536	73	1621.6	41485	100.0	14.11	31.0	18.8	1264.3
HeLa + 3...	Antibo...	C1:3	7	DNAK	536	74	7017.3	131139	100.0	33.17	41.0	370.6	1408.6
HeLa + 1...	Antibo...	C1:4	10	DNAK	536	75	5663.3	122330	100.0	23.36	41.0	219.0	1088.8
HeLa + 7....	Antibo...	C1:5	8	DNAK	536	74	3467.4	77588	100.0	21.14	37.0	115.8	1310.0
HeLa + 3....	Antibo...	C1:6	10	DNAK	536	74	2452.4	58625	100.0	17.03	35.0	71.7	1313.9
HeLa	Antibo...	C1:7	7	DNAK	533	72	1379.5	36572	100.0	14.21	32.0	17.3	1356.2
HeLa + 3...	Antibo...	C1:8	8	DNAK	537	75	6249.9	113059	100.0	29.93	39.0	306.9	1322.4
HeLa + 1...	Antibo...	C1:9	9	DNAK	537	75	5472.6	104042	100.0	21.65	36.0	162.8	1134.9



Peaks | Capillaries

Sample	Primary	Secondary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Corr. Area	Width	S/N	Baseline
Biot. Ladder	Blocking	Streptavi...	1	1	Ldr 12	243	12	3224.2	42463		455849.8	12.4	75.7	102.2
Biot. Ladder	Blocking	Streptavi...	1	2	Ldr 40	355	40	1481.0	17856		191684.3	11.3	35.9	193.6
Biot. Ladder	Blocking	Streptavi...	1	3	Ldr 66	424	66	4822.9	42202		453039.4	8.2	128.6	310.1
Biot. Ladder	Blocking	Streptavi...	1	4	Ldr 116	465	116	4418.1	39573		424820.4	8.4	118.1	360.9
Biot. Ladder	Blocking	Streptavi...	1	5	Ldr 180	504	180	3431.4	30720		329780.1	8.4	87.6	342.8
Biot. Ladder	Blocking	Streptavi...	1	6	Ldr 230	525	230	2865.2	36974		396917.2	12.1	69.9	306.1
1mg/ml hela	Primary	Secondary	2	1	14-3-3	335	35	14344.7	103842	100.0	118910.1	6.8	703.6	67.4



Peaks | Capillaries

Sample	Primary	Secondary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width	S/N	Baseline	Channel
0.2 mg/ml	HSP60	HSP60	P1:22	1	HSP60	395	60	2268.0	17937.8	100.0	7.4	203.8	135.6	NIR
0.2 mg/ml	HSP60	HSP60	P1:22	2		486	164	0.0	0.1		7.8	19.2	128.1	NIR
0.2 mg/ml	PLCy	PLCy	P1:24	1	PLCy	476	148	784.7	9302.2	100.0	11.1	78.1	154.6	NIR
0.2 mg/ml	HSP60	HSP60	P1:25	1	HSP60	397	60	2425.5	19113.6	100.0	7.4	253.7	108.7	NIR
0.2 mg/ml	bActin	mouse ...	P2:2	1		215	12	248.9	2507.0		9.5	12.6	379.3	NIR
0.2 mg/ml	bActin	mouse ...	P2:2	2	bActin	356	48	3693.0	30284.4	100.0	7.7	189.2	545.5	NIR
0.2 mg/ml	bActin	mouse ...	P2:3	1	bActin	356	48	4176.8	33954.4	100.0	7.6	154.1	645.3	NIR

NOTES:

Peaks that Compass for Simple Western names automatically using the user-defined peak name analysis parameters are color-coded.

The reported molecular weight for sample proteins detected using an Immunoassay or a Total Protein Assay in Compass for Simple Western may vary from apparent molecular weights using other techniques and based on sample and assay conditions.

- **To view all rows** - Click the **Peaks** tab, then use the scroll bar or click **Maximize** in the upper right corner.
- **To resize columns** - Click the **Peaks** tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Peak table column descriptions are as follows:

- **Sample** - Sample name. If sample names were entered in the assay template (Assay screen) or Analysis Options pane, those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Primary antibody name. For Immunoassays, if primary antibody names were entered in the assay template (Assay screen) or Analysis Options pane, those names will display here. Otherwise, Primary (default name) will display. For Total Protein Assays, Antibody Diluent will display as the default name.
- **Secondary** - Secondary antibody name. If secondary antibody names were entered in the assay template (Assay screen) or Analysis Options pane, those names will display here. Otherwise, Secondary (default name) will display.
- **Cap**
  - Sally Sue and Peggy Sue: Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.
  - Jess and Wes: Capillary number.
  - Jess RePlex Assays: Probe and capillary number. For example, P1:4 indicates Probe 1, capillary 4.

NOTE: Sally Sue and Peggy Sue run up to eight cycles in an experiment, 12 capillaries at a time. Capillary and cycle numbers are displayed in the Experiment tab.

- **Peak** - Peak number. Peaks are numbered in order of detection.
- **Name** - Peak name. Displays peaks that Compass for Simple Western named automatically using the user-defined peak name analysis parameters. Cells will be blank if Compass was not able to name the peak or if naming parameters were not entered.
- **Position** - Displays the pixel position of a peak in the image.
- **MW (kDa)** - Displays the calculated molecular weight in kDa for the peak (shown for sample data only).
- **Height** - Displays the calculated peak height.

- **Area** - Displays the calculated peak area (shown for sample data only).
- **% Area** - Reported when area is calculated using the Gaussian method (default for Immunoassays, see "Peak Find Settings" on page 301 for more information). Displays the calculated percent area for the named peak compared to all named peaks. This value results from dividing the individual peak area by the sum of all named peak areas for the capillary and multiplying by 100 (shown for named peak sample data only).
- **% Total** - Reported when area is calculated using the Dropped Line method (default for Total Protein Assays, see "Peak Find Settings" on page 301 for more information). Displays the calculated percent area for the peak compared to the total area measured in the capillary. This value results from dividing the individual peak area by the entire area under the curve for the capillary and multiplying by 100.
- **Conc (Concentration)** - Displays the calculated concentration of protein for the named peak. This column will not appear unless a standard curve is defined.
- **Corr. Area** - Displays the corrected area for the peak. This column will not appear unless a Loading Control is defined or Normalization is enabled in Analysis Preferences.
  - **For PN channel** - A linear correction calculation is applied to peak area to represent amount of protein. The linear calculation correction factor is **Corr. Area** = Area + (1e-5 * $Area^2$). The normalization factor is not used for this calculation.
  - **For Chemi, NIR, and IR channel peaks** - Corrected area is determined by first calculating the Capillary Normalization factor for each capillary and then correcting the peak area as follows:

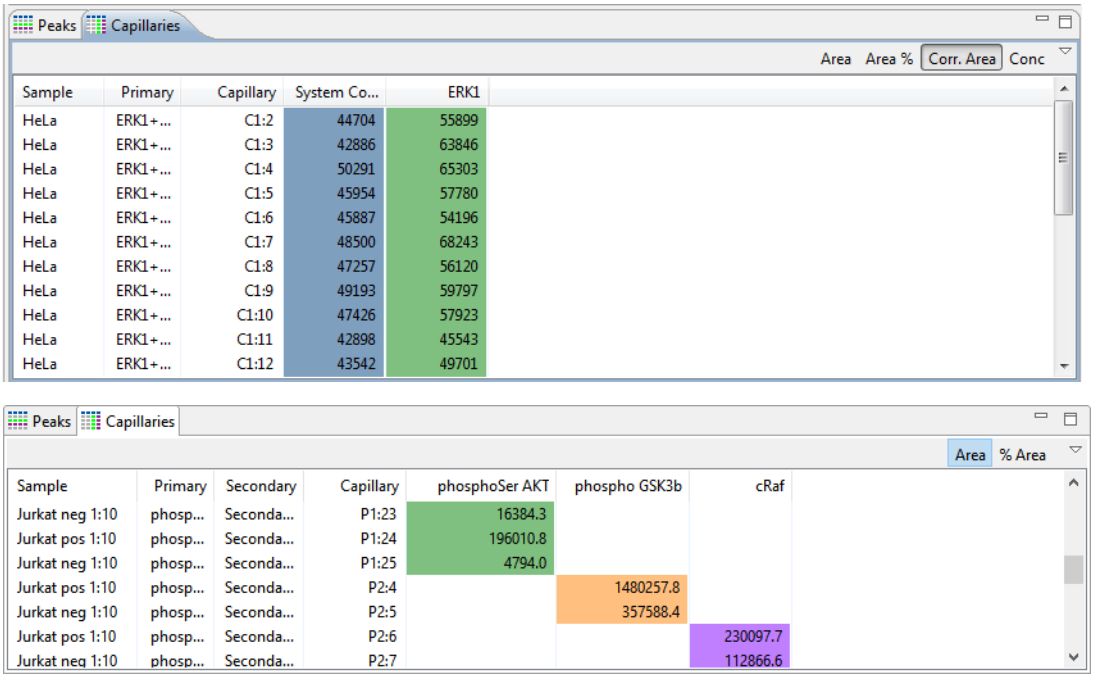
    Capillary Normalization Factor = Normalization area for chosen capillary/Normalization area for reference capillary in Analysis settings.

    Then,

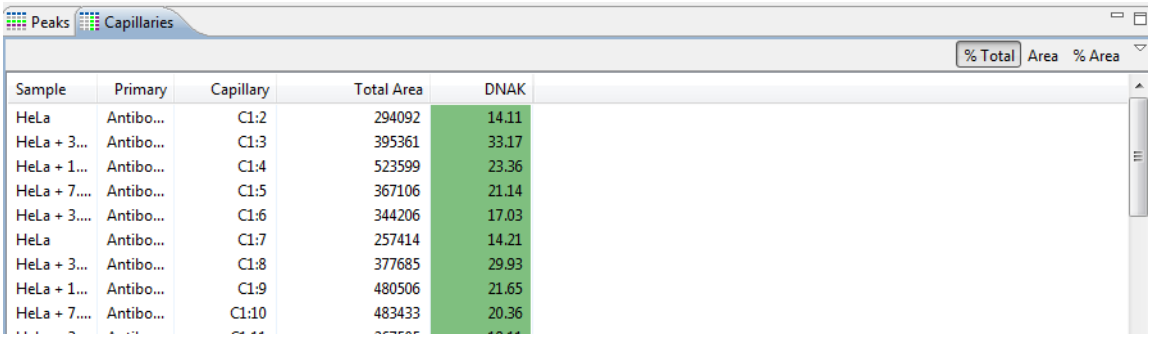
    Corr. Area = Peak area/Capillary Normalization factor.
- **Width** - Displays the calculated peak width (shown for sample data only).
- **S/N** - Displays the calculated signal to noise ratio for the peak (shown for sample data only). Please note this calculation is based on a peak finding algorithm to provide a relative ratio comparing all peaks within the electropherogram in Compass.
- **Baseline** - Displays the raw baseline signal of each peak
- **Channel** - Detection channel (Chemi, NIR, IR, PN)

Capillaries Pane: User-Specified Peak Names

Click the **Capillaries** tab to view tabulated results for sample proteins associated with specific primary antibodies, Protein Normalization area, or Total Protein area in the run data. Compass for Simple Western labels these sample peaks automatically using user-defined peak name settings. Each row in the table shows the individual results for the named peaks detected in each capillary. Data for samples in the capillaries table is shown in the following example for an Immunoassay and a RePlex Assay with two immunoassays.

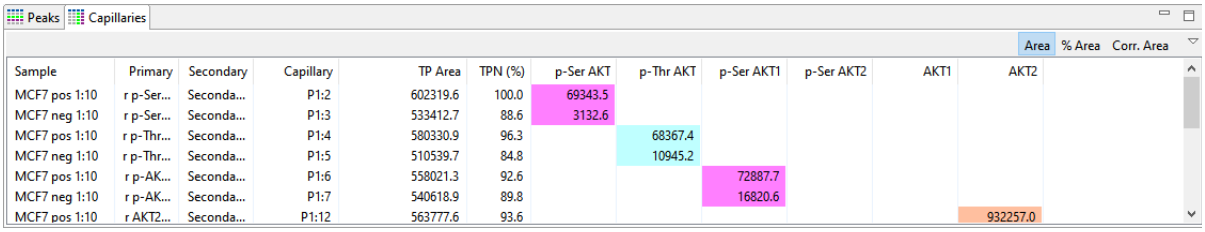


For standard Total Protein Assays where area calculation is handled via Dropped Lines by default, the Capillaries tab is shown below. Compass for Simple Western labels the sample peaks automatically using user-defined peak name settings. Each row in the table shows the individual results for the total area and named peaks detected in each capillary.



- **Total Area** - Total area measured in the capillary when using a standard Total Protein Assay.

For RePlex Assays with Total Protein enabled where the TP Area column accounts for this measurement automatically, the capillaries tab is shown below.



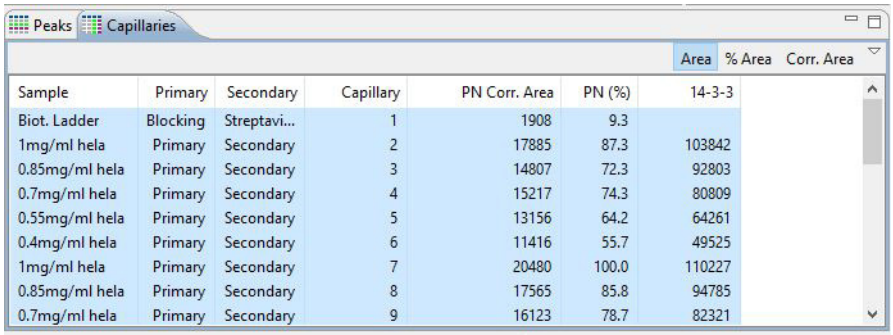
Peaks | Capillaries

Area | % Area | Corr. Area

Sample	Primary	Secondary	Capillary	TP Area	TPN (%)	p-Ser AKT	p-Thr AKT	p-Ser AKT1	p-Ser AKT2	AKT1	AKT2
MCF7 pos 1:10	r p-Ser...	Seconda...	P1:2	602319.6	100.0	69343.5					
MCF7 neg 1:10	r p-Ser...	Seconda...	P1:3	533412.7	88.6	3132.6					
MCF7 pos 1:10	r p-Thr...	Seconda...	P1:4	580330.9	96.3		68367.4				
MCF7 neg 1:10	r p-Thr...	Seconda...	P1:5	510539.7	84.8		10945.2				
MCF7 pos 1:10	r p-AK...	Seconda...	P1:6	558021.3	92.6			72887.7			
MCF7 neg 1:10	r p-AK...	Seconda...	P1:7	540618.9	89.8			16820.6			
MCF7 pos 1:10	r AKT2...	Seconda...	P1:12	563777.6	93.6						932257.0

- **TP Area** - Total area measured in the capillary for the Total Protein signal.
- **TPN (%)** - Total Protein signal for chosen capillary/Total Protein signal for reference capillary in the Analysis settings.

When using the Protein Normalization Module on Jess, where the PN Corr.Area column accounts for this measurement automatically, the capillaries tab is shown below.



Peaks | Capillaries

Area | % Area | Corr. Area

Sample	Primary	Secondary	Capillary	PN Corr. Area	PN (%)	14-3-3
Biot. Ladder	Blocking	Streptavi...	1	1908	9.3	
1mg/ml hela	Primary	Secondary	2	17885	87.3	103842
0.85mg/ml hela	Primary	Secondary	3	14807	72.3	92803
0.7mg/ml hela	Primary	Secondary	4	15217	74.3	80809
0.55mg/ml hela	Primary	Secondary	5	13156	64.2	64261
0.4mg/ml hela	Primary	Secondary	6	11416	55.7	49525
1mg/ml hela	Primary	Secondary	7	20480	100.0	110227
0.85mg/ml hela	Primary	Secondary	8	17565	85.8	94785
0.7mg/ml hela	Primary	Secondary	9	16123	78.7	82321

PN Corr.Area is determined by first calculating the normalization factor for each capillary and then correcting the peak area for each signal as follows:

Capillary Normalization Factor = Protein Normalization signal for chosen capillary/Protein Normalization signal for reference capillary in Analysis settings.

Then,

PN Corr.Area = PN Area + (1e-5 * Area^2)

PN (%) = Capillary Normalization Factor x 100, as percentage

NOTES:
Peaks that Compass for Simple Western names automatically with user-defined peak name settings are color-coded.

Information displayed for fluorescent standards and capillary registration data will be for identified standards or registration peaks.

- **To view all rows** - Click the **Capillaries** tab, then use the scroll bar or click **Maximize** in the upper right corner.
- **To resize columns** - Click the **Capillaries** tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

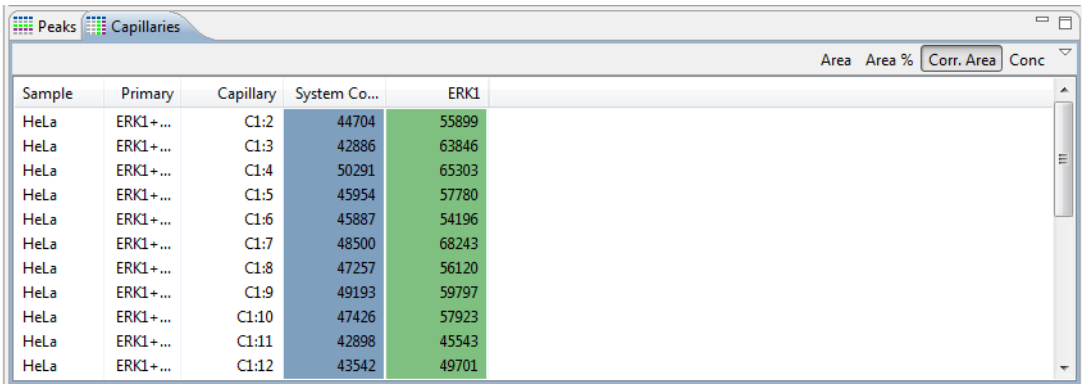
Capillaries table column descriptions are as follows:

- **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen) or Analysis Options pane, those names will display here. Otherwise, Primary (default name) will display.
- **Secondary** - Secondary antibody name. If secondary antibody names were entered in the assay template (Assay screen) or Assay Options pane, those names will display here. Otherwise, Secondary (default name) will display.
- **Capillary**
  - Sally Sue and Peggy Sue: Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.
  - Jess and Wes: Capillary number.
  - Jess RePlex Assays: Probe and capillary number. For example, P1:4 indicates Probe 1, capillary 4.

NOTE: Sally Sue and Peggy Sue run12 capillaries at a time in a cycle and are able to run up to eight cycles in an experiment. The information on cycle and capillary number is displayed in the Experiment tab.

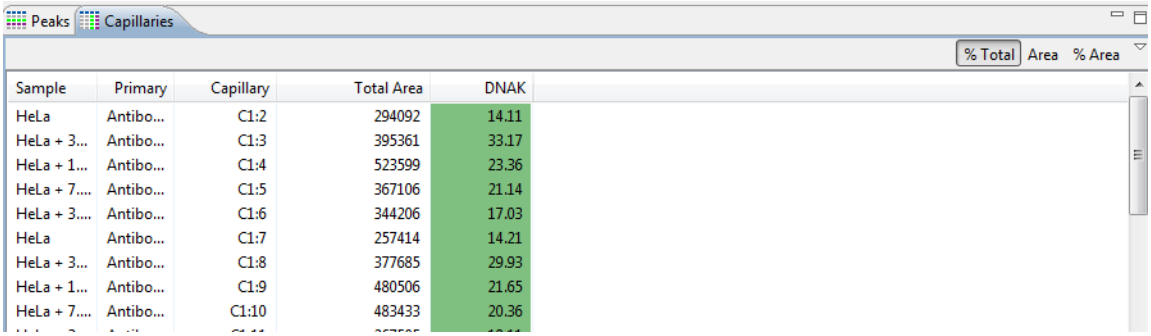
- **Peak Name Columns** - An individual column per peak name will display for every peak identified by name in the run data. Cells for capillaries in these columns will be blank if Compass for Simple Western did not find peaks automatically using the user-defined peak name analysis parameters (or none were entered).

- **To view % area in the peak name columns** - the Gaussian Fit area calculation setting must first be selected. To do this, select **Analysis** from the **Edit** menu, then select **Peak Fit** page. In the Peak Find box, select **Gaussian Fit** for the Area Calculation setting (default for Immunoassays). Next, select **Area %** in the upper right corner of the Capillaries pane. This displays the calculated percent area for the named peak compared to all named peaks. This value results from dividing the individual peak area by the sum of all named peak areas for the capillary and multiplying by 100.



Sample	Primary	Capillary	System Co...	ERK1
HeLa	ERK1+...	C1:2	44704	55899
HeLa	ERK1+...	C1:3	42886	63846
HeLa	ERK1+...	C1:4	50291	65303
HeLa	ERK1+...	C1:5	45954	57780
HeLa	ERK1+...	C1:6	45887	54196
HeLa	ERK1+...	C1:7	48500	68243
HeLa	ERK1+...	C1:8	47257	56120
HeLa	ERK1+...	C1:9	49193	59797
HeLa	ERK1+...	C1:10	47426	57923
HeLa	ERK1+...	C1:11	42898	45543
HeLa	ERK1+...	C1:12	43542	49701

- **To view peak area in the peak name columns (default)** - Select **Area** in the upper right corner of the pane. This displays calculated peak area for the individual peak only.
- **To view corrected peak area in the peak name columns** - Select Corr. Area in the upper right corner of the pane. This displays the corrected peak area for the named peak compared with the loading control peak or reference capillary when Protein Normalization is enabled in the Analysis settings.
- **To view concentration in the peak name columns** - Select Conc. in the upper right corner of the pane. This displays the calculated concentration for the named peak compared with the standard curve.
- **To view % total in the peak name columns** - The Dropped Lines area calculation setting must first be selected. To do this, select **Analysis** from the **Edit** menu, then select **Peak Fit** page. In the Peak Find box, select **Dropped Lines** for the Area Calculation setting (default for Total Protein Assays). Next, select **% Total** in the upper right corner of the Capillaries pane. This displays the calculated percent total for the named peak compared to the total area measured in the capillary. This value results from dividing the individual peak area by the entire area under the curve for the capillary and multiplying by 100.



Sample	Primary	Capillary	Total Area	DNAK
HeLa	Antibo...	C1:2	294092	14.11
HeLa + 3...	Antibo...	C1:3	395361	33.17
HeLa + 1...	Antibo...	C1:4	523599	23.36
HeLa + 7....	Antibo...	C1:5	367106	21.14
HeLa + 3....	Antibo...	C1:6	344206	17.03
HeLa	Antibo...	C1:7	257414	14.21
HeLa + 3...	Antibo...	C1:8	377685	29.93
HeLa + 1...	Antibo...	C1:9	480506	21.65
HeLa + 7....	Antibo...	C1:10	483433	20.36

Viewing Run Data

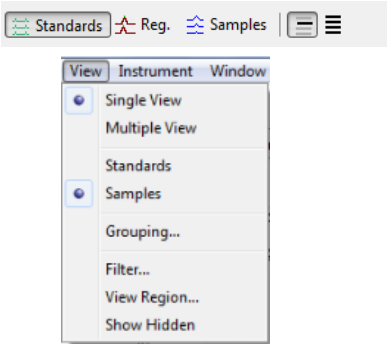
Each run file contains the following data for up to 96 capillaries:

- **Sample data** - For the proteins in the sample.
- **Standards data** - For the fluorescent standards run with each sample.
- **Registration data (Sally Sue and Peggy Sue only)** - For tracking capillaries as they are moved for various assay steps.

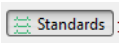
Data can be viewed for one, all, or individually selected capillaries.

Switching Between Sample, Standards and Registration Data Views

You can switch between viewing sample, standards and registration data in a run file using the View bar or View menu:

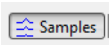


Data buttons in the View bar:



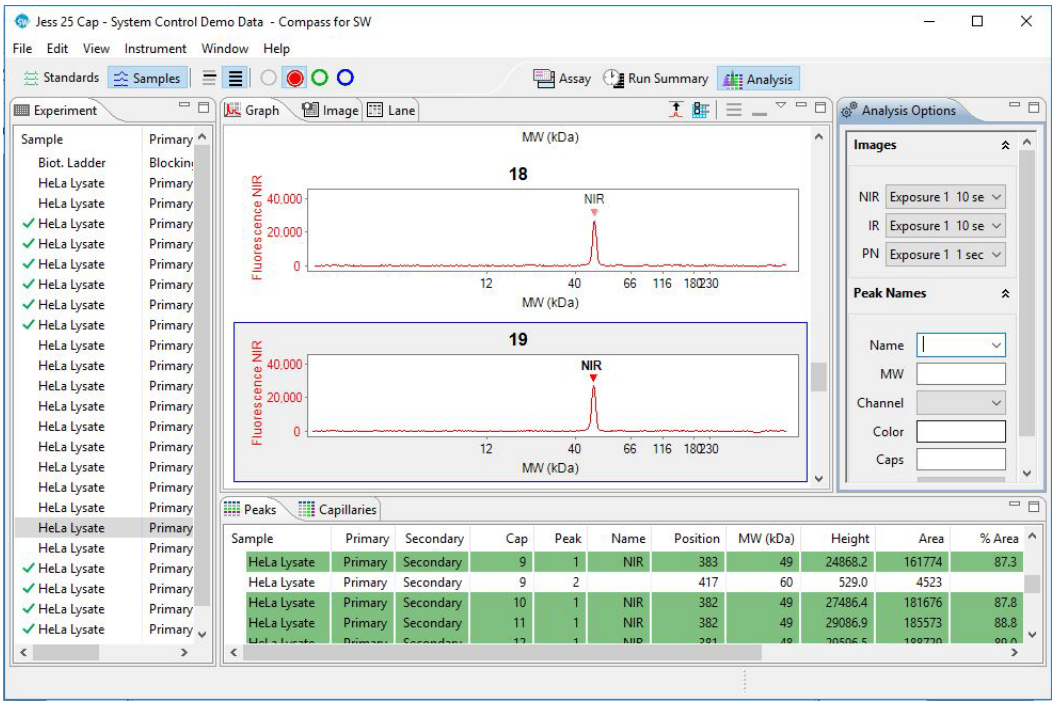
Show Standards

Show Registrations



Show Samples

- **To view sample data** - Click **Show Samples** in the View bar or select **View** in the main menu and click **Samples**:



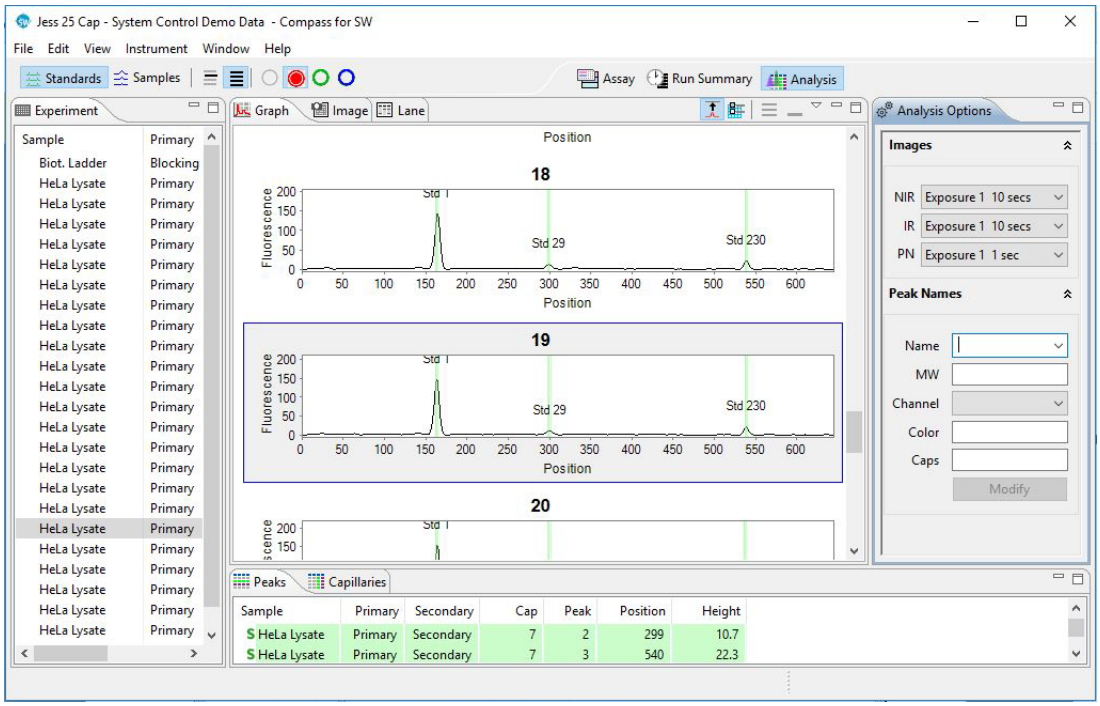
- Data in this view is for sample proteins only.
- Graph view data displays electropherograms in chemiluminescence, NIR or IR units (y-axis) and molecular weight in kDa (x-axis). If protein normalization was performed in the same immunoassay run, the overlay is also displayed in the Graph view.
- Lane view data displays sample proteins only.
- Image view data displays sample proteins only.
- Results for each protein are shown in the peaks and capillaries tables.

NOTE: The reported molecular weight for sample proteins detected by an Immunoassay or a Total Protein Assay in Compass for Simple Western may vary from apparent molecular weights using other techniques and based on sample and assay conditions.

For information on checking and identifying sample peaks, see "Step 4 – Checking the Ladder" on page 234 or "Step 5 – Checking Samples" on page 236.

- **To view standards data** - Click **Show Standards** in the View bar or select **View** in the main menu and click **Standards**:

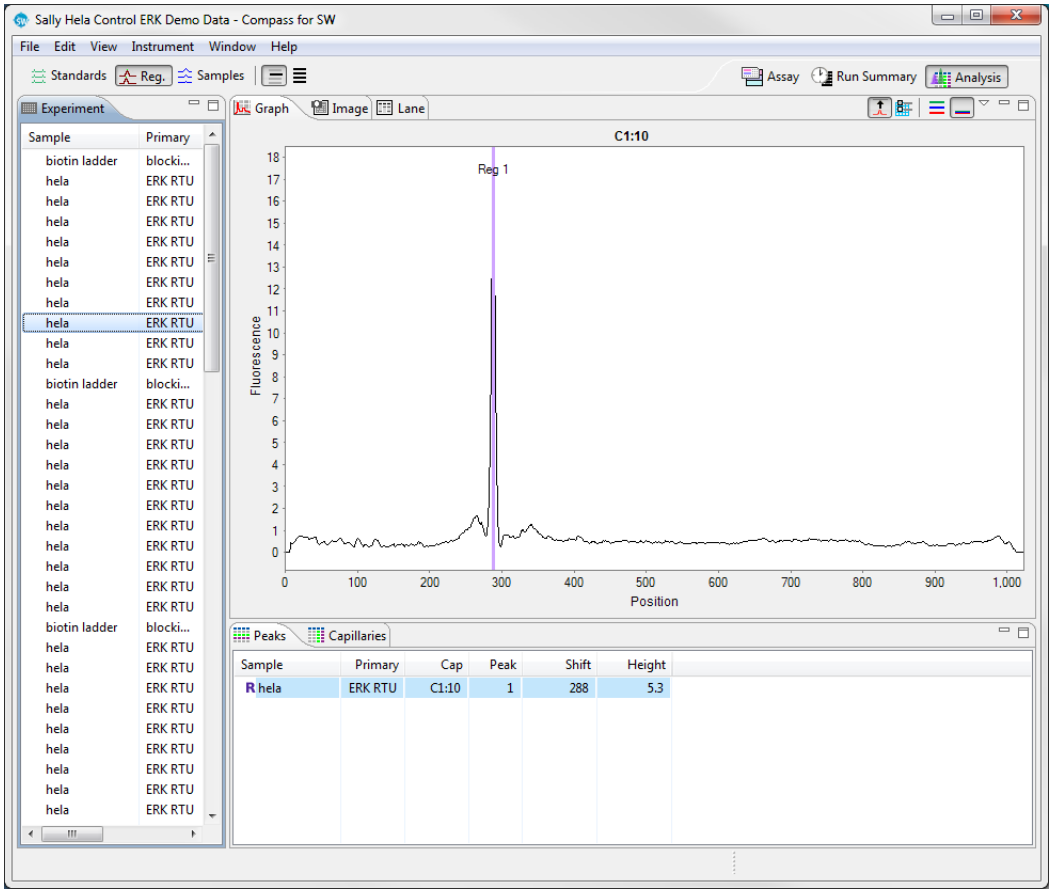
NOTE: For RePlex Assays (Jess only), standards data will be displayed regardless of what channel (Probe 1 or Probe 2) is selected.



- Data in this view is for the fluorescent standards only. These standards are premixed with each sample prior to analysis.
- Graph view data displays electropherograms in fluorescence (y-axis) and position (x-axis).
- Lane view data displays fluorescent standards only.
- Image view data displays fluorescent standards only.
- Standards are highlighted in both the peaks and capillaries tables and marked with an **S**.

For information on checking and identifying standards peaks, see "Step 2 – Checking Fluorescent Sizing Standards" on page 230.

- **To view registration data (Sally Sue and Peggy Sue only)** - Click **Show Registrations** in the View bar or select **View** in the main menu and click **Registration**:



- Data in this view shows capillary registration only.
- Graph view data displays electropherograms in fluorescence (y-axis) and position (x-axis).
- Lane view data displays capillary registration only.
- Image view data displays capillary registration only.
- Registrations are highlighted in both the peaks and capillaries tables and marked with an R.

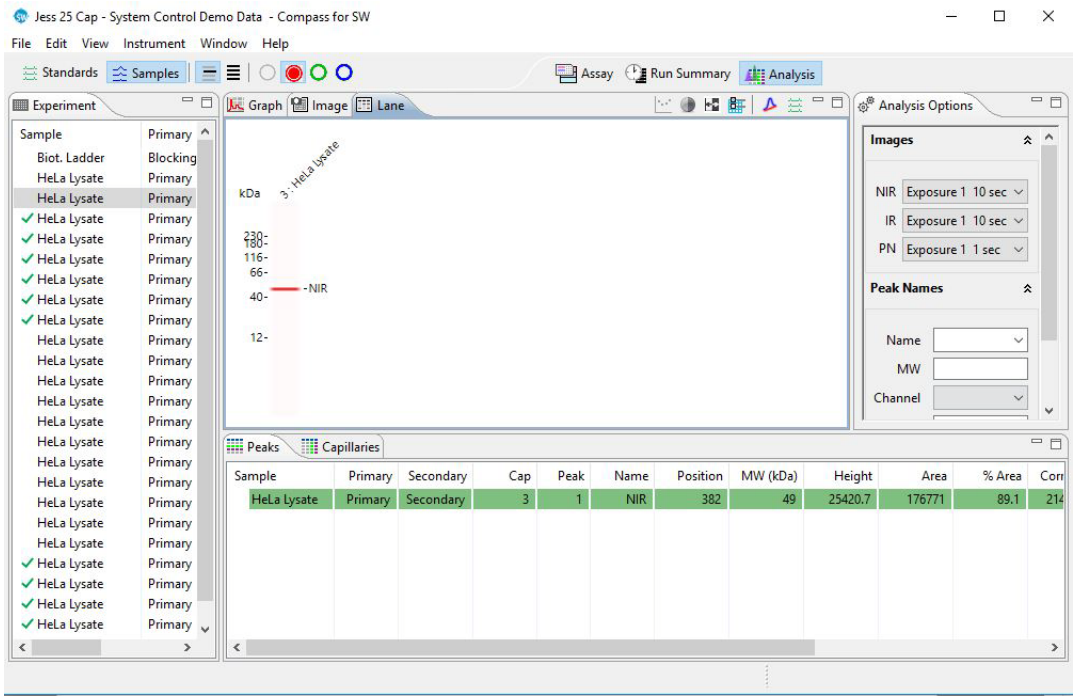
Because capillaries in Sally Sue and Peggy Sue must be moved multiple times during the run, their positions are tracked to account for potential changes in imaging position as they are moved to and from the separation block. To do this, one of the standards is used as a capillary registration. An image of the standard used for registration is taken at the end of the separation step prior to chemiluminescent imaging. The position of this peak in the registration image is then compared to the position of the same standard peak in the standards image. The shift value listed in the peaks table is the change in pixel position between the two images. Compass for Simple Western data is then aligned to account for any changes in capillary position.

For information on checking and identifying registration peaks, see "Step 3 – Checking Capillary Registrations (Sally Sue and Peggy Sue Only)" on page 233.

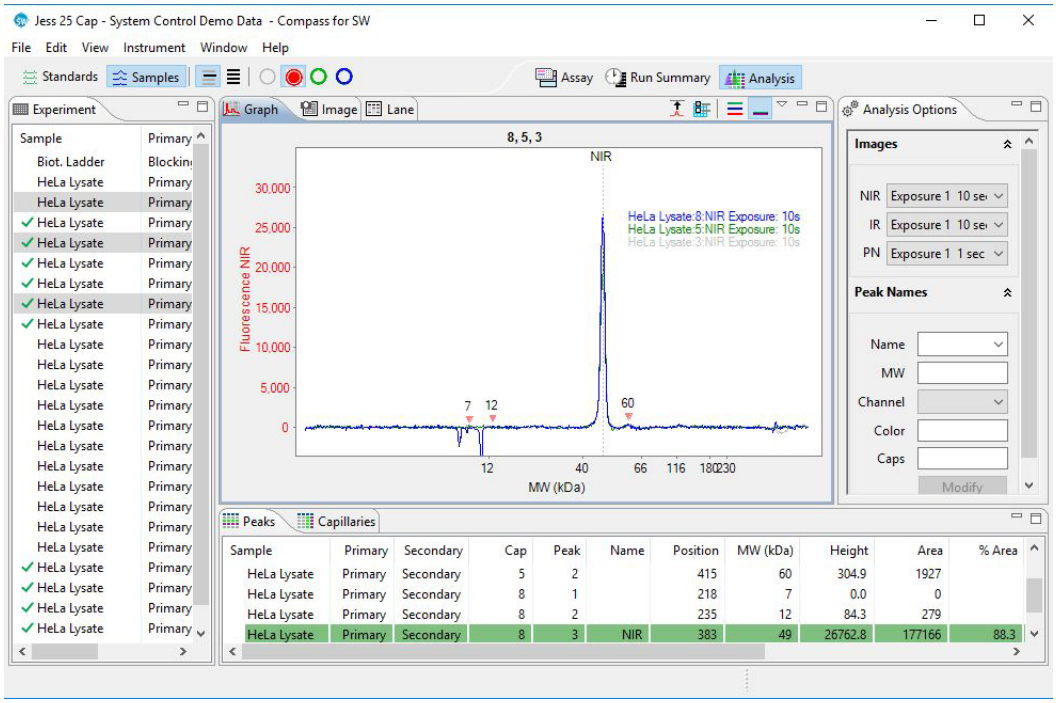
Selecting and Displaying Capillary Data

You can choose to view data from one, multiple, or all capillaries at once.

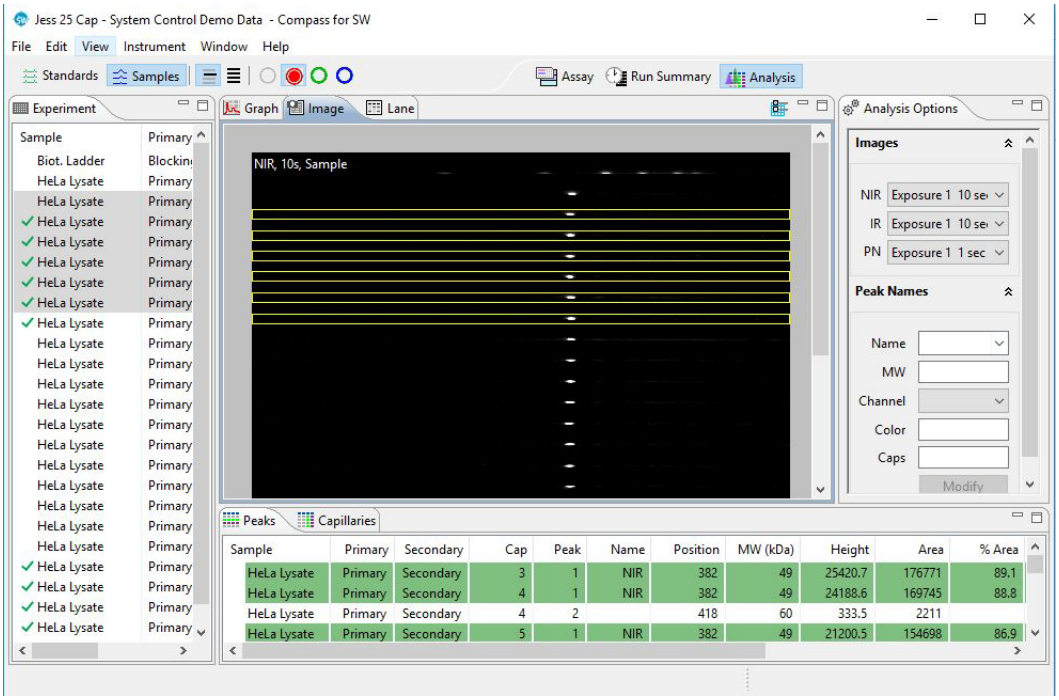
- **To look at data for one capillary** - Click a row in the experiment pane. Data for just the row selected will display in all data views and tables. The following example shows sample data displayed in the lane and peaks panes when one sample is selected:



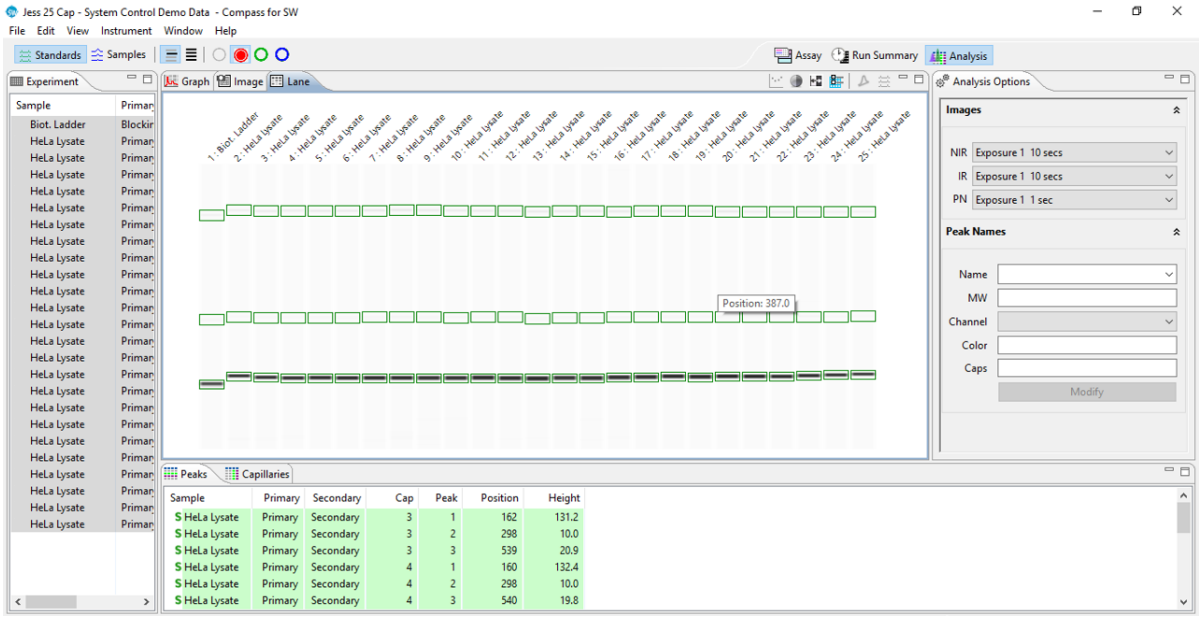
- **To look at data for multiple non-sequential capillaries** - Hold the **Ctrl** key and select the rows you want to view in the experiment pane. Data for only the rows selected will display in all data views and results tables. The following example shows sample data displayed in the graph (in multiple view) and capillaries panes when multiple rows are selected:



- **To look at data for multiple sequential capillaries** - Hold the **Shift** key and select the first and last rows you want to view in the experiment pane. Data for only the selected rows will display in all data views and results tables. The following example shows standards data displayed in the image and peaks panes when multiple sequential rows are selected:

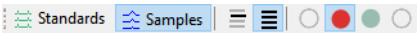


- **To look at data for all capillaries** - Hold the **Shift** key and select the first and last rows in the experiment pane. Data for all rows will display in all data views and results tables. The following example shows standards data displayed in the lane and peaks panes when all rows are selected. The pane scroll bars can be used to view all 8 cycles:



Switching Between Fluorescence Channel Views (Jess Only)

You can switch between displaying run data in the graph, image and lane panes for samples run using HRP-tagged or fluorophore dye-containing secondaries in the View bar. Protein normalization overlay data can also be selected here. This view is selected in the View bar:



Detection channels in the View bar:

Overlay chemiluminescent channel

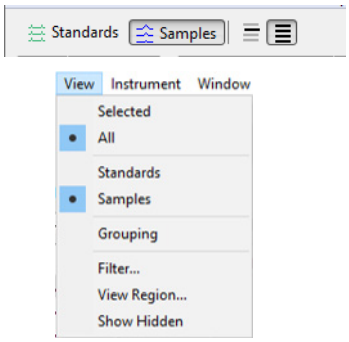
Overlay near infrared channel

Overlay infrared channel

Overlay protein normalization channel

Switching Between Single and Multiple Views of the Capillaries

You can switch between displaying run data in the graph, image and lane panes in a per capillary (single) format or up to 96 capillary format. This view is selected in the View bar or the View menu.

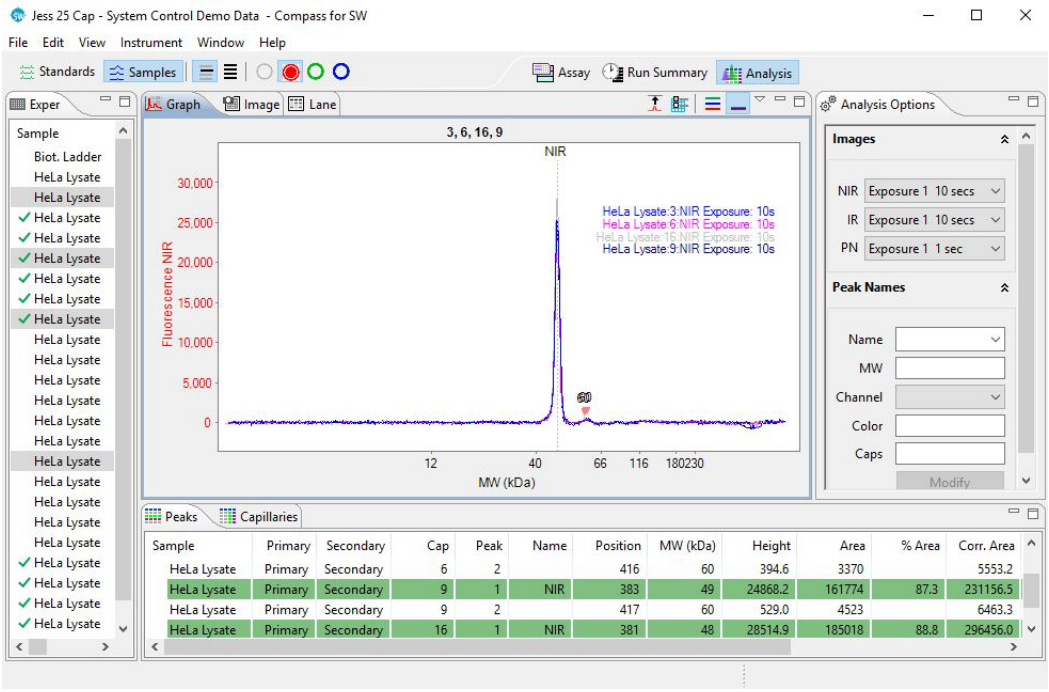


Capillary view buttons in the View bar:

Single View

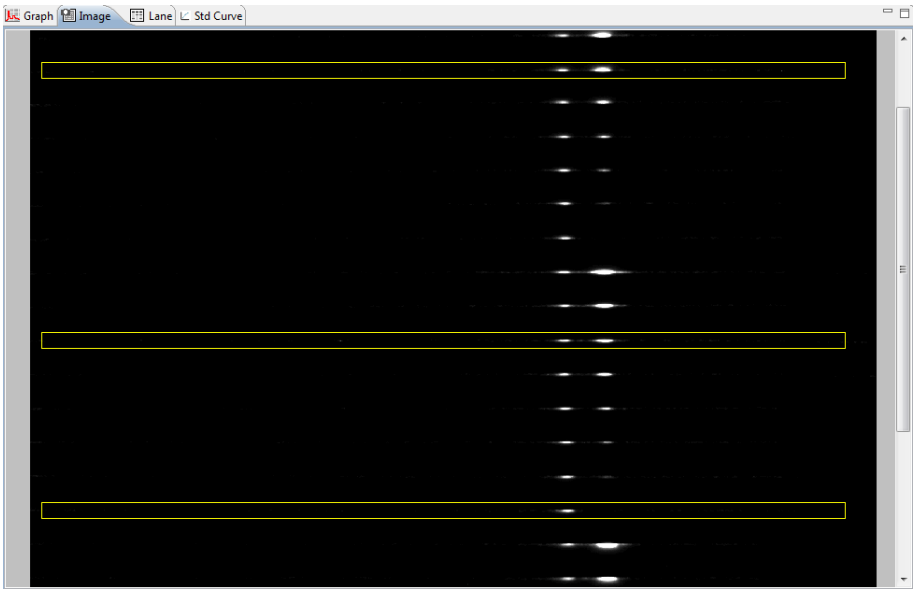
Multiple View

- **To view data in a per capillary format** - Click **Single View** in the View bar or select **View** in the main menu and click **Single View**:



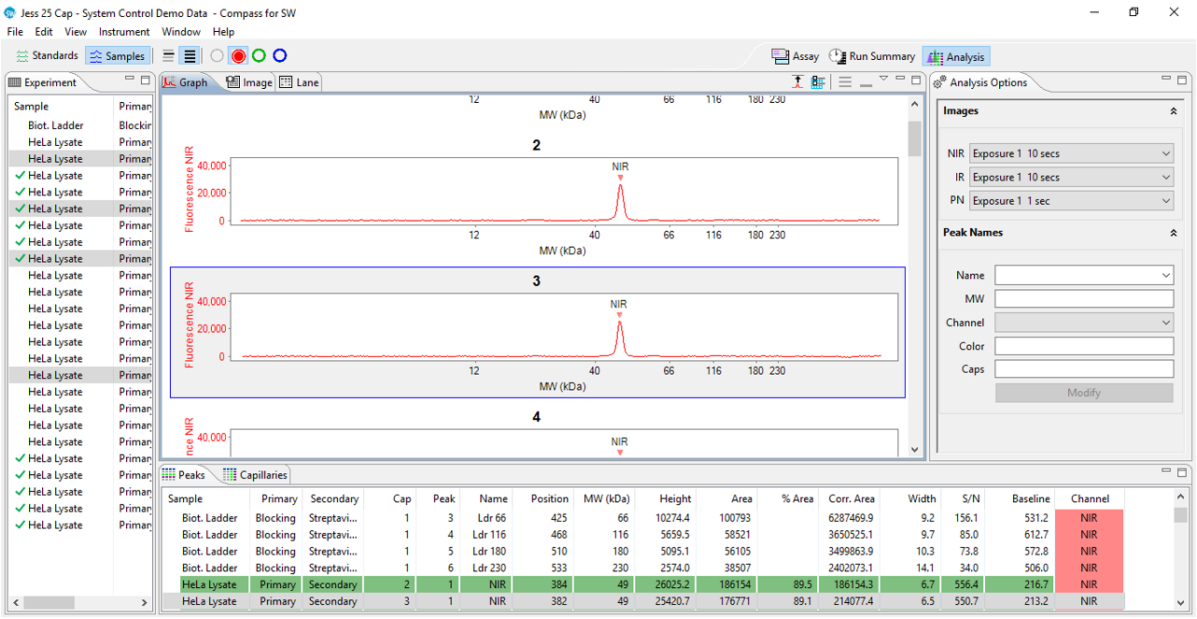
Data for the row(s) selected in the experiment pane will display as follows:

- Electropherograms in the graph pane display overlaid or stacked (depending on the graph option chosen).
- Results for only the selected row(s) will display in the peaks and capillaries tables.
- The selected row(s) are highlighted in the image pane:



- Lanes for only the selected row(s) are displayed in the lane pane:

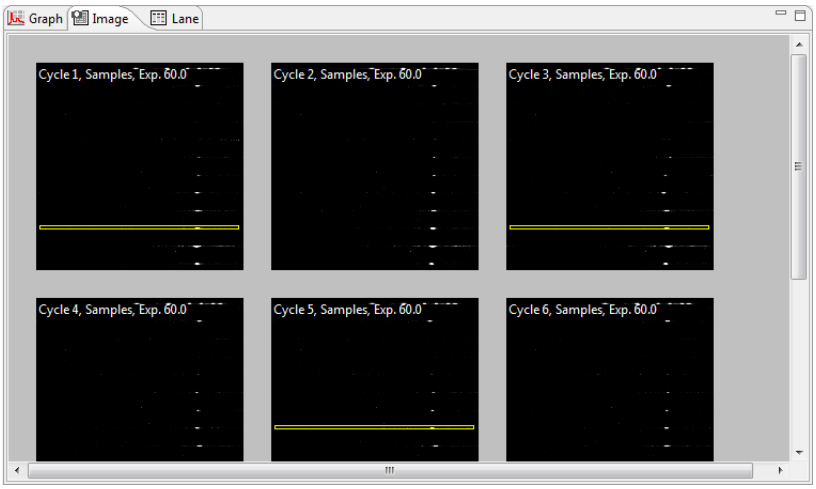
- **To view data in a multiple capillary format** - Click **Multiple View** in the View bar or select **View** in the main menu and click **Multiple View**:



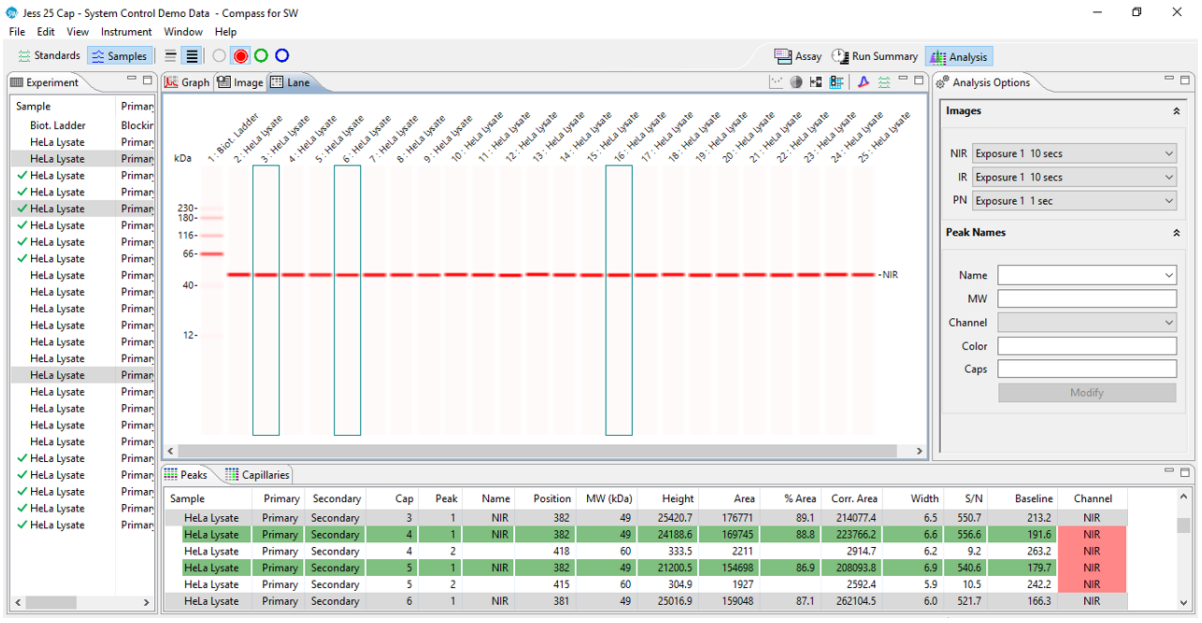
Sample	Primary	Secondary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Corr. Area	Width	S/N	Baseline	Channel
Biot. Ladder	Blocking	Streptavi...	1	3	Ldr 66	425	66	10274.4	100793		6287469.9	9.2	156.1	531.2	NIR
Biot. Ladder	Blocking	Streptavi...	1	4	Ldr 116	468	116	5659.5	58521		3650525.1	9.7	85.0	612.7	NIR
Biot. Ladder	Blocking	Streptavi...	1	5	Ldr 180	510	180	5095.1	56105		3499863.9	10.3	73.8	572.8	NIR
Biot. Ladder	Blocking	Streptavi...	1	6	Ldr 230	533	230	2574.0	38507		2402073.1	14.1	34.0	506.0	NIR
HeLa Lysate	Primary	Secondary	2	1	NIR	384	49	26025.2	186154	89.5	186154.3	6.7	556.4	216.7	NIR
HeLa Lysate	Primary	Secondary	3	1	NIR	382	49	25420.7	176771	89.1	214077.4	6.5	550.7	213.2	NIR

Data for the row(s) selected in the experiment pane will display as follows:

- Electropherograms corresponding to the selected row(s) are highlighted and displayed in the graph pane.
- Results for selected capillaries display in the peaks and capillaries tables and are highlighted.
- The selected row(s) are highlighted in the image pane:

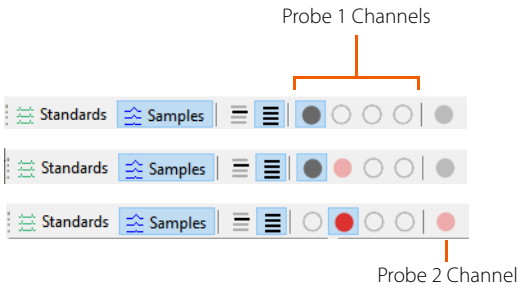


- All selected lanes display in the lane pane, and lanes corresponding to the selected row(s) are highlighted.



Viewing RePlex Channel Views (Jess Only)

Probe 1 data displays by default when you open a RePlex run file. To display a second Probe 1 or Probe 2 channel data, click the channel icon in the View bar. Channel designations are shown below:



Detection channels in the View bar:


Chemiluminescent channel (grey)

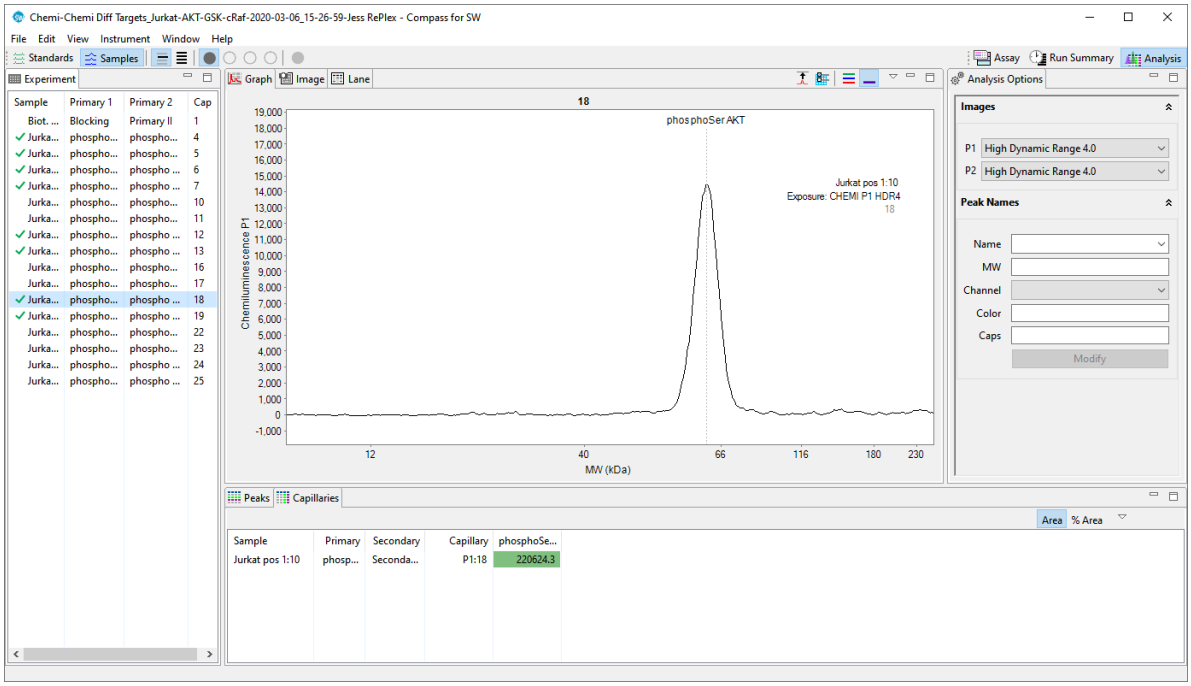

Near infrared channel (red)


Unused channel (no color)


Channel data is displayed

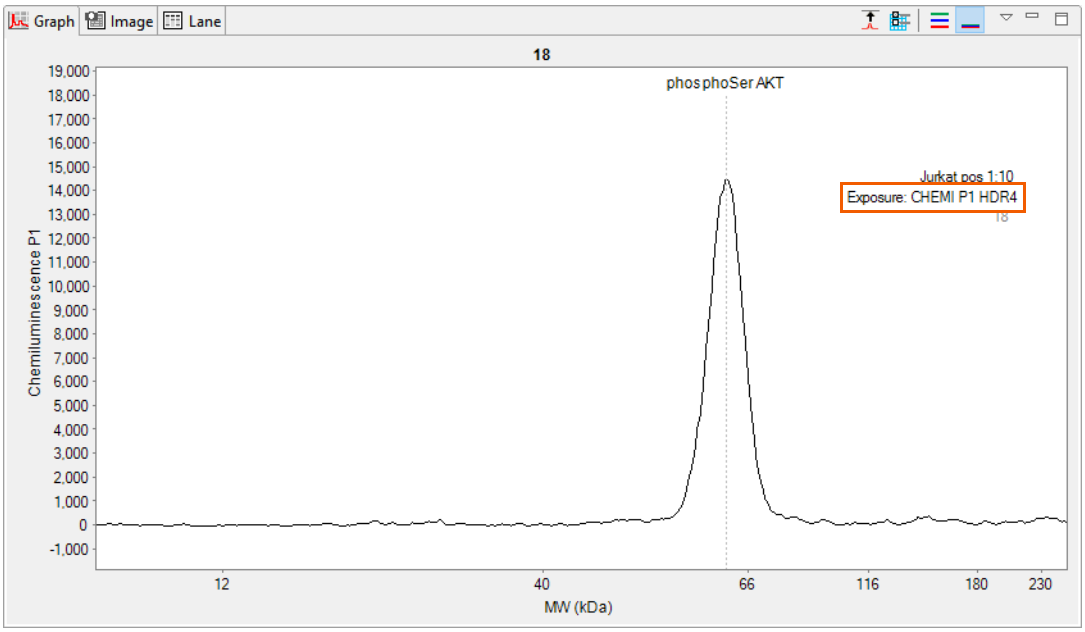

Channel data is available but not displayed

The initial view displays Probe 1 data only. In this example, the Probe1 channel is chemiluminescence.

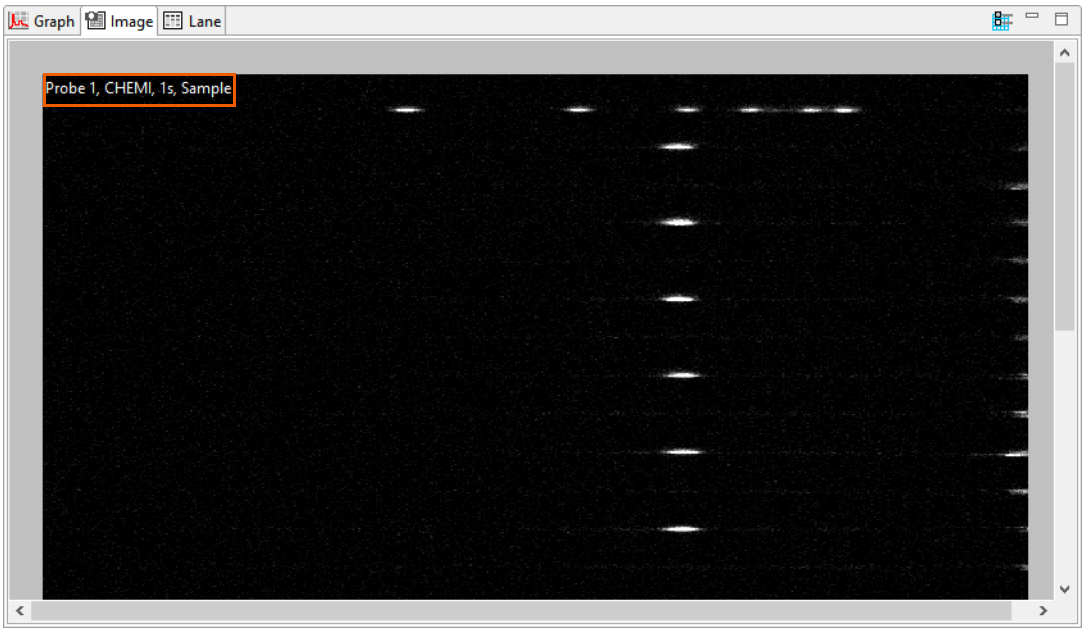


Probe 1 data is labeled in all data views as P1 or Probe 1:

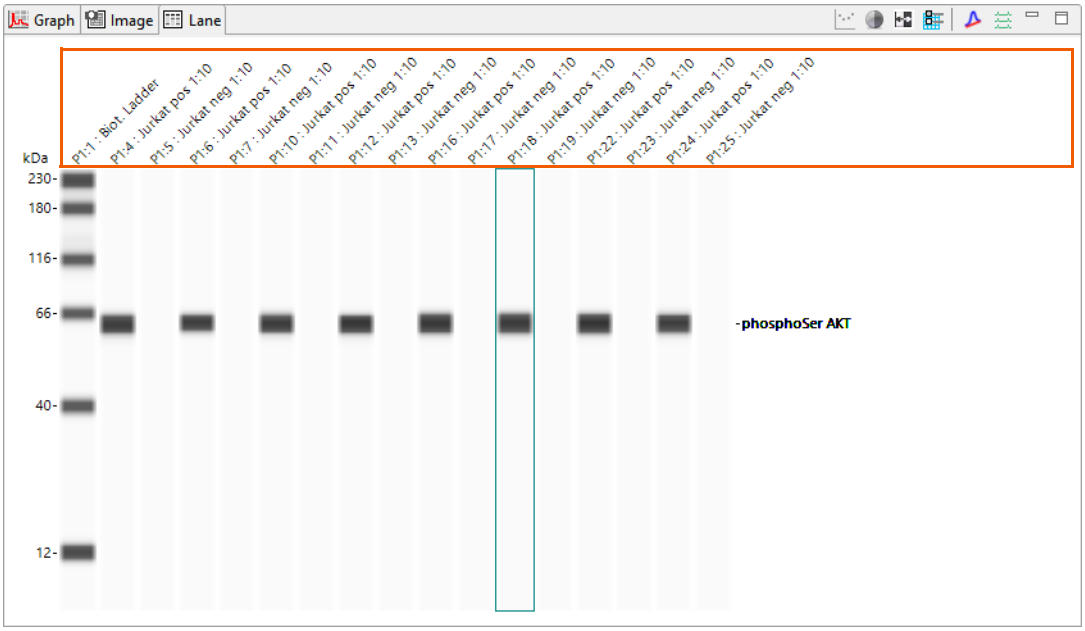
- Graph view:



- Image view:



- Lane view - sample lanes are labeled probe number: capillary number



- Peaks and Capillaries table: rows are labeled probe number: capillary number

Peaks | Capillaries

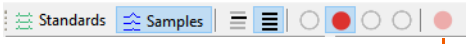
Sample	Primary	Secondary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width	S/N	Baseline	Channel
Jurkat pos 1:...	phosp...	Seconda...	P1:4	1	phosp...	417	63	14026.4	212153.9	100.0	14.2	551.7	185.6	CHEMI
Jurkat neg 1...	phosp...	Seconda...	P1:5	1	phosp...	422	64	130.4	2767.2	100.0	19.9	4.2	249.3	CHEMI
Jurkat pos 1:...	phosp...	Seconda...	P1:6	1	phosp...	417	63	13738.5	185454.1	100.0	12.7	502.4	321.4	CHEMI
Jurkat neg 1...	phosp...	Seconda...	P1:7	1	phosp...	413	62	100.8	2060.6	100.0	19.2	9.4	258.5	CHEMI
Jurkat neg 1...	phosp...	Seconda...	P1:7	2		534	232	347.5	3416.3		9.2	25.5	194.7	CHEMI
Jurkat pos 1:...	phosp...	Seconda...	P1:10	1	phosp...	419	63	14133.1	208185.9	100.0	13.8	441.9	293.2	CHEMI
Jurkat neg 1...	phosp...	Seconda...	P1:11	1	phosp...	416	62	289.2	9157.2	100.0	29.7	11.8	232.6	CHEMI
Jurkat pos 1:...	phosp...	Seconda...	P1:12	1	phosp...	419	63	15392.0	212526.5	100.0	13.0	594.2	307.3	CHEMI

Peaks | Capillaries

Sample	Primary	Secondary	Capillary	phosphoSe...
Jurkat pos 1:10	phosp...	Seconda...	P1:4	212153.9
Jurkat neg 1:10	phosp...	Seconda...	P1:5	2767.2
Jurkat pos 1:10	phosp...	Seconda...	P1:6	185454.1
Jurkat neg 1:10	phosp...	Seconda...	P1:7	2060.6
Jurkat pos 1:10	phosp...	Seconda...	P1:10	208185.9
Jurkat neg 1:10	phosp...	Seconda...	P1:11	9157.2
Jurkat pos 1:10	phosp...	Seconda...	P1:12	212526.5

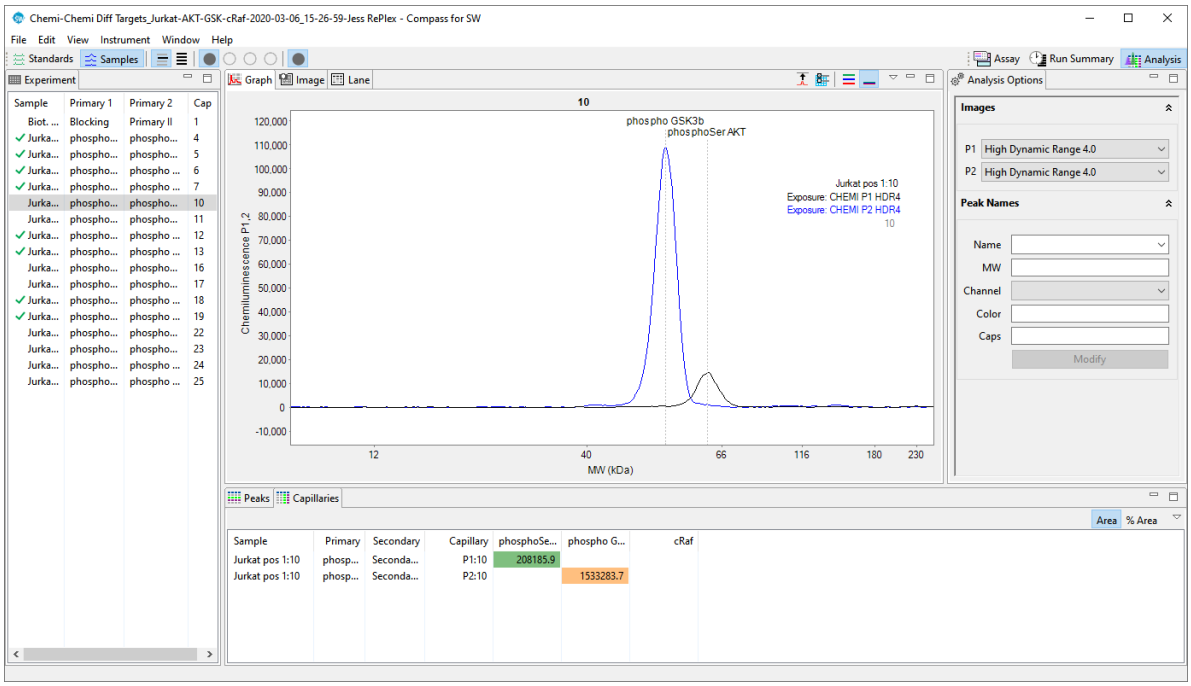
Viewing Data for Probe 2

To view chemiluminescent or NIR data for Probe 2, select it's channel in the view bar:

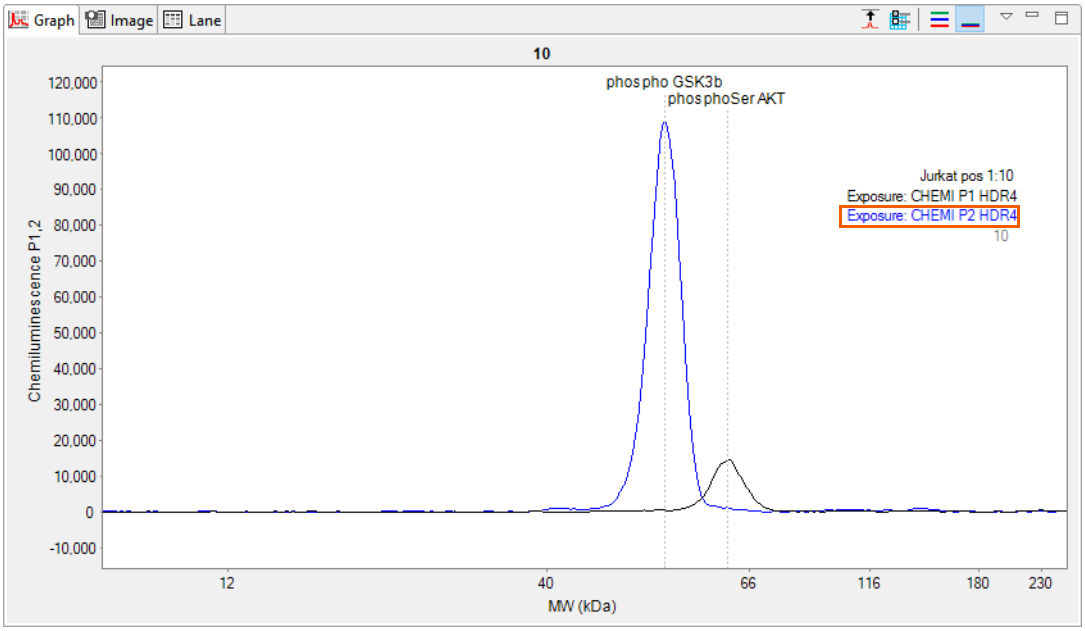


Probe 2 Channel

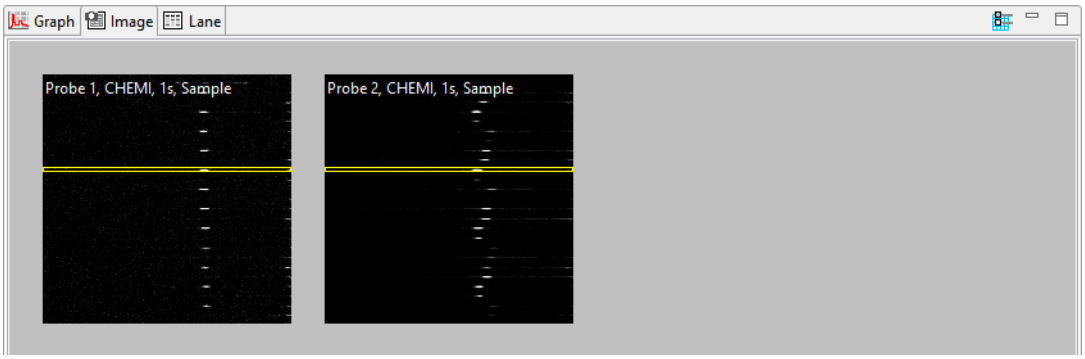
Probe 2 data is labeled P2 or Probe 2:



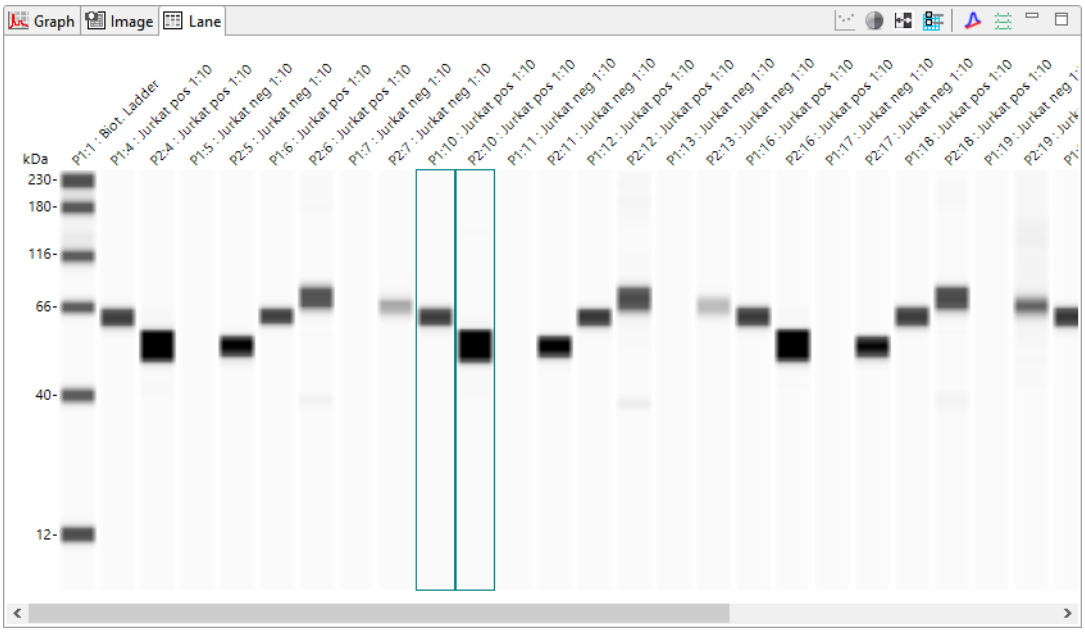
- Graph view will display data stacked or overlaid depending on the option chosen:



- Image view will display separate images for each probe:



- Lane view will display P1 and P2 lanes side by side:



  - Peaks and Capillaries tables will display all row data for P1 first, then P2:

Peaks | Capillaries

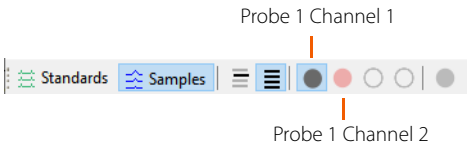
Sample	Primary	Secondary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width	S/N	Baseline	Channel
Jurkat pos 1:...	phosp...	Seconda...	P1:10	1	phosp...	419	63	14133.1	208185.9	100.0	13.8	441.9	293.2	CHEMI
Jurkat pos 1:...	phosp...	Seconda...	P2:10	1	phosp...	397	55	108450.8	1533283.7	100.0	13.3	1539.5	923.6	CHEMI

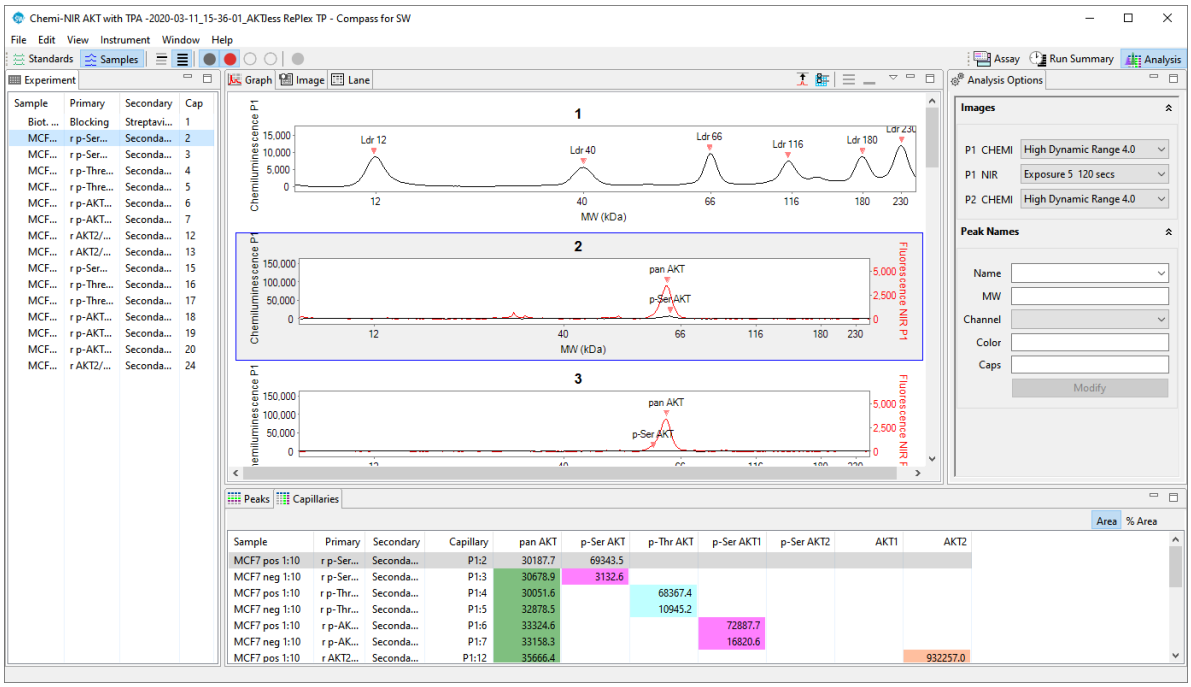
Peaks | Capillaries

Sample	Primary	Secondary	Capillary	phosphoSe...	phospho G...	cRaf
Jurkat pos 1:10	phosp...	Seconda...	P1:10	208185.9		
Jurkat pos 1:10	phosp...	Seconda...	P2:10		1533283.7	

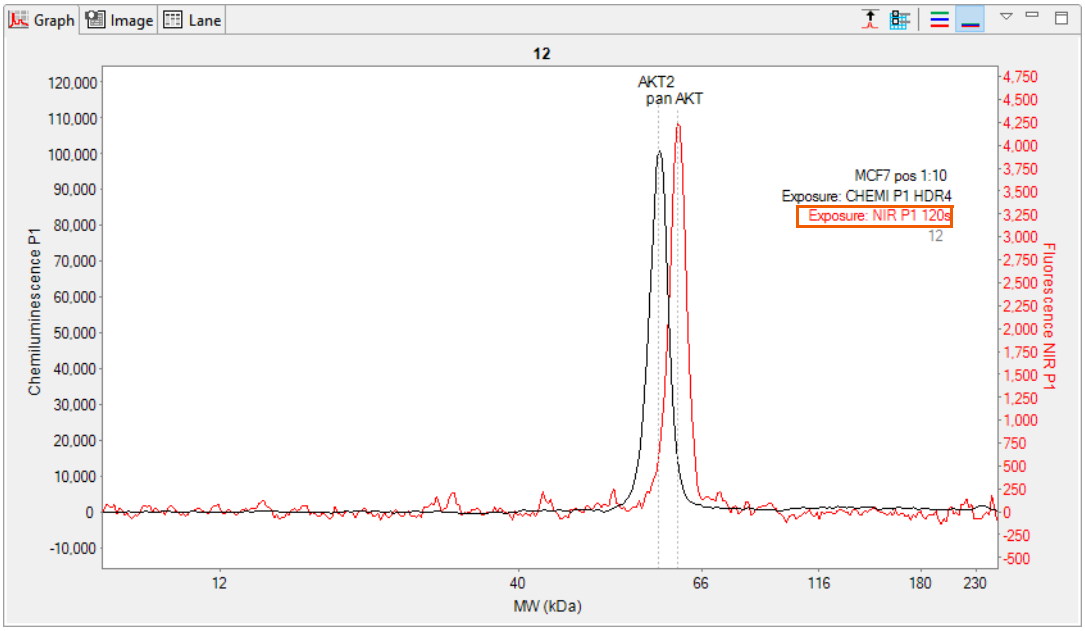
Viewing Data for a Second Channel in Probe 1

To view the second channel data for Probe 1, for example in a RePlex Chemiluminescence/NIR fluorescence + Total Protein assay, select it's channel in the view bar:

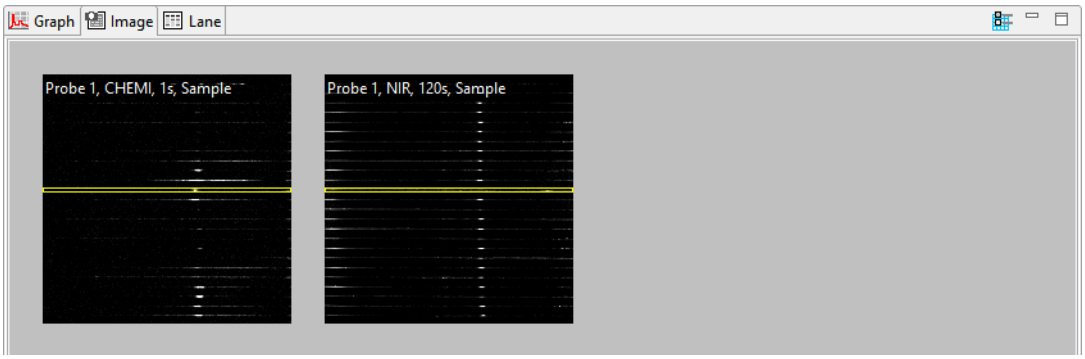




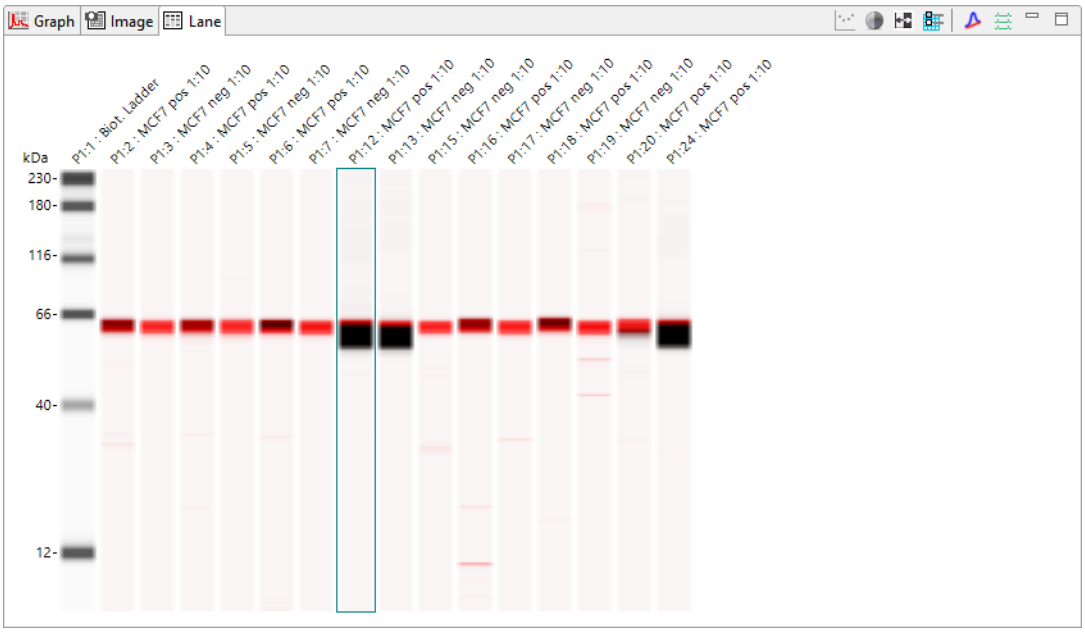
- Graph view will display data stacked or overlaid channel data depending on the option chosen:



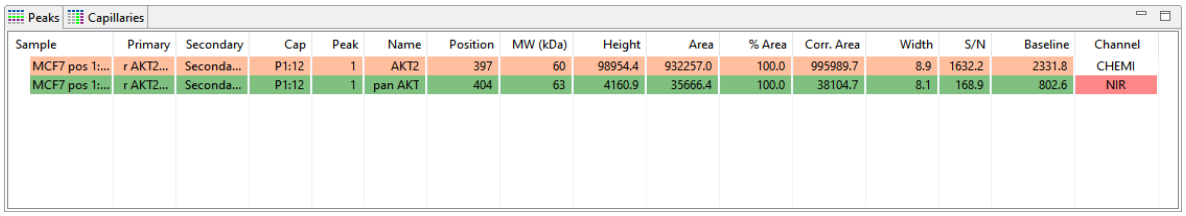
- Image view will display individual images for each channel:



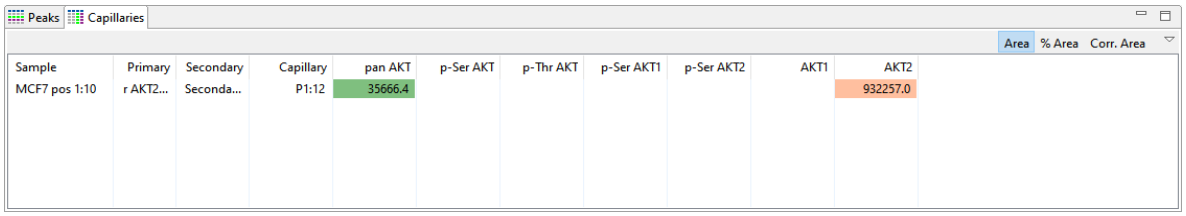
- Lane view will display lanes for each channel overlaid:



  - Peaks and Capillaries tables will list data for both channels:



Sample	Primary	Secondary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Corr. Area	Width	S/N	Baseline	Channel
MCF7 pos 1:...	r AKT2...	Seconda...	P1:12	1	AKT2	397	60	98954.4	932257.0	100.0	995989.7	8.9	1632.2	2331.8	CHEMI
MCF7 pos 1:...	r AKT2...	Seconda...	P1:12	1	pan AKT	404	63	4160.9	35666.4	100.0	38104.7	8.1	168.9	802.6	NIR



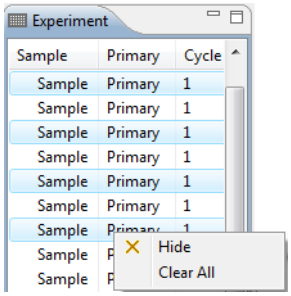
Sample	Primary	Secondary	Capillary	pan AKT	p-Ser AKT	p-Thr AKT	p-Ser AKT1	p-Ser AKT2	AKT1	AKT2
MCF7 pos 1:10	r AKT2...	Seconda...	P1:12	35666.4						932257.0

Hiding Capillary Data

Capillary data can be hidden from view if needed. To do this:

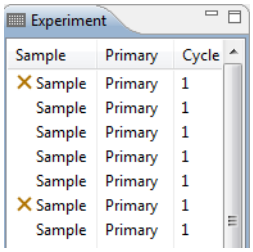
1. Click the **Experiment** tab.

2. Select the rows you want to hide, then right click on one of the selected rows and click **Hide**:



Data for selected rows will be hidden in all data views and results tables (except for the image pane).

- **To view hidden rows** - Select **View** in the main menu and click **Show Hidden**. Hidden rows will become visible again in all panes, and hidden rows will be marked with an X in the experiment pane:



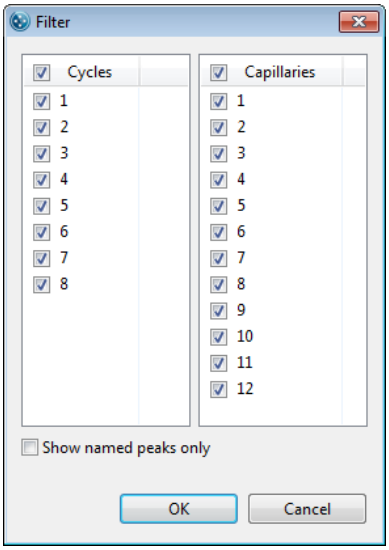
- **To unhide rows** - Select the hidden row(s). Right click on one of the selected rows and click **Unhide.**

Setting Run Data Display Filters

The filter lets you auto-hide specific capillaries in the run file data, or only show data for peaks that Compass for Simple Western identified automatically using the user-defined peak name analysis parameters.

NOTE: When more than one run file is open, filter settings will be applied to all files.

- **To filter data to show specific capillaries only** - Select **View** in the main menu and click **Filter**. Uncheck the boxes for the capillaries you do not want shown, then click **OK**.



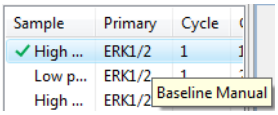
Only data for the checked capillaries will display in data views and results tables, unchecked capillaries will be hidden.

- **To filter data to show named peaks only** - Select **View** in the main menu and click **Filter**. Select **Show named peaks only** then click **OK**. Only data for peaks that Compass for Simple Western identified automatically using the user-defined peak name analysis parameters will display in data views and results tables, all other peak data will be hidden.

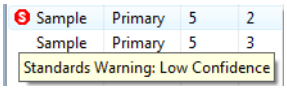
Run Data Notifications and Warnings

If Compass for Simple Western detects a potential data issue, a notification or warning will display next to the row in the experiment pane. A list of warnings, notifications and corrective actions are as follows:

- **Manual correction of sample data notification** - Indicates the user modified sample data manually, such as adding or removing a sample peak. Rolling the mouse over the icon displays the type of data modification made.

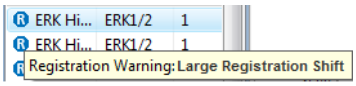


- **Standards warning -** Indicates that one of the standards may not be identified properly. This can be resolved by manually identifying the standards. Please refer to "Step 2 – Checking Fluorescent Sizing Standards" on page 230 for details. Rolling the mouse over the icon displays warning details.



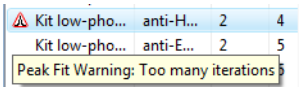
- **Manual correction of standards data notification** - Indicates the user modified the standards data manually. Rolling the mouse over the icon displays the type of data modification made.

- **Registrations warning -** Indicates that a capillary registration was not identified properly. This can be resolved by manually identifying the registration peak. Please refer to "Step 3 – Checking Capillary Registrations (Sally Sue and Peggy Sue Only)" on page 233 for details. Rolling the mouse over the icon displays warning details.



- **Manual correction of registrations notification -** Indicates the user modified the capillary registration data manually. Rolling the mouse over the icon displays the type of data modification made.

- **Peak fit warning -** Indicates that a peak cannot be fit properly. This is typically caused either by a very small peak in the electropherogram or a peak that is very close to the end of the molecular weight range. This can be resolved by removing the peak. Please refer to "Step 4 – Checking the Ladder" on page 234 or "Step 5 – Checking Samples" on page 236 for details. Rolling the mouse over the icon displays warning details.



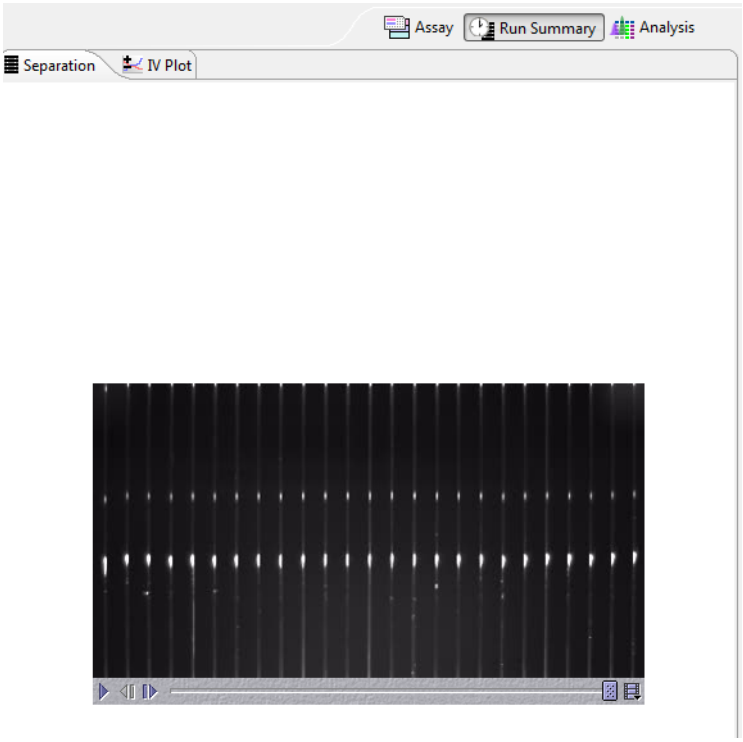
Checking Your Results

Compass for Simple Western detects proteins, fluorescent standards and capillary registrations and reports results automatically with no user-interaction. However, we recommend you review the data as outlined in this section as good general practice to ensure accurate results. If a data warning is presented in the experiment pane, the following steps will also help you identify and correct any issues.

Step 1 – Review the Fluorescent Sizing Standards Movie

All capillaries should have three fluorescent sizing standards. To verify the standards separated in the capillary correctly:

1. When the run has completed, click the **Run Summary** screen tab.
2. Click the **Separation** tab and play the movie (this will be the fluorescent standards separation).



3. For each capillary, verify that the standards visibly separated. Each fluorescent point in the capillary represents one of the three sizing standards.

Step 2 – Checking Fluorescent Sizing Standards

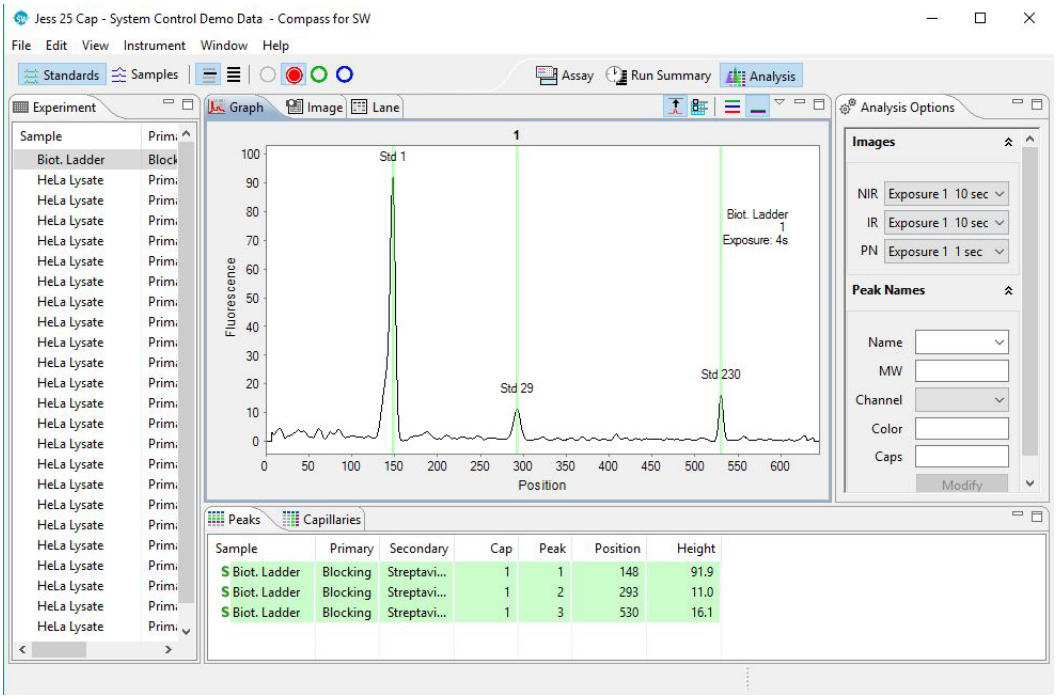
To verify the standards are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Standards** in the View bar. Verification that the standards have been correctly identified can be done in either the graph or lane panes.

Graph Pane:

a. Click **Single View** in the View bar.

b. Click on the first row in the experiment pane, then click the **Graph** tab. Check that the electropherogram has three standard peaks labeled Std 1, Std 29 and Std 230 (for 12-230 kDa size assays), Std 57 and Std 280 (for 66-440 kDa size assays) or Std 1 and Std 29 (for 2-40 kDa size assay). They will also be identified with a green **S** in the peaks table.



If standards are not identified correctly, they can be manually corrected as follows:

- **If an incorrect peak is identified as a standard** - Right click the peak in the electropherogram or peaks table and select **Not a Standard**. Compass for Simple Western should correctly reassign the remaining peaks as standards, and update the peaks table.
- **To set an unidentified peak as a standard** - Right click the peak in the electropherogram or peaks table and select **Force Standard**. Compass for Simple Western will assign the peak as a standard, and correctly reassign the remaining standard peaks. A lock icon indicating the standard was set manually will display next to the peak in the peaks table.

---

*NOTE: To remove standards peak assignments that were made manually, right click on the peak in the electropherogram or peaks table and click **Clear**.*

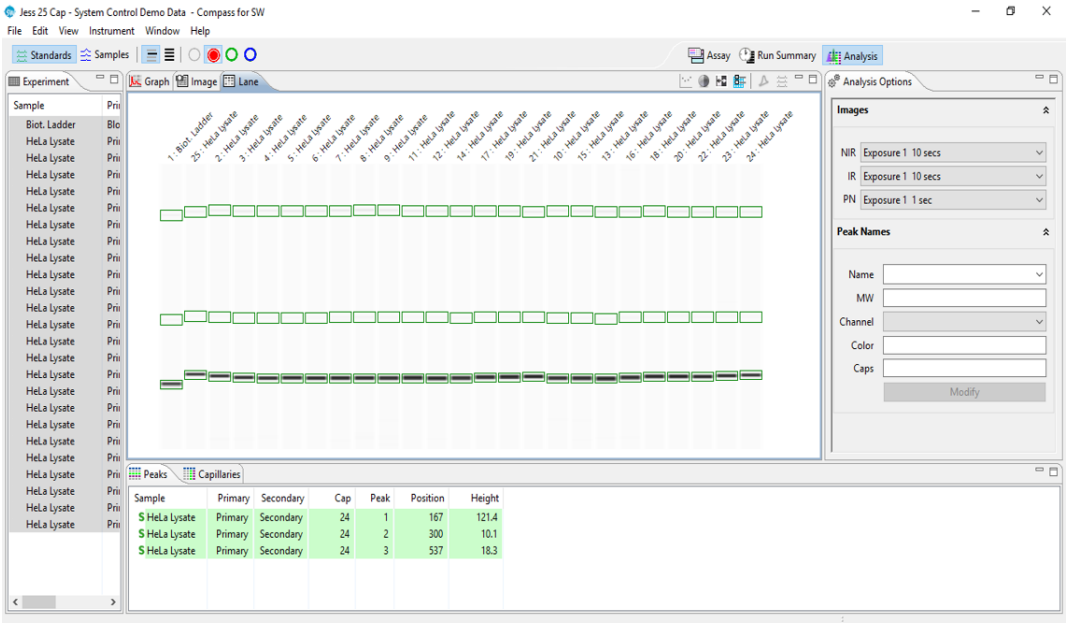
---

c. Repeat the previous steps for the remaining rows in the experiment pane to make sure all standards are identified correctly.

Lane Pane:

a. Click **Multiple View** in the View bar.

b. Click on the first row in the experiment pane, then click the **Lane** tab. Standards will be bands and identified with a green outline. Check that all standard bands are labeled: Std 1, Std 12 and Std 230 (for 12-230 kDa size assays) or Std 57 and Std 280 (for 66-440 kDa size assays). They will also be identified with a green **S** in the peaks table. To view band labels, roll the mouse over the individual bands.



If standards are not identified correctly, they can be manually corrected as follows:

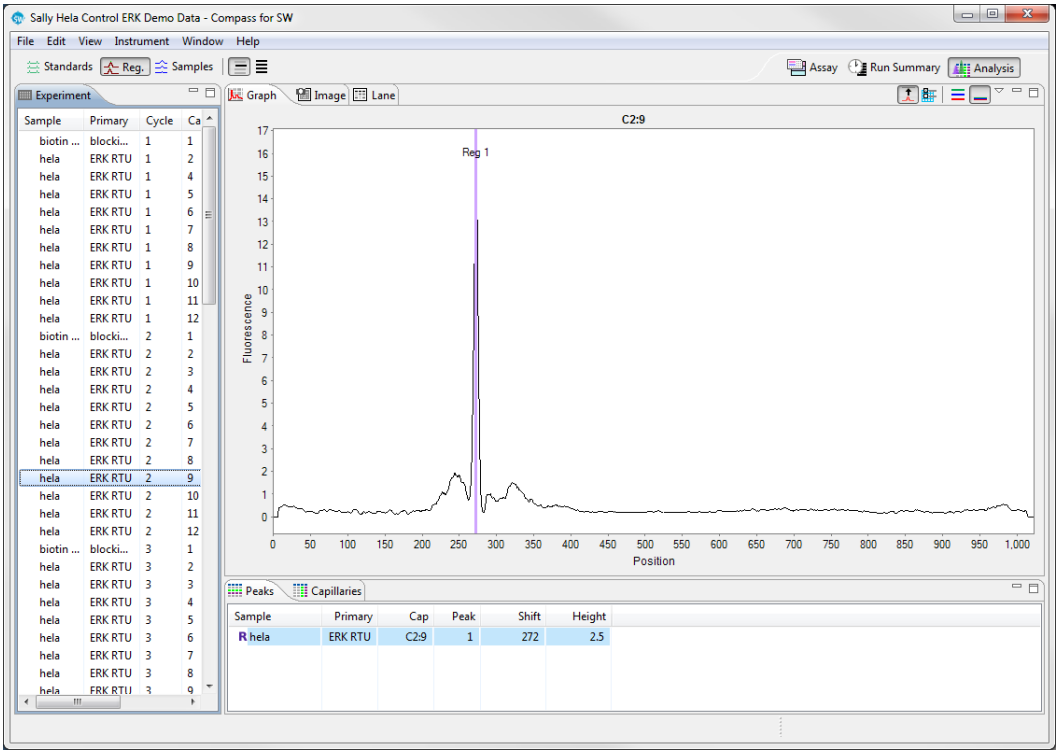
- **If an incorrect band is identified as a standard** - Right click the band in the lane or peaks table and select **Not a Standard**. Compass for Simple Western should correctly reassign the remaining bands as standards.
- **To set an unidentified band as a standard** - Right click the band in the lane or peaks table and select **Force Standard**. Compass for Simple Western will assign the band as a standard, and correctly reassign the remaining standard bands.

c. Repeat the previous steps for the remaining rows in the experiment pane to make sure all standards are identified correctly.

Step 3 – Checking Capillary Registrations (Sally Sue and Peggy Sue Only)

All capillaries should have a single capillary registration peak. To verify the registrations are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Registrations** and **Single View** in the View bar.
3. Click the **Graph** tab.
4. Click on the first row in the experiment pane. The registration peak will be the first and largest peak in the electropherogram. Check that the registration peak is identified and labeled Reg 1 in the electropherogram. It will also be identified with a purple **R** in the peaks table.



5. If the registration peak is not identified correctly, right click the correct registration peak in the electropherogram or peaks table and select **Registration Force**. Compass for Simple Western will assign the new peak as the capillary registration. A lock icon indicating the registration was set manually will display next to the peak in the peaks table.

*NOTE: To remove registration peak assignments that were made manually, right click on the peak in the electropherogram or peaks table and click **Registration Clear**.*

6. Repeat the previous steps for the remaining rows in the experiment pane to make sure all registrations are identified correctly.

Step 4 – Checking the Ladder

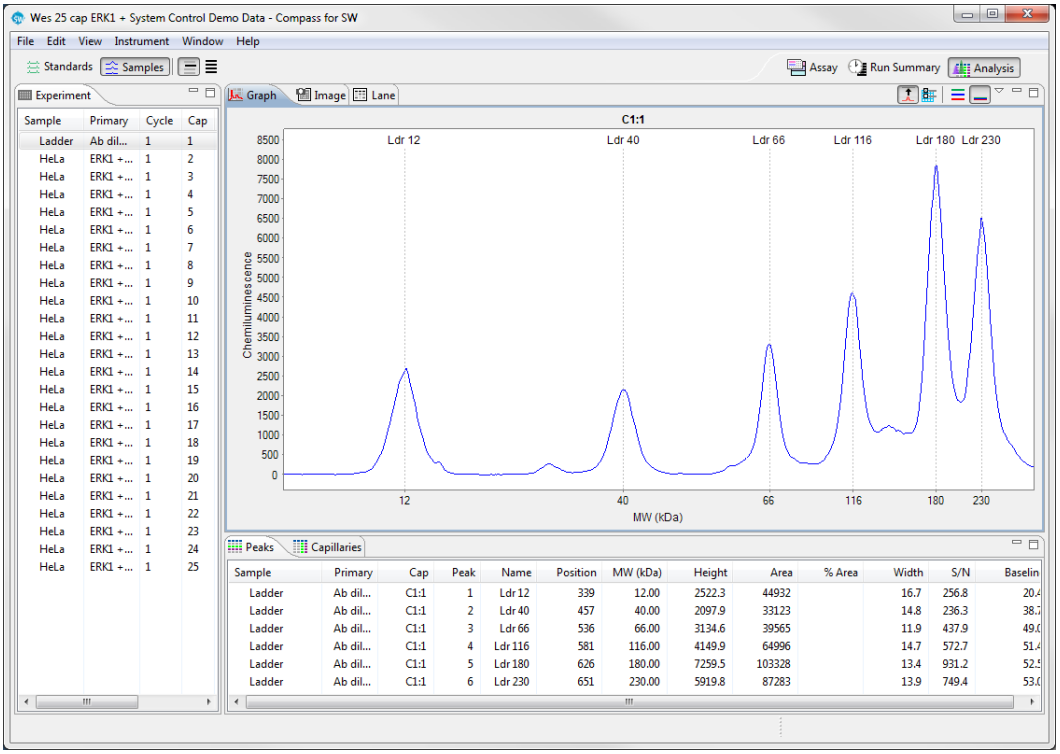
Depending on the size assay you are running, the biotinylated ladder should have the following sizing standards: 12, 40, 66, 116, 180 and 230 kDa (for 12-230 kDa size assays), or 66, 116, 200, 280 and 440 kDa (for 66-440 kDa assays). To verify the ladder standards are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Samples** in the View bar. Verification that the ladder standards have been correctly identified can be done in either the graph or lane panes, but manual corrections must be done in the graph pane.

   Graph Pane:

   a. Click **Single View** in the View bar.

   b. Click on the row in the experiment pane that contains the ladder (typically row 1), then click the **Graph** tab. Check that the electropherogram has either six ladder peaks (for 12-230 kDa size assays) or five ladder peaks (for 66-440 and 2-40 kDa size assays). In the example below, the electropherogram has six peaks labeled Ldr 12, Ldr 40, Ldr 66, Ldr 116, Ldr 180 and Ldr 230.



If ladder peaks are not identified correctly, they can be manually corrected as follows:

- **If an incorrect peak is identified as a ladder peak** - Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass for Simple Western should correctly reassign the remaining peaks as ladder standards.
- **To set an unidentified peak as a ladder peak** - Right click the peak in the electropherogram or peaks table and select **Add Peak**. Compass for Simple Western will assign the peak as a ladder standard, and correctly reassign the remaining ladder standards peaks.

---

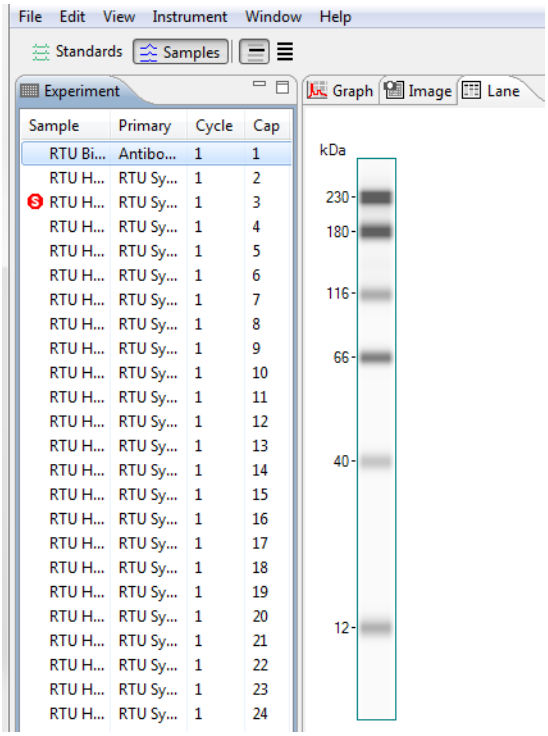
*NOTE: To remove ladder peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.*

Lane Pane:

a. Click either **Single View** or **Multiple View** in the View bar.

b. Click on the row in the experiment pane that contains the ladder (typically row 1), then click the **Lane** tab. Check that the lane has either six ladder bands (for 12-230 kDa size assays) or five ladder bands (for 66-440 kDa and 2-40 kDa size assays). In the example below, the lane has six peaks labeled

Ldr 12, Ldr 40, Ldr 66, Ldr 116, Ldr 180 and Ldr 230. To view band labels, roll the cursor over the individual bands. If ladder bands are not identified correctly, they must be corrected in the graph pane as described in the previous section.



Step 5 – Checking Samples

All sample proteins in the graph and lane panes will be labeled automatically with the calculated protein size.

NOTE: The reported molecular weight for sample proteins detected by an Immunoassay or a Total Protein Assay in Compass for Simple Western may vary from apparent molecular weights using other techniques and based on sample and assay conditions.

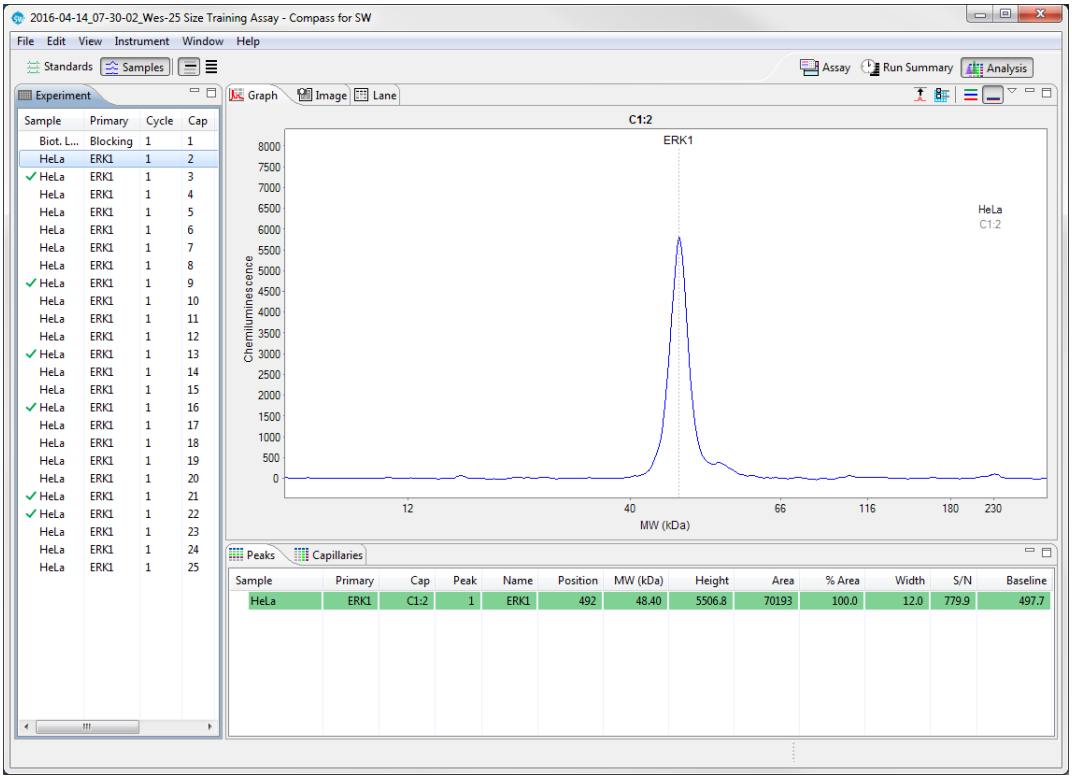
To verify that sample proteins are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Samples** in the View bar. Verification that sample proteins have been correctly identified can be done in either the graph or lane panes, but manual corrections must be done in the graph pane.

Graph Pane:

a. Click **Single View** in the View bar.

b. Click on the row in the experiment pane that contains the sample you wish to check, then click the **Graph** tab.



If sample peaks are not identified correctly, they can be manually corrected as follows:

- **If an incorrect peak is identified as a sample peak** - Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass for Simple Western will no longer identify it as a sample peak in the electropherogram and the peak data will be removed in the results tables.
- **To set an unidentified peak as a sample peak** - Right click the peak in the electropherogram or peaks table and select **Add Peak**. Compass for Simple Western will calculate and display the results for the peak in the results tables and identify the peak in the electropherogram.

---

*NOTE: To remove sample peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.*

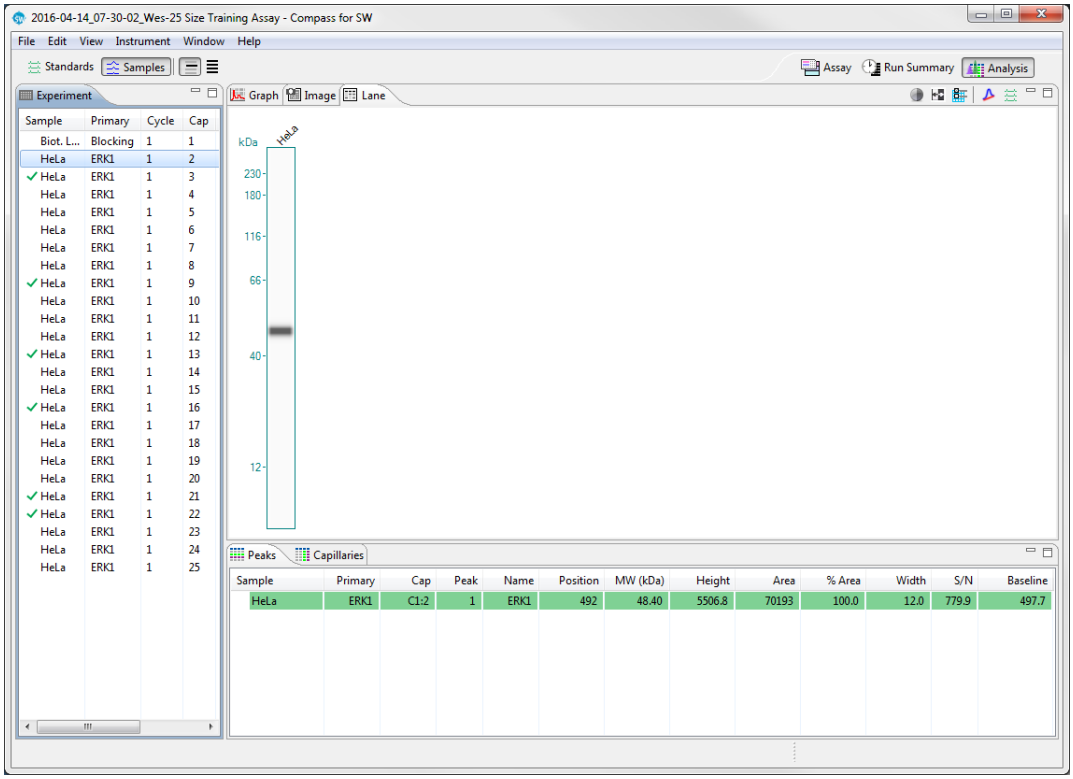
---

c. Repeat the previous steps for the remaining sample rows in the experiment pane to make sure all sample proteins are identified correctly.

Lane Pane:

a. Click either **Single View** or **Multiple View** in the View bar.

b. Click on the row in the experiment pane that contains the sample you wish to check, then click the **Lane** tab. To view sample protein band labels, roll the cursor over the individual bands. If sample bands are not identified correctly, they must be corrected in the graph pane as described in the previous section.



Step 6 – Assigning Peak Names (Optional)

Compass for Simple Western can identify and automatically name sample proteins associated with specific primary antibodies in the run data using user-specified peak name settings. For more information on how to do this see "Peak Names Settings" on page 308.

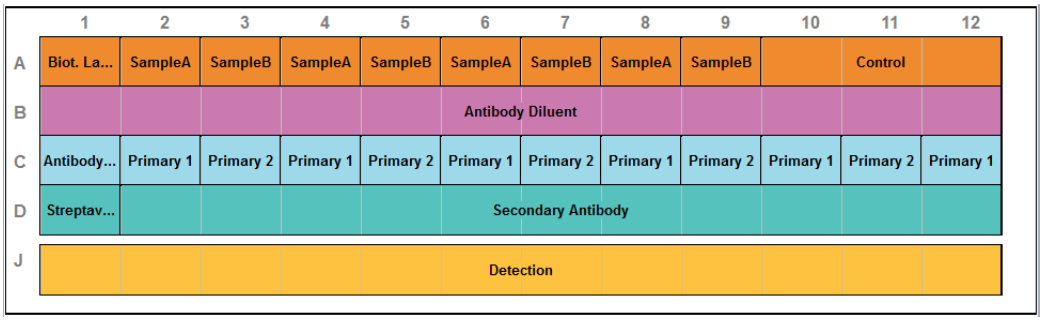
Group Statistics

The Grouping View is used to analyze replicates by calculating the mean, standard deviation and CV of named proteins (see "Peak Names Settings" on page 308 for detailed information on entering named proteins). Statistics for each protein are also plotted for easy comparison between samples, antibodies, and proteins.

Using Groups

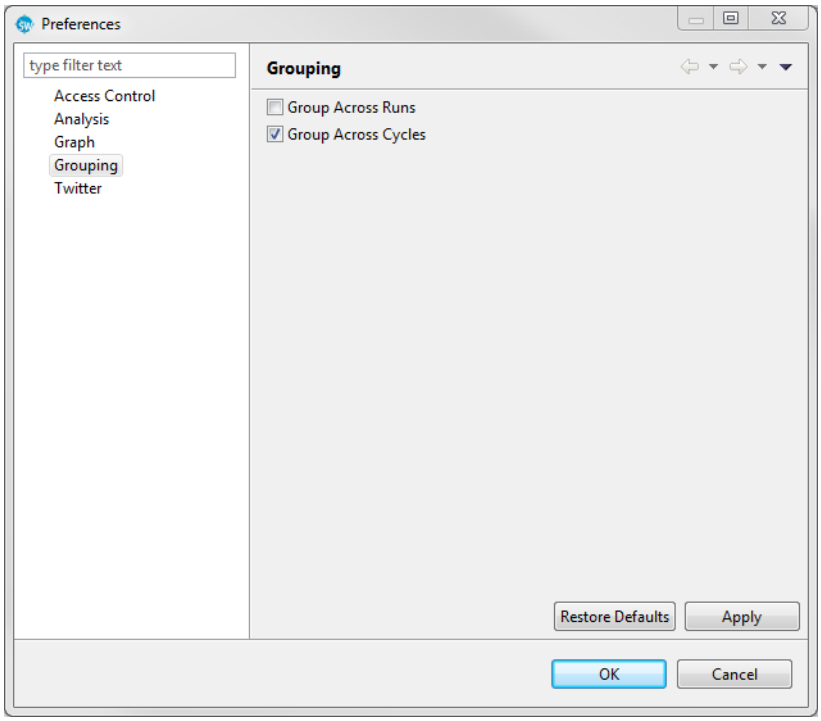
1. A group is automatically created for capillaries with the same sample and primary antibody name, so to use this feature, this information must first be entered into the Assay template. To do this:
   a. Click the **Assay** tab and go to the Template pane.
   b. Enter sample names and primary antibody names as described in "Step 6 - Add Assay Plate Annotations (Optional)" on page 43. Be sure to enter the same sample and/or primary antibody names for the groups of samples you want to calculate statistics for.

      In this example there are two sample types, **Sample A** and **Sample B** which were run with two different antibodies, **Primary 1** and **Primary 2**.



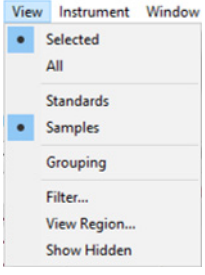
      Each of the two samples were run with each of the two antibodies twice in every cycle. This creates four groups for the combination of two samples and two antibodies.

2. To set a grouping option, go to **Edit** >**Preferences** and select the **Grouping** page. Then check the option you want to use. These options allow you to group capillaries in multiple ways:



- **Group across runs** - Groups capillaries from multiple runs. This option creates fewer groups and generates statistics across multiple runs.
- **Group across cycles** - Groups capillaries run in different cycles. You can use this for Jess/Wes runs too.
- **No option selected** - When only one run is open, groups will only contain capillaries from the same cycle. When more than one run is open, groups are created for each run.

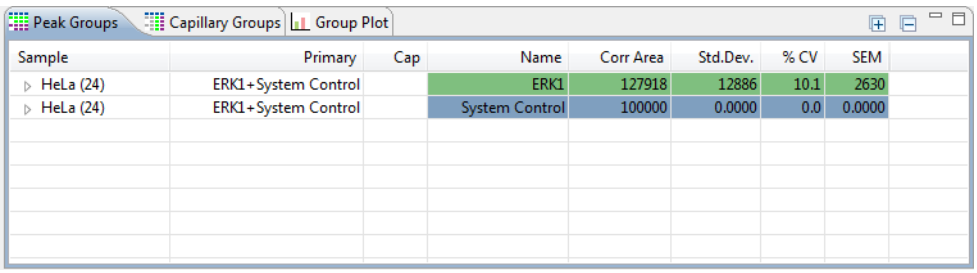
3. Click **Apply** and then select **OK**.
4. Group your results and view the associated statistics by selecting **View** and clicking on **Grouping**.



Viewing Statistics

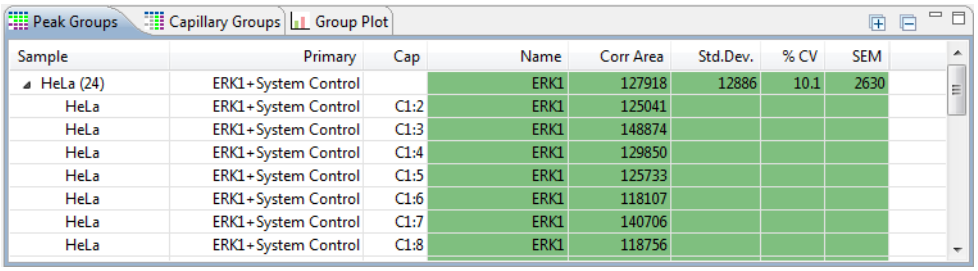
Peak and Capillary Groups

The Peak Groups pane reports statistics for each named protein in every group. Each group shows the statistics for named proteins which includes average area, standard deviation and %CV. The number in parenthesis after the sample name indicates the number of capillaries in the group.



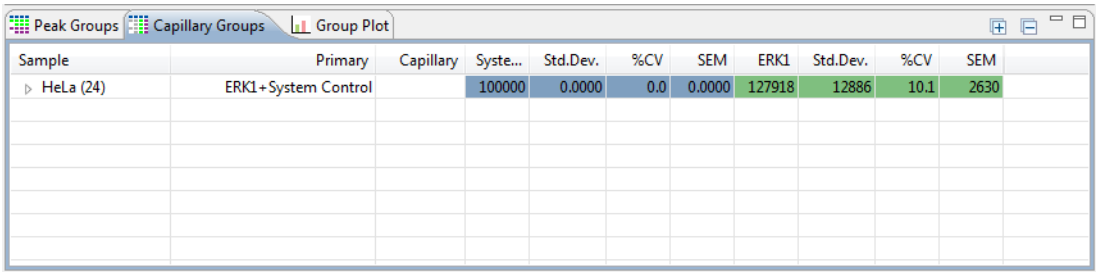
Sample	Primary	Cap	Name	Corr Area	Std.Dev.	% CV	SEM
HeLa (24)	ERK1+System Control		ERK1	127918	12886	10.1	2630
HeLa (24)	ERK1+System Control		System Control	100000	0.0000	0.0	0.0000

Clicking the arrow next to a group lists the individual capillaries in the group and reported data for each capillary:



Sample	Primary	Cap	Name	Corr Area	Std.Dev.	% CV	SEM
HeLa (24)	ERK1+System Control		ERK1	127918	12886	10.1	2630
HeLa	ERK1+System Control	C1:2	ERK1	125041			
HeLa	ERK1+System Control	C1:3	ERK1	148874			
HeLa	ERK1+System Control	C1:4	ERK1	129850			
HeLa	ERK1+System Control	C1:5	ERK1	125733			
HeLa	ERK1+System Control	C1:6	ERK1	118107			
HeLa	ERK1+System Control	C1:7	ERK1	140706			
HeLa	ERK1+System Control	C1:8	ERK1	118756			

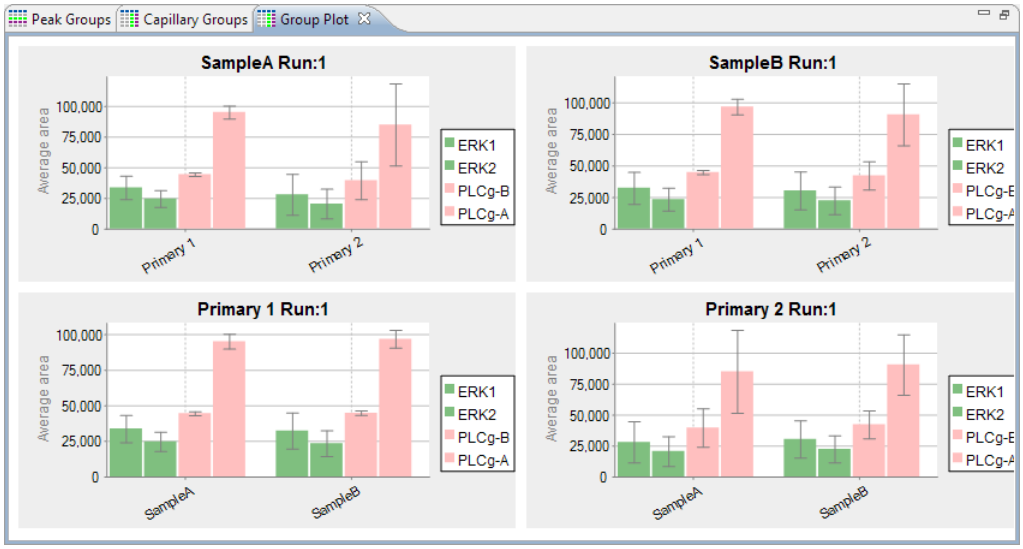
The **Capillary Groups** pane pivots the **Peak Groups** results to show statistics for named protein peaks in individual columns.



Sample	Primary	Capillary	Syste...	Std.Dev.	%CV	SEM	ERK1	Std.Dev.	%CV	SEM
HeLa (24)	ERK1+System Control		100000	0.0000	0.0	0.0000	127918	12886	10.1	2630

Group Plots

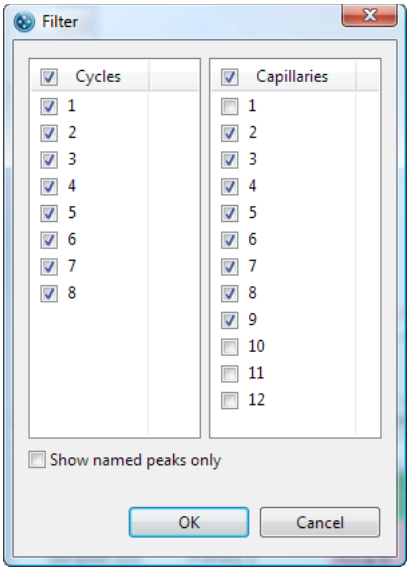
The mean values for named peaks in each group are plotted in bar graphs with error bars showing the standard deviation. The plots compare different antibodies for the same sample and different samples for the same antibody to allow a choice of presentation. The y-axis on the plot will be concentration if a standard curve is defined.



Hiding or Removing Capillaries in Group Analysis

Hidden capillaries are not included in groups. However, hiding capillaries provides an easy way to reject individual capillaries from the statistical analysis. See "Viewing RePlex Channel Views (Jess Only)" on page 216 for details on how to do this.

Larger groups of capillaries can also be excluded from analysis. To do this, select **View** and click **Filter**.



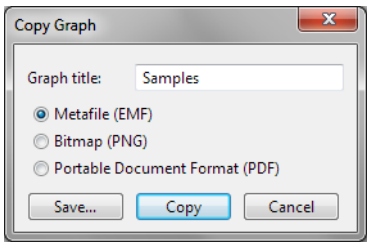
Uncheck the box next to the cycles or capillaries you wish to remove and click **OK**. This data will now be removed from the grouped view statistics.

Copying Data Views and Results Tables

You can copy and paste data and results tables into other documents, or save a data view as a graphic file.

Copying Data Views

1. Click in the graph or lane pane.
2. Select **Edit** in the main menu and click **Copy**, or right click and select **Copy**.
3. If you selected copy from the graph pane the following window will display, click **Copy**. If you selected copy from the lane pane skip to the next step.



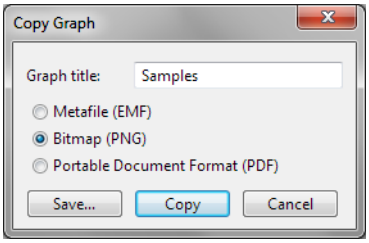
4. Open a document (Microsoft® Word®, Excel®, PowerPoint®, etc.). Right click in the document and select **Paste**. A graphic of the copied data view will be pasted into the document.

Copying Results Tables

1. Click in the peaks or capillaries pane.
2. Select one or multiple rows.
3. Select **Edit** in the main menu and click **Copy**, or right click and select **Copy**.
4. Open a document (Microsoft® Word®, Excel®, PowerPoint®, etc.). Right click in the document and select **Paste**. Data for the rows selected will be pasted into the document.

Saving the Graph View as an Image File

1. Click in the graph pane.
2. Select **Edit** in the main menu and click **Copy**, or right click and select **Copy**.
3. Select an image option (EMF, PNG or PDF) in the pop-up window, then click **Save**.



4. Select a directory to save the file to and enter a file name, then click **OK**.

Exporting Run Files

Results tables and raw plot data can be exported for use in other applications.

Exporting Results Tables

To export the information in the peaks and capillaries tables:

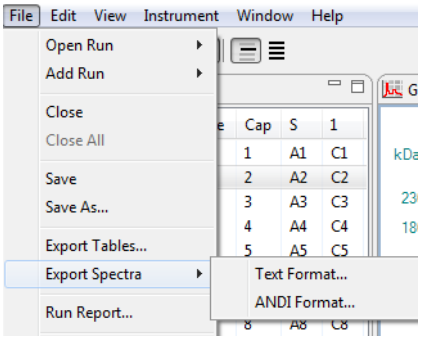
1. Click **File** in the main menu and click **Export Tables**.
2. Select a directory to save the files to and click **OK**. Data will be exported in .txt format.

NOTE: To exclude export of standards data or export results table data in .csv format, see "Setting Data Export Options" on page 465.

Exporting Raw Sample Electropherogram Data

To export raw sample plot data:

1. Click **File** in the main menu and click **Export Spectra**.



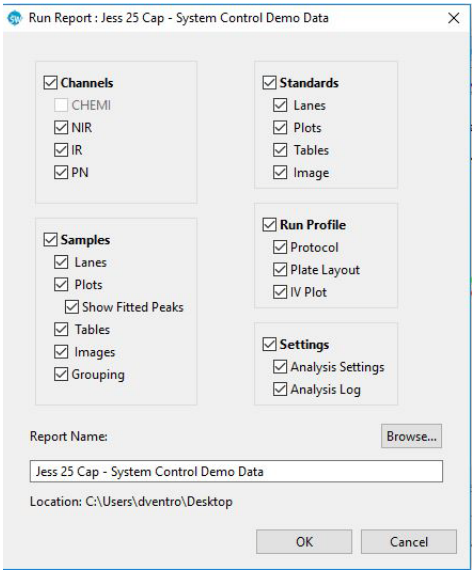
- **To export data in .txt format** - Select **Text Format**. Plots will be exported in one file for all capillaries.
- **To export data in .cdf format** - Select **Andi Format**. Plots will be exported in one file per capillary.

2. Select a directory to save the files to and click **OK**. Data will be exported in the selected format.

Running Reports

Compass for Simple Western v3.0 and higher can create and export run reports in .pdf format.

1. Open your run file, then select **File** > **Run Report**. The Run Report window displays.



2. Select or deselect the information you want included in your report.

3. Enter a file name for the report. The report file is saved in the same location as the run file is by default.
4. Click **OK**. When the report is done, the directory containing the report will display so you can open the report.

A full report includes the following:

- **Run information**

Run

Run	2018-04-27_15-43-31_Jess Alpha 2 SL
Path	C:\Users\Desktop
Assay	Jess
Kit Info	Regular: 12-230 kDa
Instrument	Jess : Jess JS3006 - js3006
Firmware Version	1.1.34338
Plate S/N	8532502129
Cartridge S/N	0133490027
Cartridge Expires	N/A
Started	Fri 3:43 PM Apr 27, 2018 EDT
Completed	Fri 7:32 PM Apr 27, 2018 EDT
Error	None

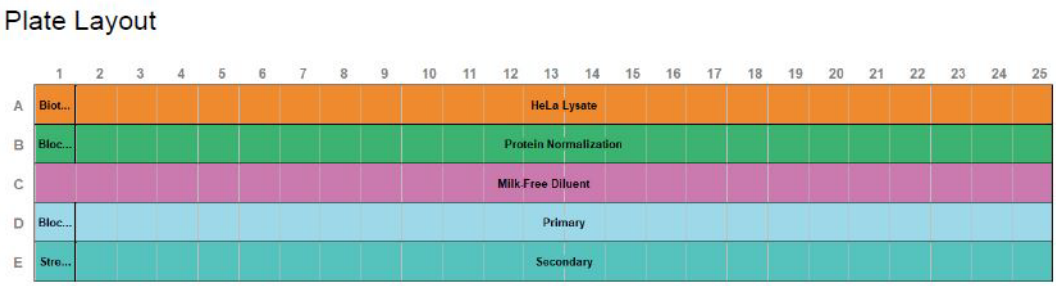
- **Protocol details**

Protocol

Separation Matrix	
Well Row	L1
Load Time (sec)	200.0
Stacking Matrix	
Well Row	M1
Load Time (sec)	15.0
Sample	
Well Row	A1
Load Time (sec)	9.0
Separation Time (min)	25.0
Separation Voltage (volts)	375
Standards Exposure (sec)	4.0
EE Immobilization Time (sec)	200.0
Matrix Removal	
Matrix Removal Time (sec)	230.0
Matrix Washes	3
Matrix Wash Soak Time (sec)	150.0
Wash Soak Time (sec)	150.0
Protein Normalization Time (min)	40.0

Well Row	B1
Washes	2
Wash Soak Time (sec)	150.0
Antibody Diluent Time (min)	5.0
Well Row	C1
Washes	0
Wash Soak Time (sec)	0.0
Primary Antibody Time (min)	30.0
Well Row	D1
Washes	2
Wash Soak Time (sec)	150.0
Secondary Antibody Time (min)	30.0
Well Row	E1
Washes	1
Wash Soak Time (sec)	150.0
Detection	
NIR Detection Profile	
Exposure 1 (sec)	10.0
Exposure 2 (sec)	30.0
Exposure 3 (sec)	60.0
Exposure 4 (sec)	120.0
Exposure 5 (sec)	300.0
IR Detection Profile	
Exposure 1 (sec)	10.0
Exposure 2 (sec)	30.0
Exposure 3 (sec)	60.0
Exposure 4 (sec)	120.0
Exposure 5 (sec)	300.0
Ladder Channel	NIR

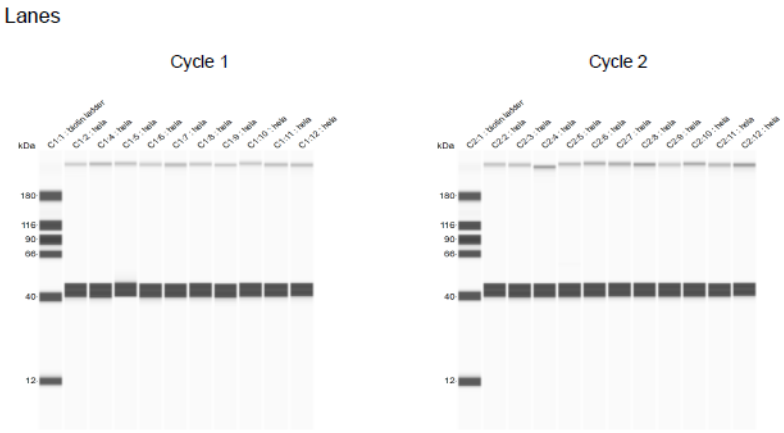
- **Plate layout**



- **Analysis settings**

Hidden Capillaries	None
Images	
NIR Fluorescence	10.0
IR Fluorescence	10.0
PN Fluorescence	1.0
Lane Contrast	Slider
NIR White Level	-68.8
NIR Black Level	27623.7
IR White Level	-27.4
IR Black Level	2792.1
PN White Level	-69.4
PN Black Level	552.1
Peak Fit	fit
Apply To	Default
Range Min (MW kDa)	1.0
Range Max (MW kDa)	250.0
Range View	Full
Baseline Threshold	1.0
Baseline Window (pixels)	15.0
Baseline Stiffness	1.0
Peak Find Threshold	10.0
Peak Find Width (pixels)	9.0
Peak Find Area Calculation	Gaussian Fit
Peak Name	Peak Group 1
Apply To	[1:1+2+3+4+5+6+7+8+9+10+11+12+13+14+15+16+17+18+19+20+21+22+23+24+25]
IR (MW kDa)	60
IR Range (%)	10
IR Channel	IR
NIR (MW kDa)	49
NIR Range (%)	10
NIR Channel	NIR
Ladder Settings	Cap: 1 Fluorescent Channel: NIR
Apply To	Default
(MW kDa)	Position
12	250
40	400
66	500
116	550
180	600
230	650
Protein Normalization	Reference Capillary: 2
Control Area	N/A
Start	12.0
End	220.0
Standards	Biotinylated Ladder (12kDa-230kDa)
Apply To	Default
Standard 1 (MW kDa)	1
Standard 1 Position (pixels)	170
Standard 1 Fit	no
Standard 1 Registration	yes
Standard 2 (MW kDa)	29
Standard 2 Position (pixels)	350
Standard 2 Fit	yes
Standard 2 Registration	no
Standard 3 (MW kDa)	230
Standard 3 Position (pixels)	650
Standard 3 Fit	yes
Standard 3 Registration	no

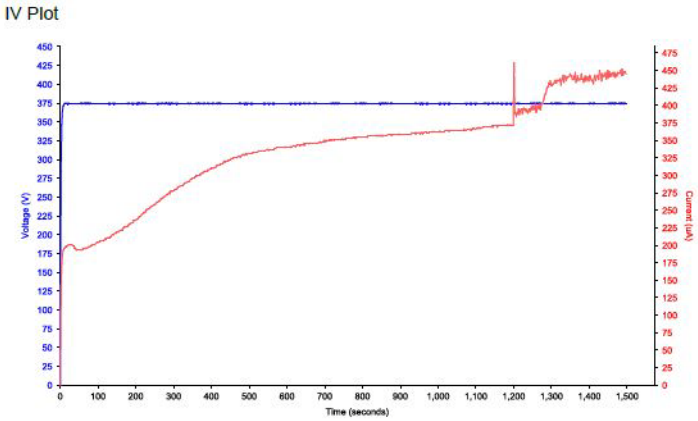
- **Lane views (for all cycles on Peggy Sue and Sally Sue):**



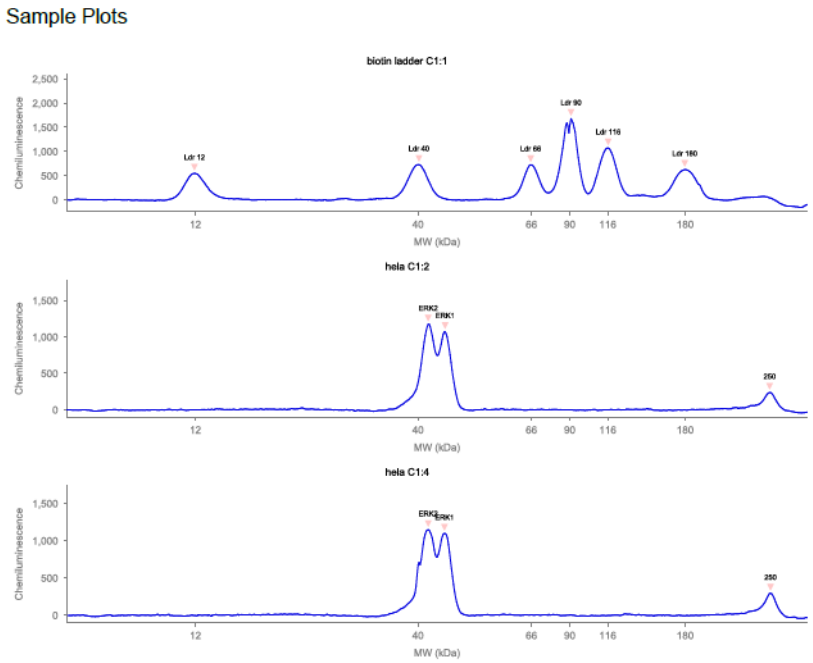
- **Lane views (Channel Lanes Overlay for Jess Only):**

NOTE: The channel lanes overlay is also divided in the report as individual NIR, IR and Standard channel lanes.

- **IV plot:**

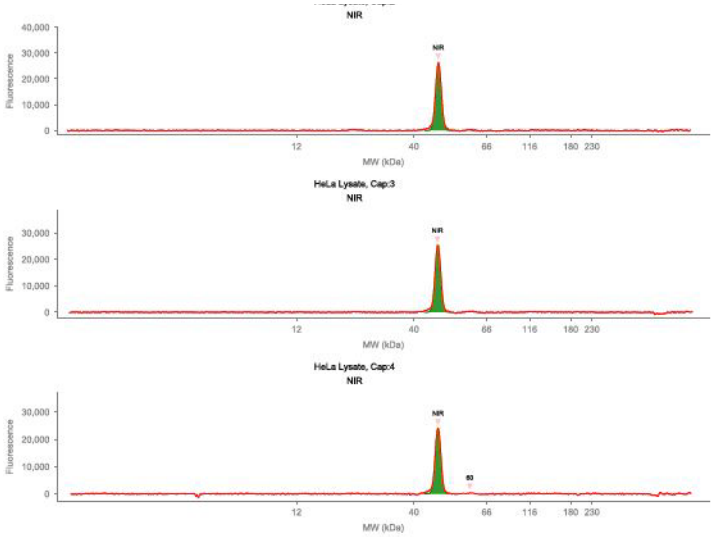


- **Sample plots for each capillary of each cycle (for Peggy Sue and Sally Sue only):**



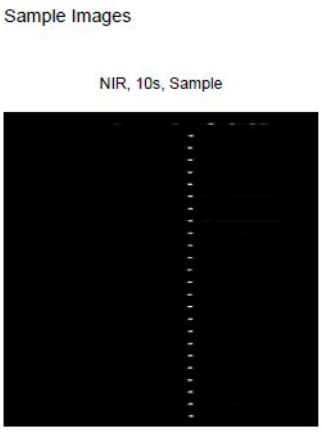
You can also include the fluorescent standards graphs for each capillary, registration graphs (Peggy Sue and Sally Sue only) and the standard peaks position and registration peak offsets in pixels in the report.

- **Sample plots for each capillary by channel (Jess only):**



NOTE: The example shown is for the NIR channel on Jess only. The report can also include the IR channel, fluorescent standards graphs and protein normalization for each capillary, in addition to sample peak area data, the standard peaks position and registration peak offsets in pixels.

- **Sample images:**



NOTE: The report can also include images of other channels and standards.

- **Analysis log:**

Analysis Log

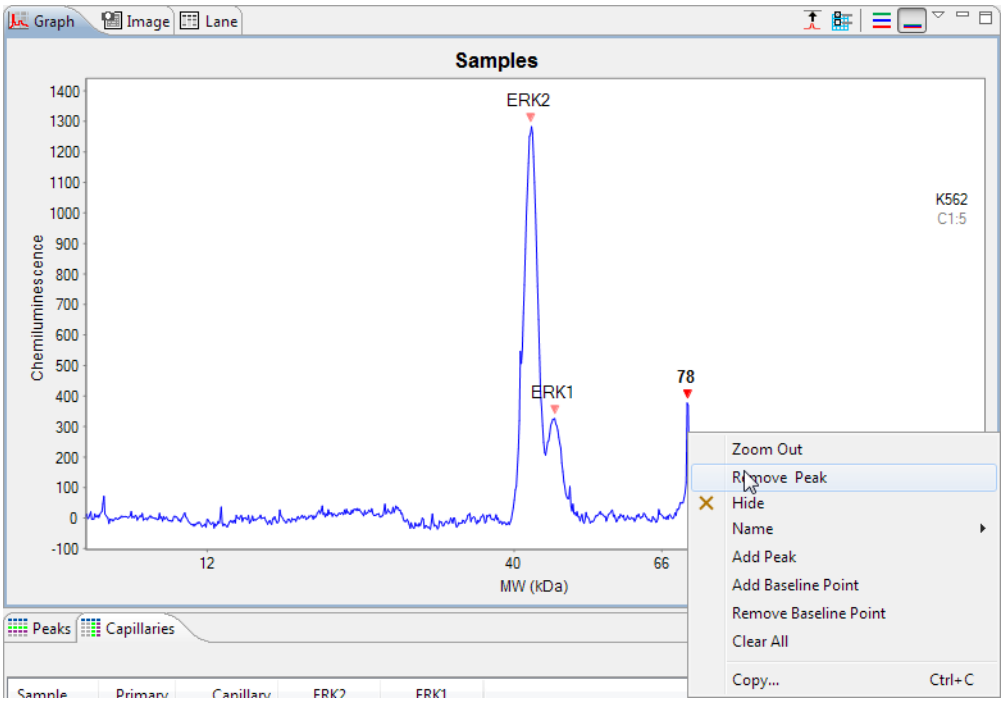
Date	User Name	Message	Comment
2018-04-27 15:43:52		Started run: 2018-04-27_15-43-31_Jess Alpha 2 SL Assay: Jess.assay	
2018-04-30 17:23:53		Saved analysis and template changes from Compass for SW v4.0.0-0423	
		Added Peak Names Apply Settings "apply Peak Group 1 to 1:1+2+3+4+5+6+7+8+9+10+11+12+13+14+15+16+17+18+19+20+21+22+23+24+25"	
		Added Peak Names Group Peak Group 1	
		Protein name: IR MW: 60.0 Color: 32512 Range: 10.0 channel: IR	
		Protein name: NIR MW: 49.0 Color: 32512 Range: 10.0 channel: NIR	
		Changed Peak Fit Analysis Settings fit: Range View from Analysis to Full	
		Changed Lane for NIR channel: Black Level from 10000.0 to 27623.7	

Changing Sample Protein Identification

Compass for Simple Western allows you to customize what sample proteins are reported in the results tables by making manual adjustments in the electropherogram or peaks table.

Adding or Removing Sample Data

1. Click **Show Samples** in the View bar.
2. Click **Single View** in the View bar.
3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.
    - **To remove a peak from the data -** Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass for Simple Western will no longer identify it as a sample peak in the electropherogram and the peak data will be removed in the results tables.



- **To add an unidentified peak to the data -** Right click the peak in the electropherogram or peaks table and select **Add Peak**. Compass for Simple Western will calculate and display the results for the peak in the results tables and identify the peak in the electropherogram.

NOTES:

*To remove sample peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.*

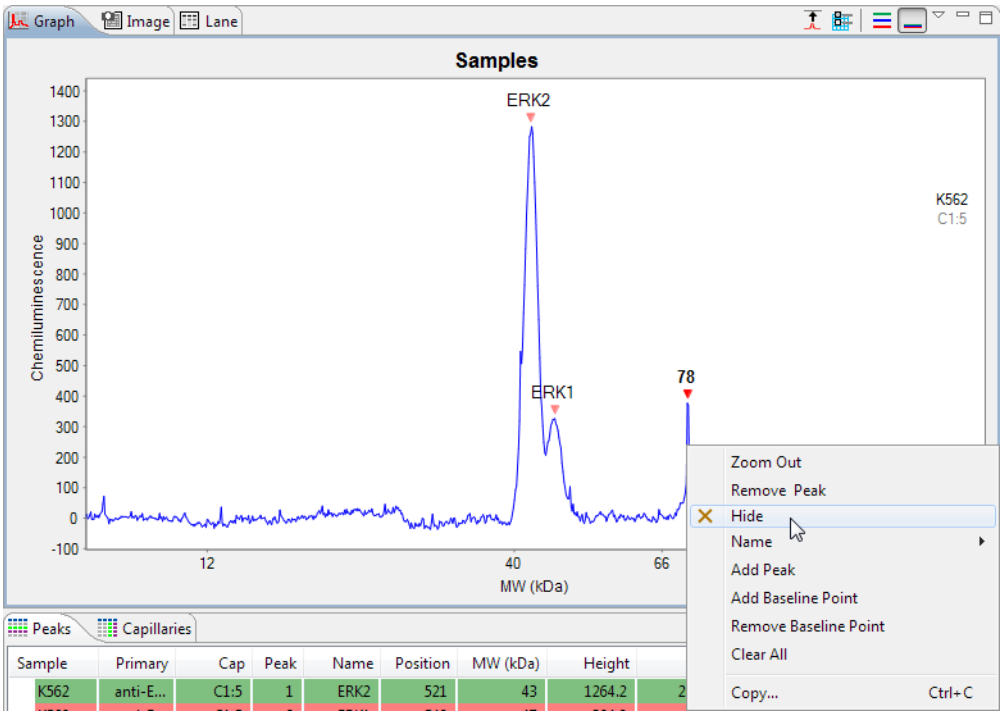
Virtual blot data in the lane pane will also update to reflect changes made in the graph pane.

Hiding Sample Data

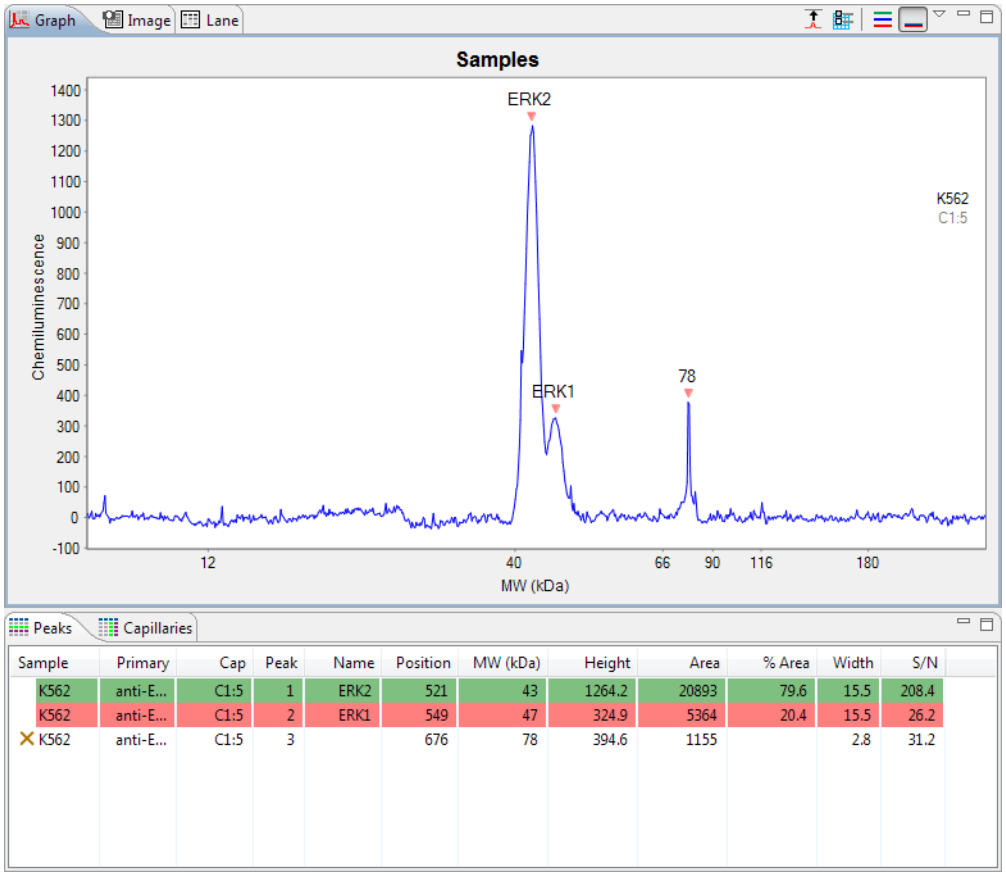
You can hide the results for a sample protein in the results tables without completely removing it from the reported results. To to this:

1. Click **Show Samples** in the View bar.
2. Click **Single View** in the View bar.

3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.
4. Right click the peak in the electropherogram or peaks table and select **Hide peak**. Compass for Simple Western will hide the peak data in the results tables.



5. To view hidden peak data, click **View** in the main menu and click **Show Hidden**. Hidden peak data will display in the results table and be marked with an **X**.

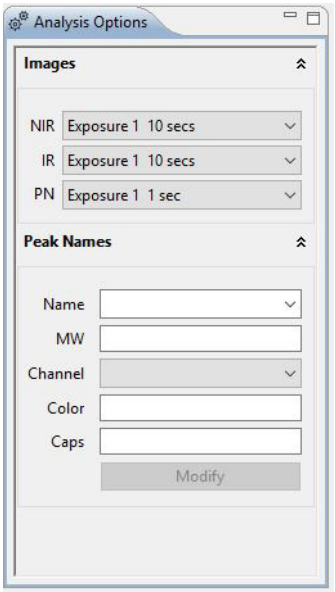


Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width	S/N
K562	anti-E...	C1:5	1	ERK2	521	43	1264.2	20893	79.6	15.5	208.4
K562	anti-E...	C1:5	2	ERK1	549	47	324.9	5364	20.4	15.5	26.2
✕ K562	anti-E...	C1:5	3		676	78	394.6	1155		2.8	31.2

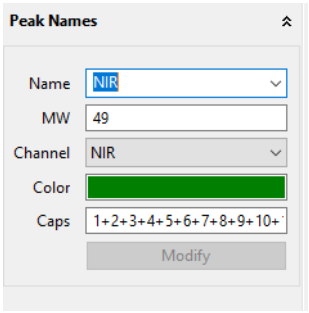
6. To unhide a peak, right click on the peak in the electropherogram or peaks table and select **Unhide Peak**.

Changing Peak Names for Sample Data

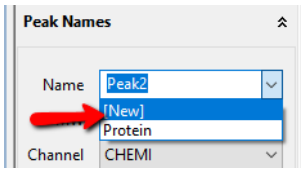
If Compass for Simple Western did not automatically name a sample protein associated with a specific primary antibody, this can be adjusted manually. You can do this in the Analysis Options pane.



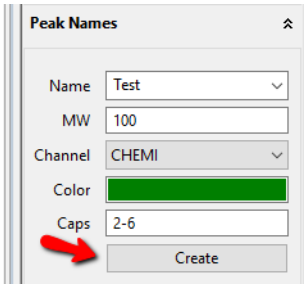
You can change the name or molecular weight of a peak, the channel and its associated color, or designate corresponding capillaries. Click the desired peak in the Graph view, lane in the Lane view or row in the Peaks Table. Then in the Analysis Options pane, Peak Names menu, click **Modify**:



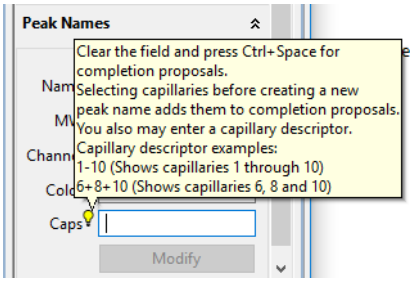
The New option in the Name dropdown box creates a new default Peak Name using the current channel and capillaries that are selected.



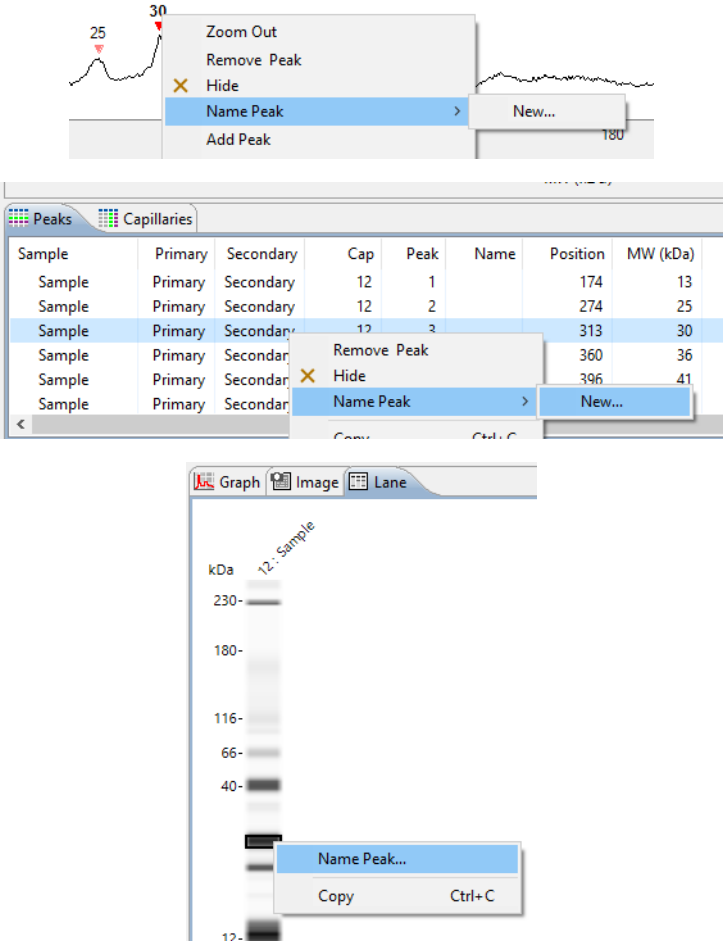
Rename the peak and modify the parameters, then click **Create**.



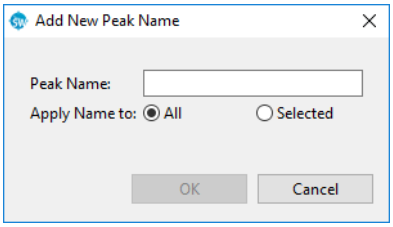
The tooltip next to the Caps field has useful information on acceptable nomenclature when designating capillaries to apply a Peak Name to:



Alternatively, right click the peak in the electropherogram, peaks table or lane view and click **Name**, then click a name in the list. Compass for Simple Western will change the peak name in the electropherogram and results tables, and adjust peak names for other sample proteins accordingly.



Next, enter the appropriate peak name in the Peak Name box:



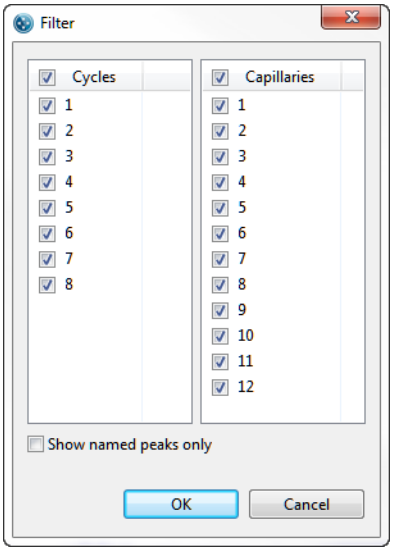
NOTES:
For details on how to specify peak name settings, see "Peak Names Settings" on page 308.

Virtual blot data in the lane pane will also update to reflect changes made in the graph pane.

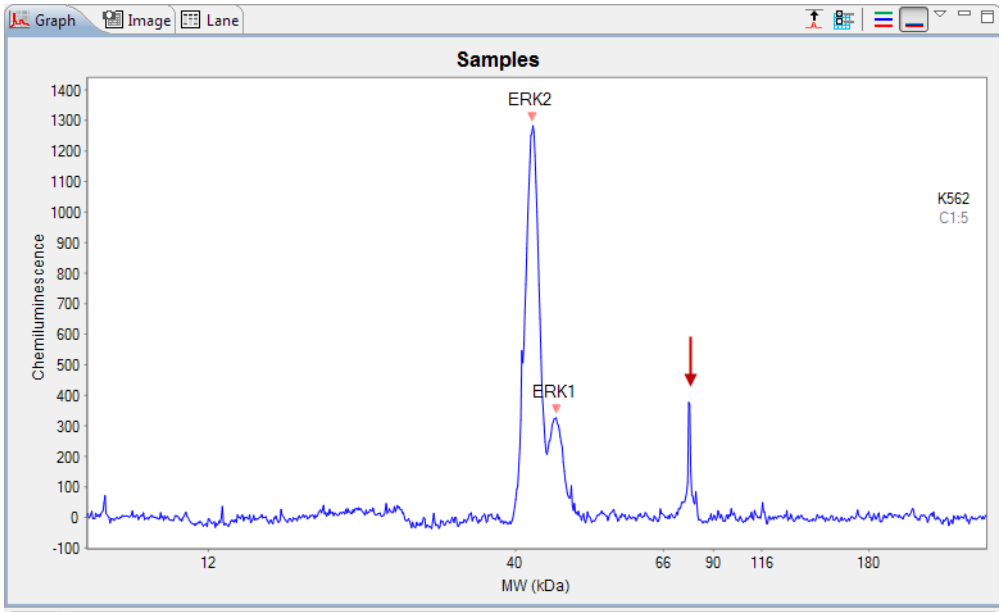
Displaying Sample Data for Named Peaks Only

You can adjust the sample data to display results only for user-specified named peaks. To do this:

1. Click **Show Samples** in the View bar.
2. Click **View** in the main menu and click **Filter**.
3. Check the **Show Named Peaks only** box and click **OK**.



Compass for Simple Western will hide peak data in the electropherogram and results tables for all unnamed peaks, and instead only display data for named peaks in the electropherogram and results tables.



Peaks | Capillaries

Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width	S/N
K562	anti-E...	C1:5	1	ERK2	521	43	1264.2	20893	79.6	15.5	208.4
K562	anti-E...	C1:5	2	ERK1	549	47	324.9	5364	20.4	15.5	26.2

Changing the Virtual Blot View

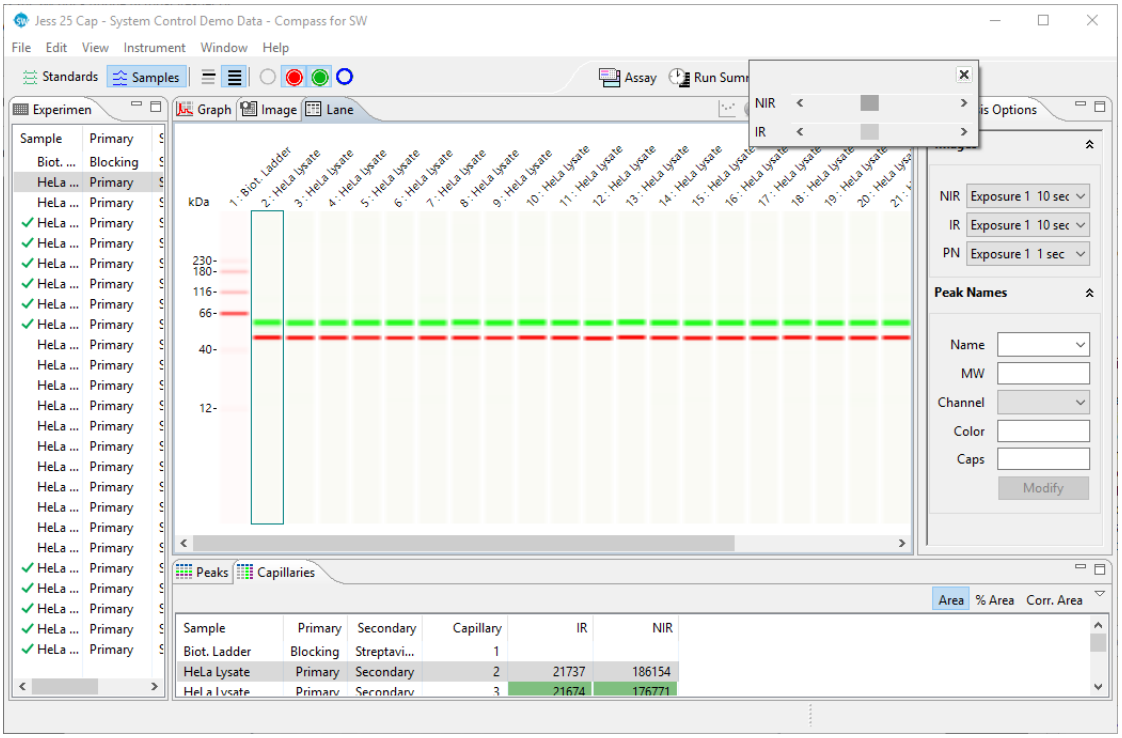
Options in the lane pane let you change the contrast or invert the virtual blot, remove baseline noise, change lane labels or overlay standards data on sample lanes.

The Lane pane toolbar has the following options:

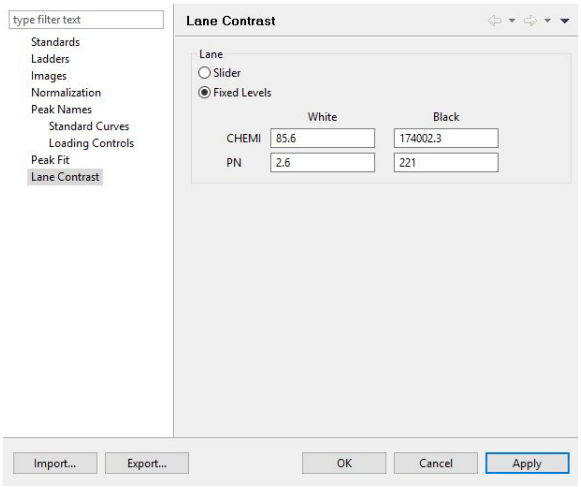
- Contrast Adjustment
- Invert
- Lane Options
- Remove Baseline
- Overlay Standards Data

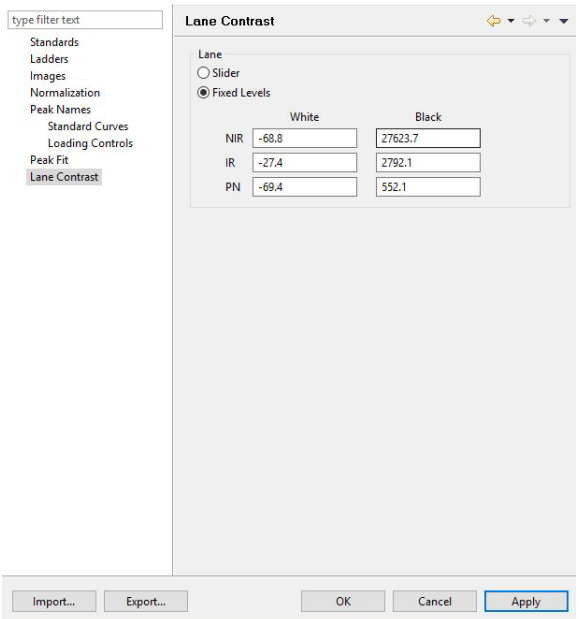
Adjusting the Contrast

1. Click the **Contrast Adjustment** button. The contrast tool will display:



2. There are two ways you can use the Contrast Adjustment tool. The slider is unlocked by default, so you can slide it left or right until the desired contrast is reached. You can also lock the slider at a fixed position and save your desired contrast levels. This lets you reproduce the same contrast level across data files.
3. To lock the slider, select **Edit** > **Analysis**, and click the **Lane Contrast** page. Select **Fixed Levels**. The numbers displayed refer to the current White and Black levels for the chemiluminescence or fluorescence (Jess only) run being analyzed. Change these settings and click **Apply** to see how they impact the contrast on the lane view. Once you're satisfied with the changes, click **OK.**





- When the you select Fixed Level, the slider won't be available in the lane view.
- The Lane Contrast setting can be also be determined and saved in the Default Analysis of the Assay. That way, any data obtained by running the assay will all have the same contrast settings.
- Lane Contrast settings from the assay or run analysis windows can be exported to be applied to other assays or runs.

Inverting the Virtual Blot

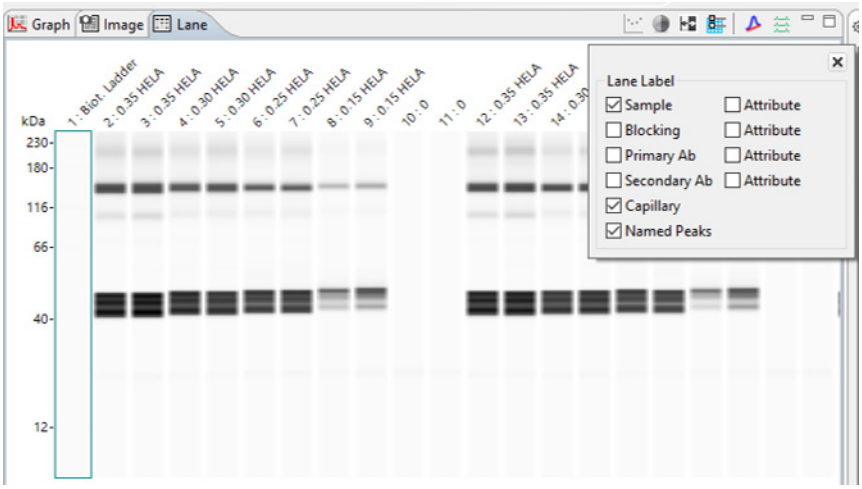
1. Click the **Invert** button. The virtual blot image will invert:

2. Click the **Invert** button again to return to the default view.

Selecting Lane Labels

The labels shown above the lanes in the virtual blot can be customized. To do this:

1. Click the **Lane Options** button. The label box will display. Lane labels for a standard Immunoassay and a RePlex Assays are shown:



2. The following lane display options are available:

   - **Independent Probe Scales (RePlex Assays only)** - Checking this box allows the user to have independent control of contrast scales for Probe 1 and Probe 2 data for RePlex assays when using the Contrast Adjustment button.
   - **Lane order by Probe (RePlex Assays only)** - Checking this box displays lanes based on the probe number. All Probe 1 data will be displayed side by side, followed by Probe 2 data.

   The following label options are available for all assays. Check one or multiple label boxes, and uncheck those you don't want to display. To remove labels completely, uncheck all boxes.

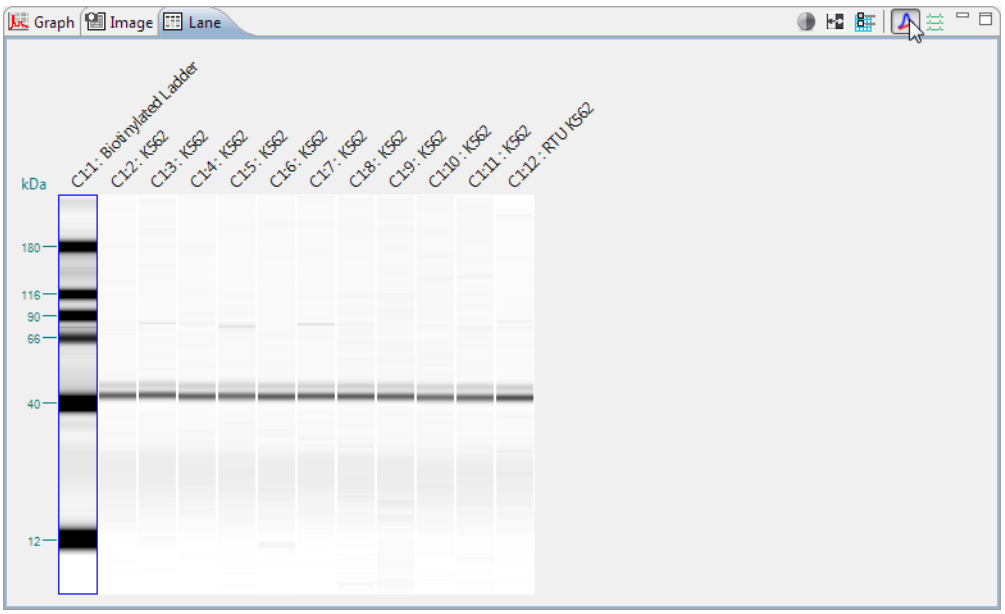
   - **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
   - **Blocking** - Blocking reagent name. If a name was entered in the assay template (Assay screen), that name will display here. Otherwise, Blocking (default name) will display.
   - **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
   - **Secondary Ab** - Secondary antibody name. If a secondary antibody name was entered in the assay template (Assay screen), those names will display here. Otherwise, Secondary (default name) will display.
   - **Capillary**
     - Sally Sue and Peggy Sue: Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.
     - Jess and Wes: Capillary number.
     - Jess RePlex Assays: Probe and capillary number. For example, P1:4 indicates Probe 1, capillary 4.

NOTE: Sally Sue and Peggy Sue run up to eight cycles (12 capillaries at a time), so the cycle number displayed will always be C1-C8 depending on the number of cycles programmed.

- **Attributes** - Attribute text. If attribute information was entered for any of the rows or wells in the assay template (Assay screen), up to four can be selected and displayed as lane labels.

Viewing the Uncorrected Sample Baseline

1. Click the **Remove Baseline** button (active for sample data only). This will remove the automatic baseline correction.

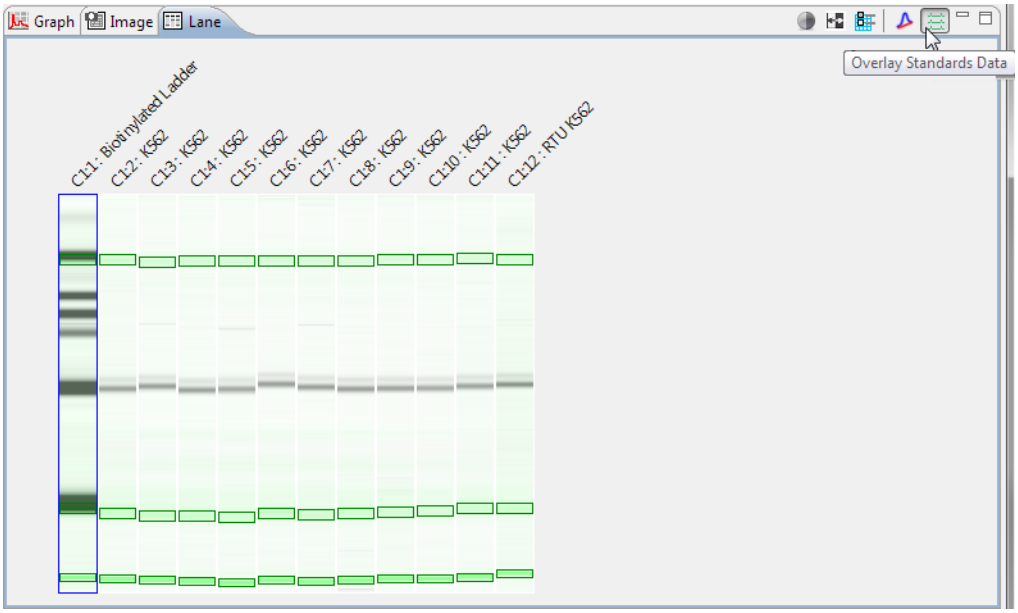


2. Click the **Remove Baseline** button again to return to the default, baseline corrected view.

Overlaying Standards Data on Sample Lanes

Data for the standards can be overlaid on the sample data in the virtual blot so you can view the raw, unaligned (uncorrected) lane data. To do this:

1. Click the **Overlay Standards Data** button (active for sample data only). An overlay of the raw sample and standards data will display:



2. Click the **Overlay Standards Data** button again to return to the default, auto-aligned view.

Moving Lanes in the Virtual Blot View

Lanes in the virtual blot view can be moved to make it easier to compare specific lanes of data. To do this:

1. Click a lane in the virtual blot. Hold the mouse button down and drag the lane to a new position.

2. Release the mouse button. The lane will now be repositioned in the virtual blot view.

Changing the Electropherogram View

Options in the graph pane let you zoom and scale electropherograms, overlay or stack plots and change the peak and plot information displayed.

The graph pane toolbar has the following options:


Auto Scale

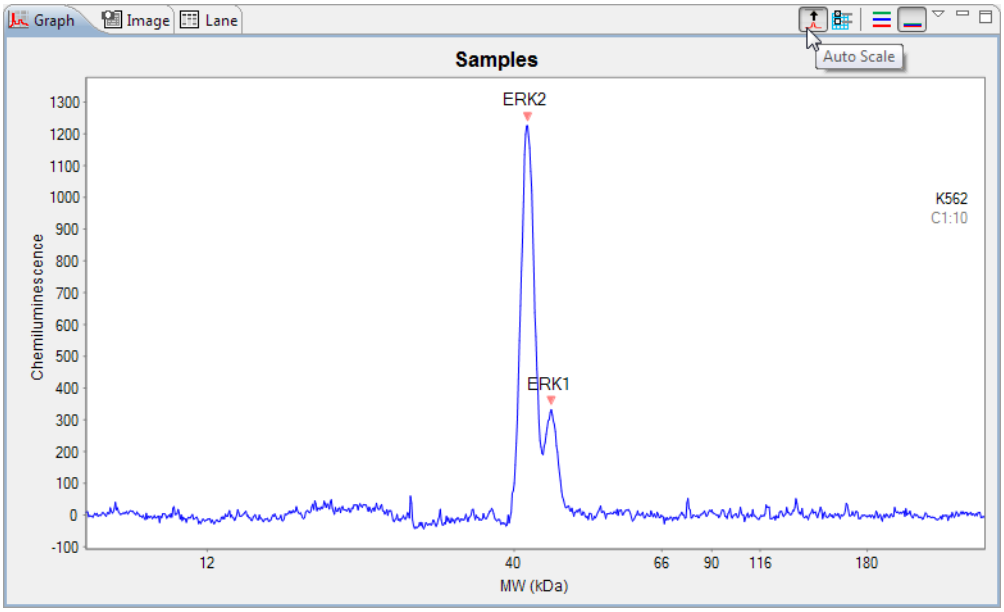

Graph Options


Stack the Plots

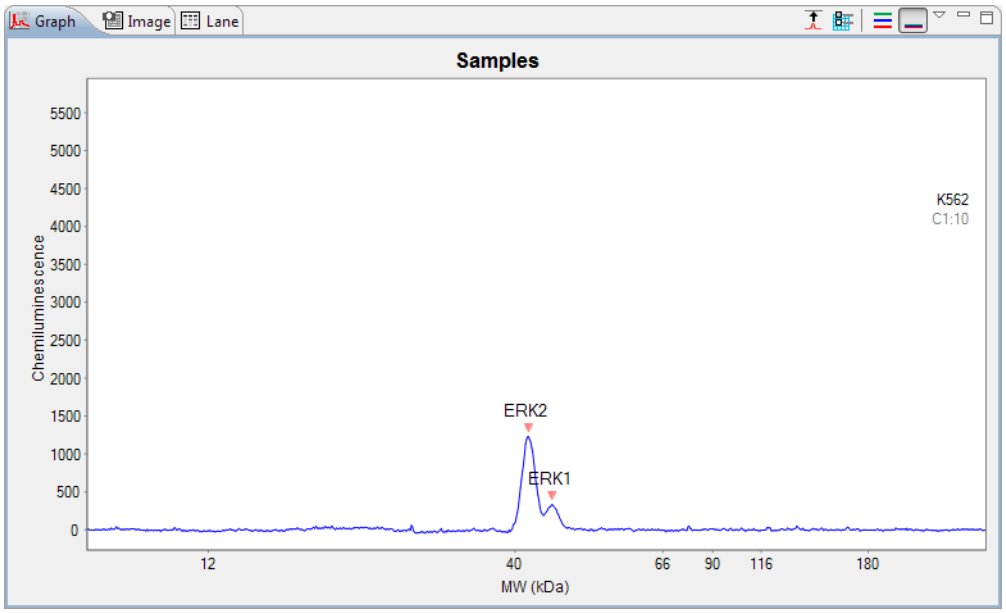

Overlay the Plots

Autoscaling the Electropherogram

Click the **Autoscale** button to scale the y-axis to the largest peak in the electropherogram.



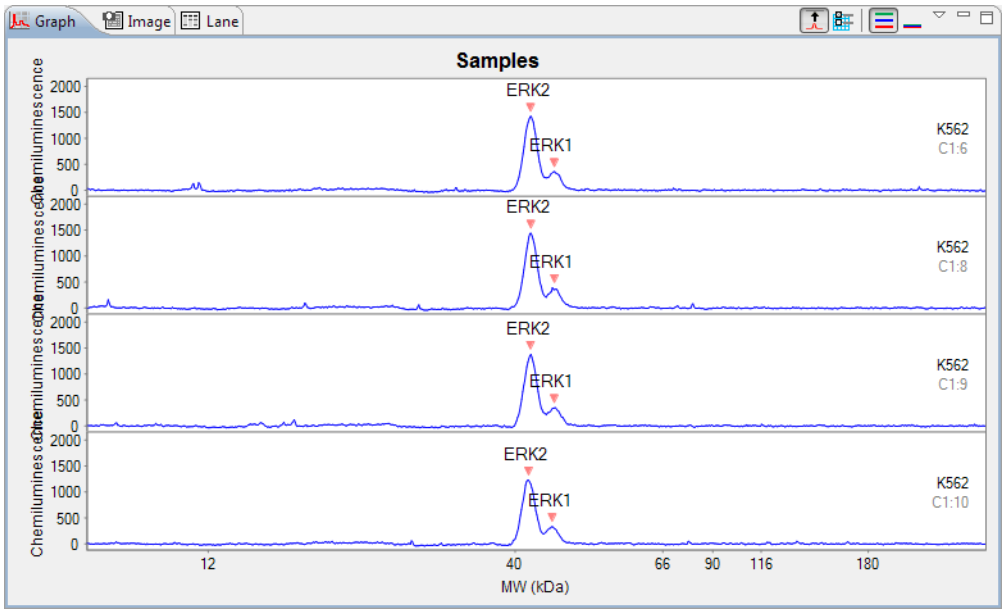
Click the **Autoscale** button again to return to default scaling.



Stacking Multiple Electropherograms

Electropherogram data for multiple capillaries can be stacked vertically in the graph pane for comparison. To do this:

1. Click **Single View**.
2. Select multiple rows in the experiment pane.
3. Click the **Stack the Plots** button. The individual electropherograms for each row selected will stack in the graph pane.

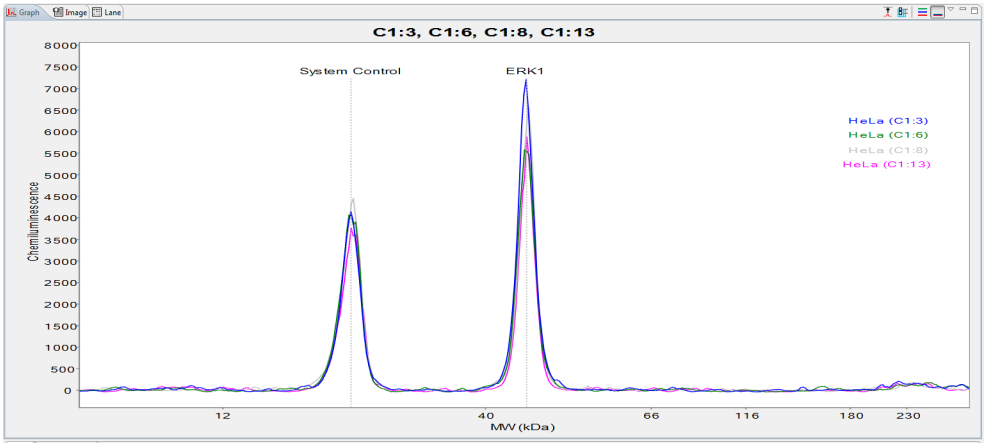


You can customize the colors used for the stacked plot display. For more information see "Selecting Custom Plot Colors for Graph Overlay" on page 466.

Overlaying Multiple Electropherograms

The electropherogram data for multiple capillaries can also be overlaid on top of each other for comparison in the graph pane. To do this:

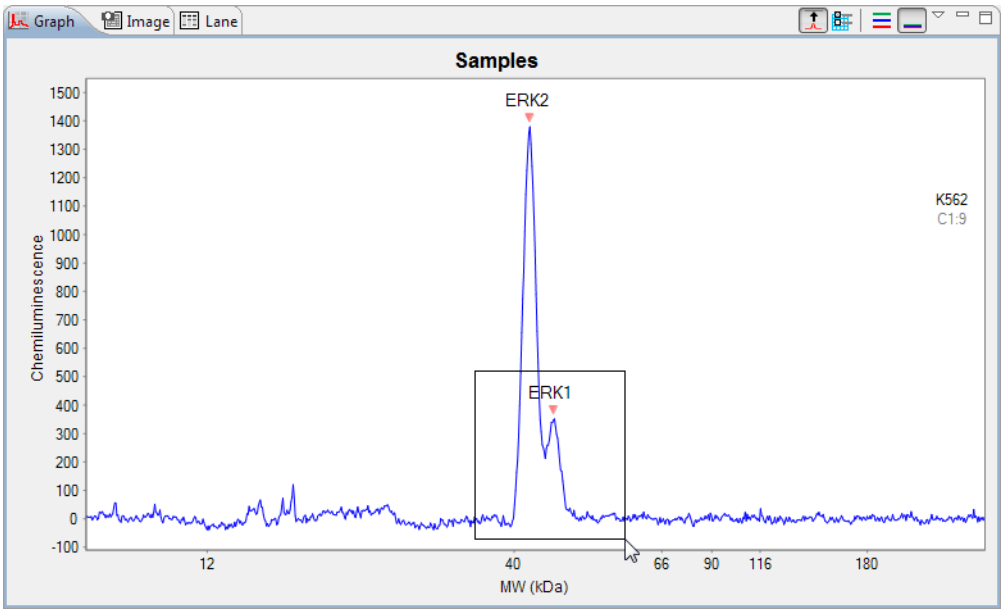
1. Click **Single View**.
2. Select multiple rows in the experiment pane.
3. Click the **Overlay the Plots** button. The individual electropherograms for each row selected will overlay in the graph pane.



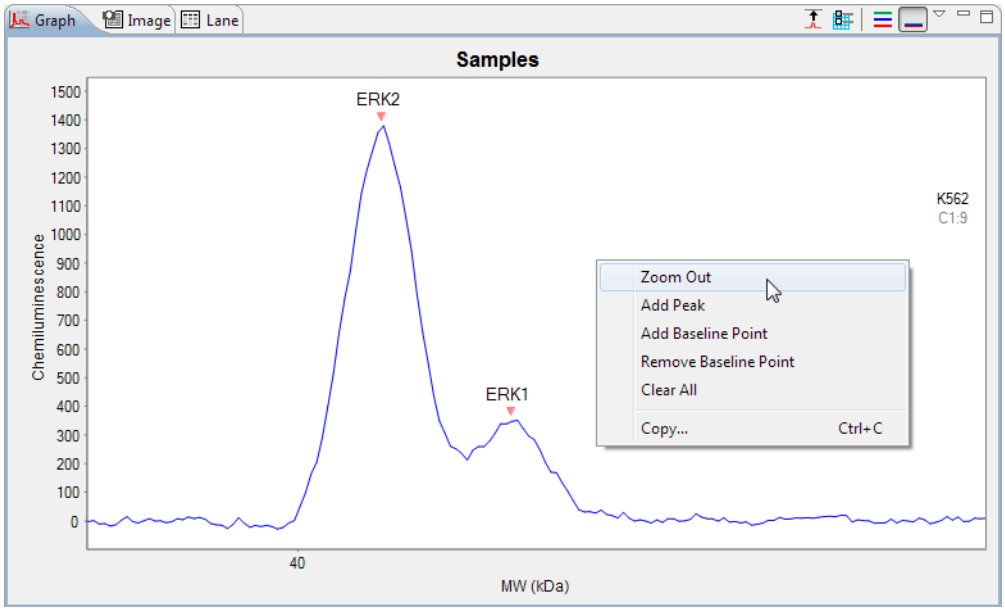
You can customize the colors used for the overlay plot display. For more information see “Selecting Custom Plot Colors for Graph Overlay” on page 466.

Zooming

To zoom in on a specific area of the electropherogram, hold the mouse button down and draw a box around the area with the mouse:

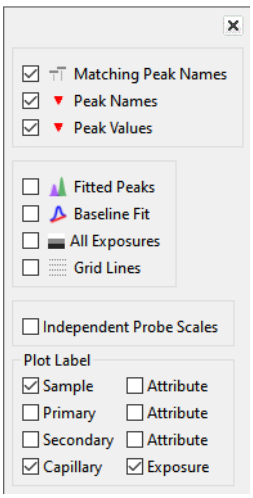


To return to default scaling, right click in the electropherogram and click **Zoom Out**.



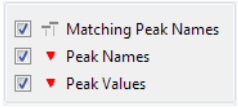
Customizing the Data Display

You can customize electropherogram peak labels, plot labels and display options. To do this, select the **Graph Options** button.

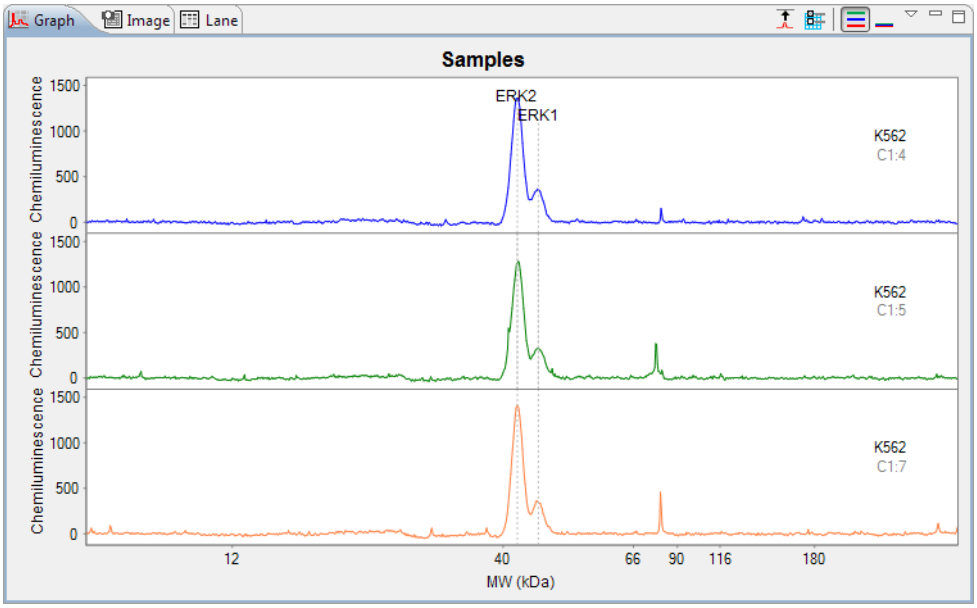


Peak Labels

You can customize the labels used to identify peaks in the electropherogram with these options:

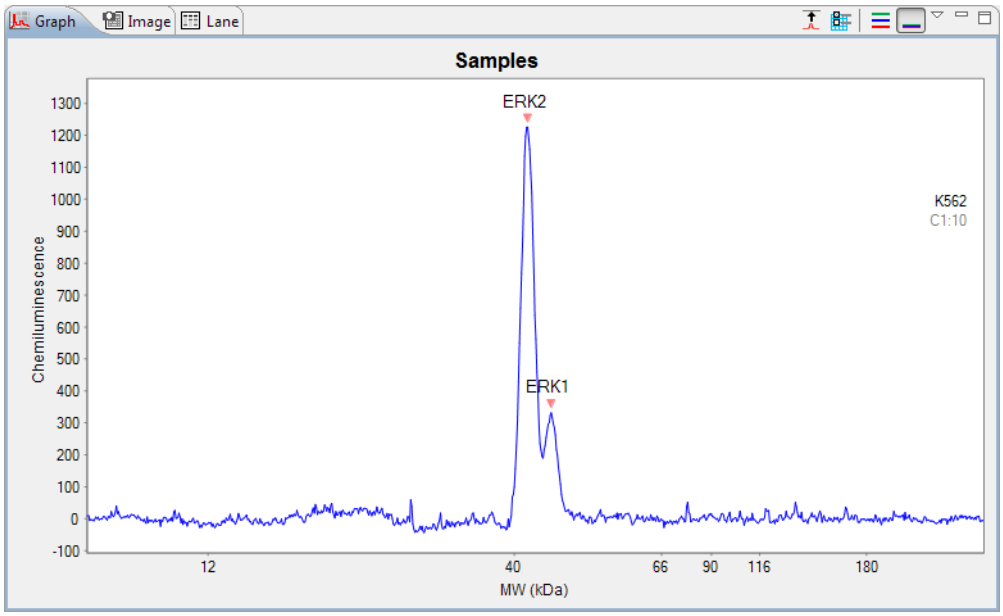


Matching Peak Names - Checking this box will draw vertical lines through each named peak. Using this option with Stack the Plots or Overlay the Plots features is useful for visual comparison of named peaks across multiple capillaries.



- **Peak Names** - Checking this box will display peak name labels on all named peaks in the electropherogram.

NOTE: If more than one peak label option is selected, peak name labels will always be used for named peaks.

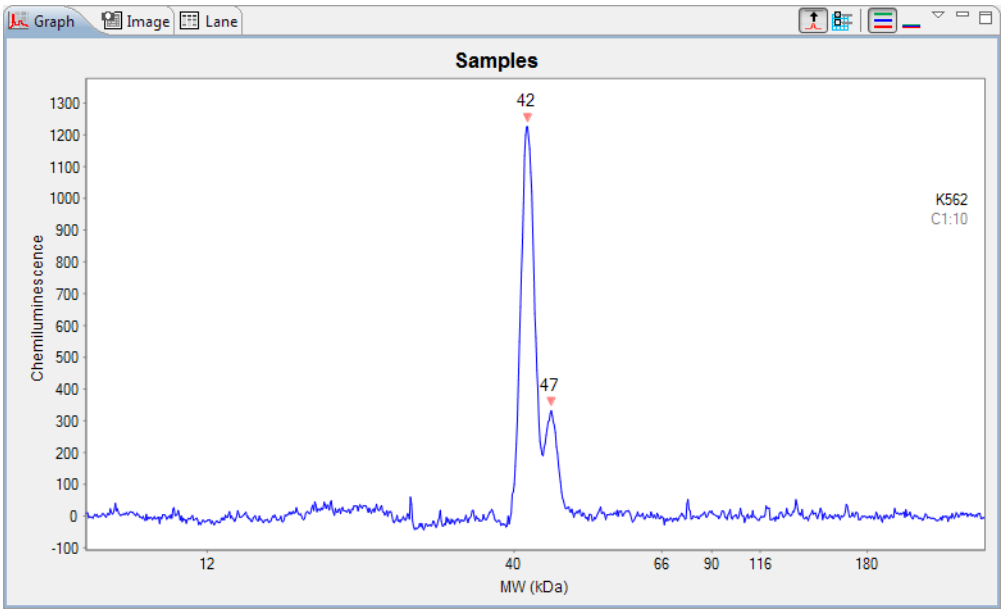


- **Peak Values** - Checking this box will display the molecular weight labels on all peaks in the electropherogram.

NOTES:
When viewing standards and registration data, this option is called Peak Positions. Labels displayed are peak positions rather than molecular weight.

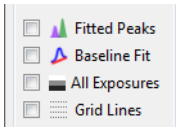
If more than one peak label option is selected, peak name labels will always be used for named peaks.

The reported molecular weight for sample proteins detected by an Immunoassay or a Total Protein Assay in Compass for Simple Western may vary from apparent molecular weights using other techniques and based on sample and assay conditions.



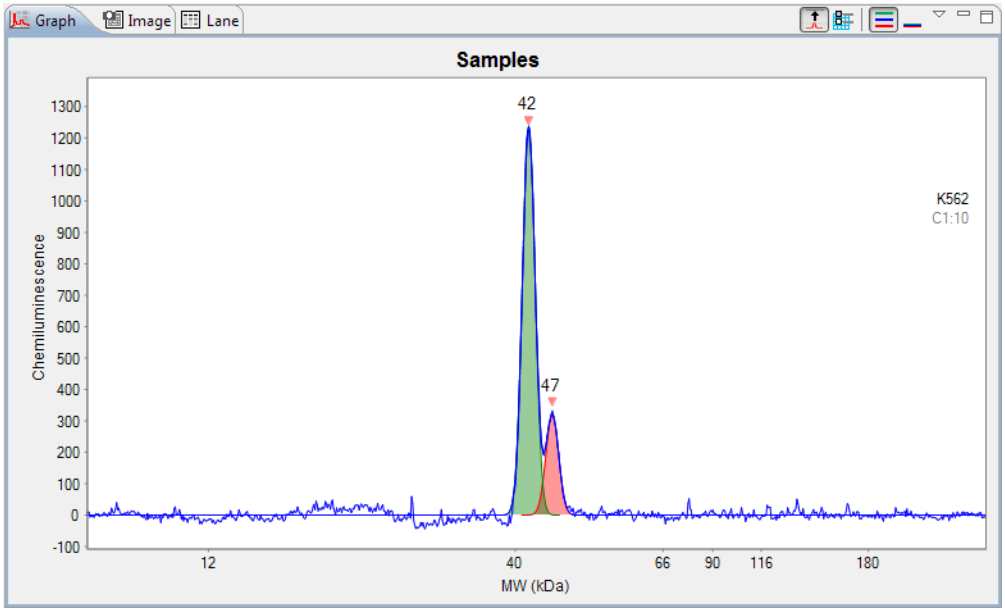
Baseline, All Exposures and Grid Options

You can view the calculated baseline fit, peak integration, view all exposures and show grid lines with these options.

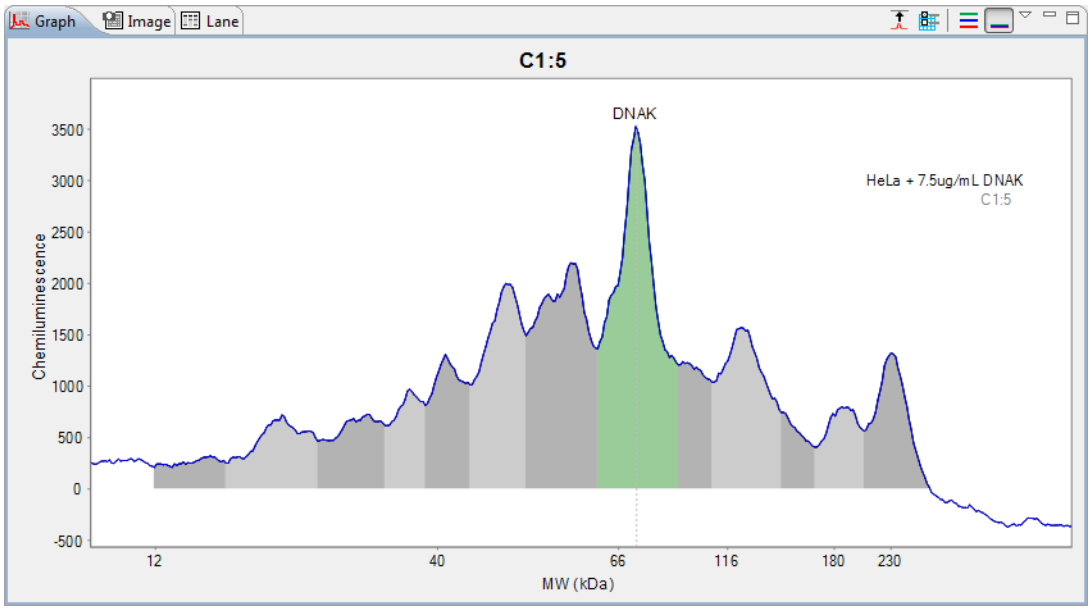


- **Fitted peaks** - Checking this box will display how the peaks were fit by the software.
  - For Immunoassays, the software uses Gaussian fit by default:

NOTE: This option is only available for sample data.

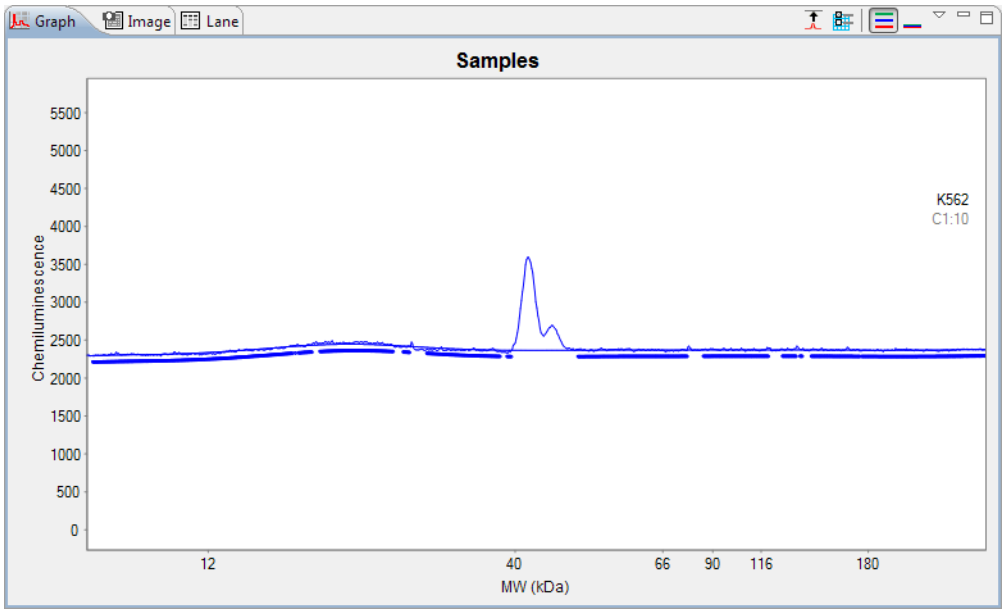


- For Total Protein Assays, the software uses Dropped Lines fit by default:



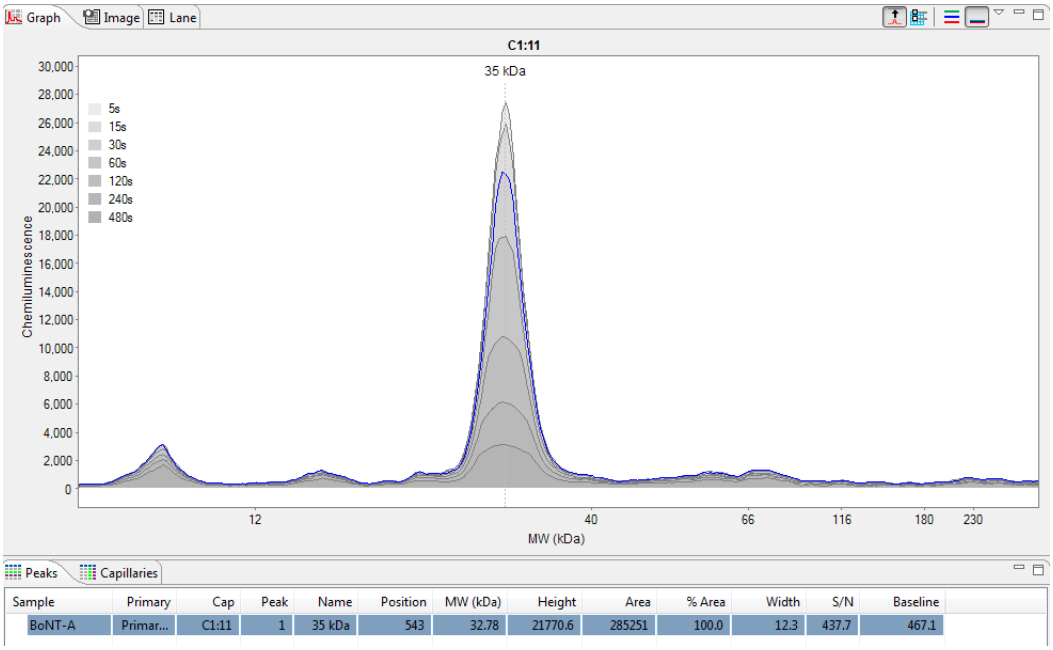
- **Baseline Fit** - Checking this box will display the calculated baseline for the peaks. Baseline points will also display for regions of the electropherogram considered to be at baseline.

NOTE: This option is only available for sample data.



- **All Exposures** - Checking this option will overlay all the chemiluminescence or fluorescence (Jess only) exposure times acquired for a particular run.The peak that corresponds to the exposure time selected in the Analysis window on the Images page will be outlined in blue and the Peaks table below the graph window displays the data corresponding to the selected exposure time.

  In the image below, the All Exposures option is displayed and the 30 second exposure selected in the Analysis window is outlined in blue.



Viewing all the exposures in a single image can aid in spotting potential signal burnout.

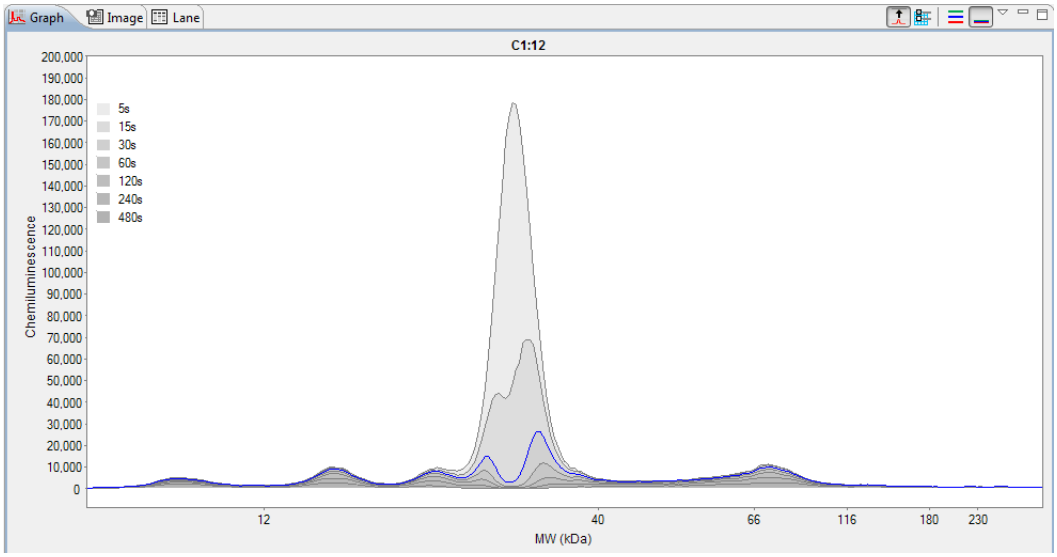
Exposure times are sequential, so longer exposures may have less signal. Since the y-axis in the graph view reports signal/time, the peak heights for the different exposures should be fairly similar. The peak heights and areas will decrease over time with normal HRP enzyme or fluorophore decay, which is expected to occur in the late exposures (longer than 120 or 240 seconds).

If on the other hand you observe a quicker signal (peak height) decline over time in the tall peaks vs. smaller peaks for a chemiluminescence run, then this decline is what we refer to as burnout and is a result of local Luminol depletion.

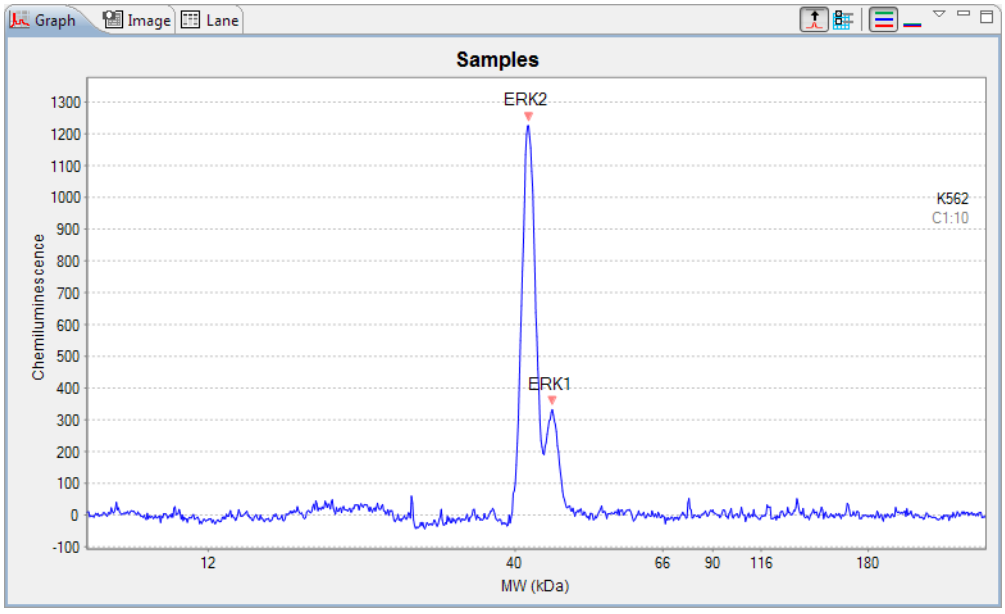
As a rule of thumb, start by looking at the shortest exposure times (typically 5 to 30 seconds). If the peak heights from each exposure have similar values, then there is likely no signal burnout. If the peak height decreases significantly during those early exposure times, this is an indication of potential signal burnout. Another hallmark of signal burnout is peaks developing shoulders and eventually splitting as exposure time increases.

If you experience burnout, the first thing to do is to reduce the signal level by lowering the sample concentration in the assay. We do not recommend lowering the primary or secondary antibody concentration. Keeping the primary antibody concentration at saturation conditions keeps your assay linear and reproducible.

The image below shows a chemiluminescence detection example where the peak height from 5 to 30 seconds declines significantly and it is split in two peaks by 30 seconds. In this case we would recommend that you start by lowering the sample concentration.



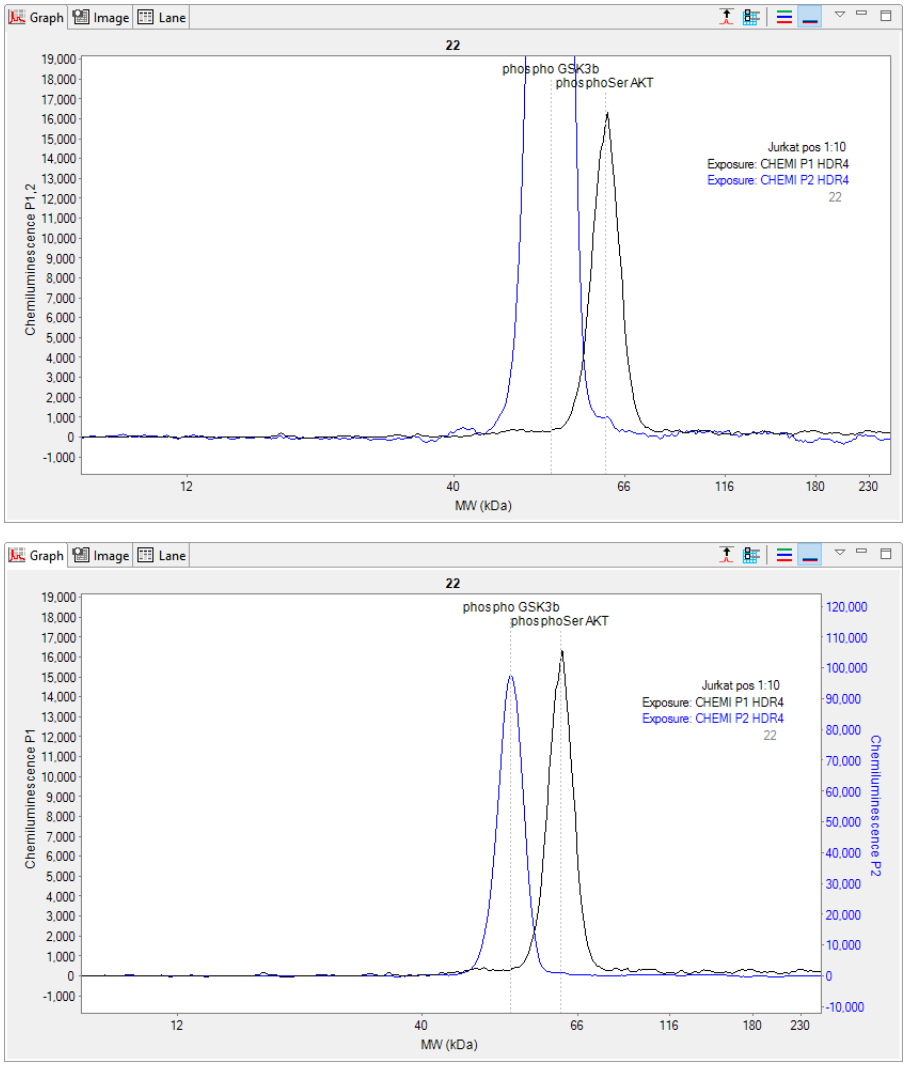
- **Grid Lines** - Checking this box will display grid lines in the graph area.



Independent Probe Scales (RePlex Assays Only)

Checking this option displays Probe 1 and Probe 2 data using the same detection channel on independent y-axis scales. The Probe 1 y-axis will display on the left and the Probe 2 y-axis will display on the right. Y-axis labels will indicate P1 and/or P2. The following example show the graph display with the Independent Probe Scales off (top) and on (bottom).

NOTE: When more than one channel is used for Probe 1, all data for a channel is displayed on the same y-axis when viewing three or more channels.



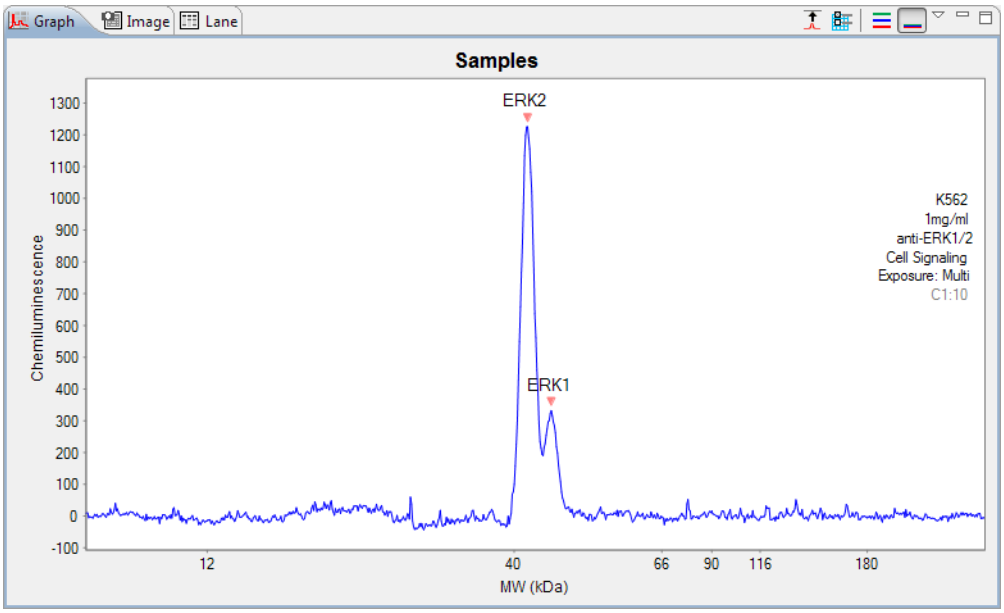
Plot Labels

You can customize the plot labels displayed on the electropherogram with these options.

Plot labels are shown on the right side of the graph pane.

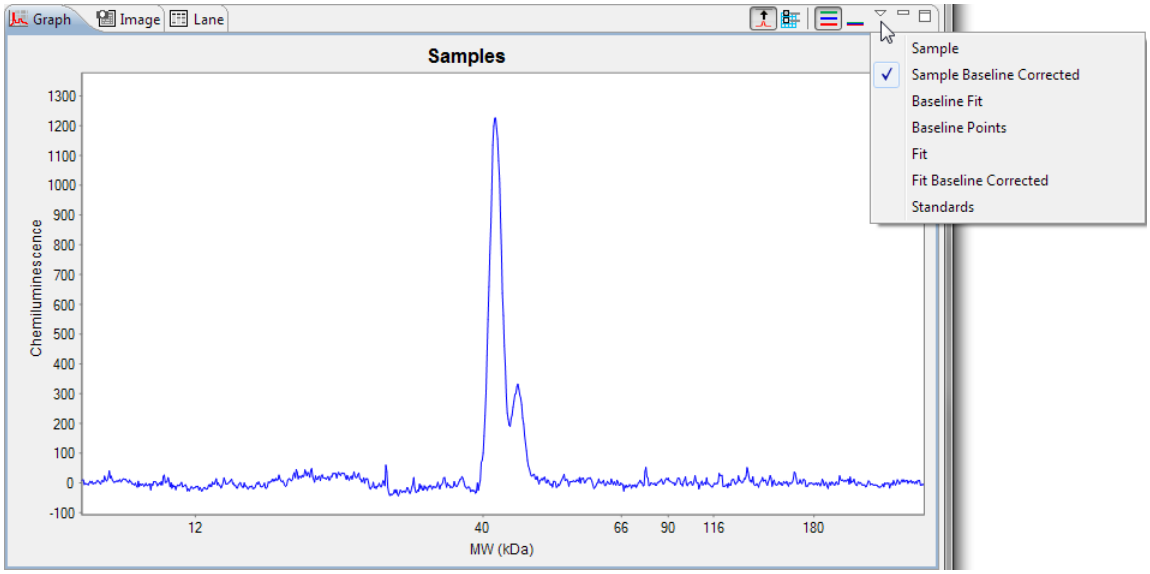
- **Sample** - Checking this box will display the sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Checking this box will display the primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Capillary** - Checking this box will display the cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.
- **Attributes** - Checking this box will display attribute text. If attribute information was entered for samples or primary antibodies in the assay template (Assay screen), they can be selected as plot labels.
- **Exposure** - Checking this box will display the exposure time(s) used for the data.

The following example shows an electropherogram with all plot labels selected:



Selecting Data Viewing Options

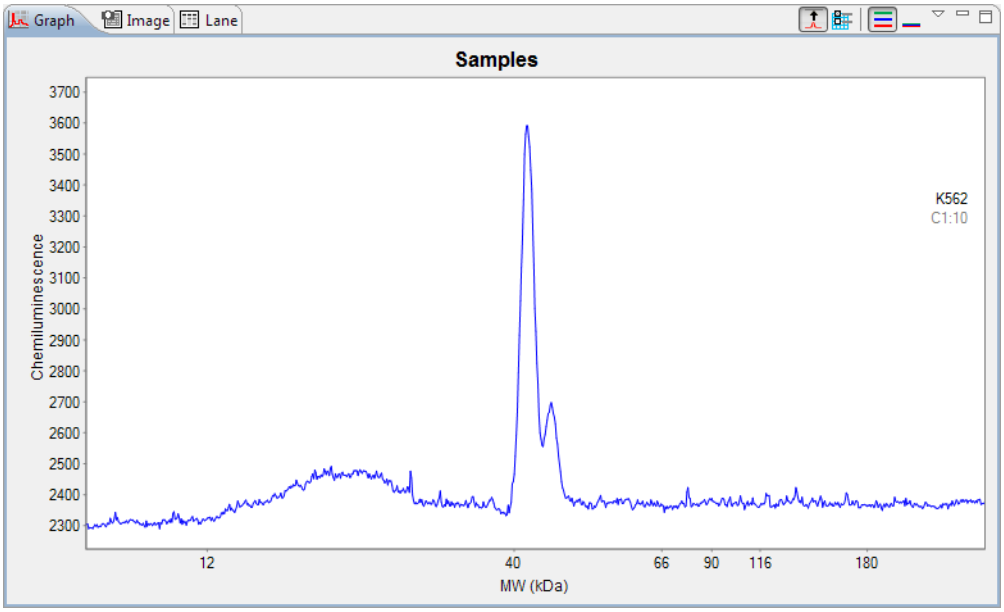
The graph view menu provides you with multiple options for changing what type of electropherogram data is displayed. To access the view menu, click the down arrow next to the graph pane toolbar:



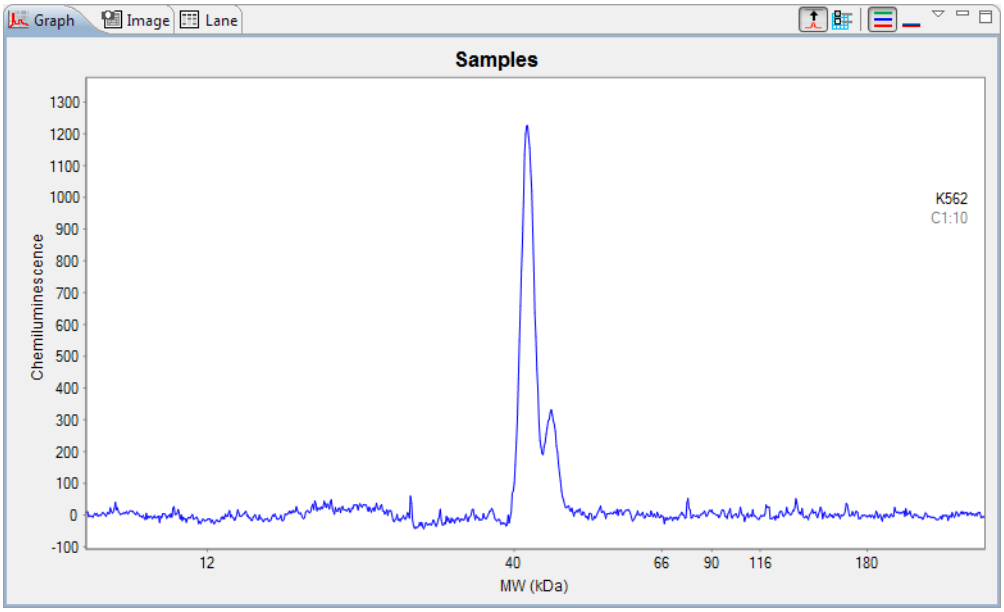
A check mark next to the menu option indicates it is currently selected. Multiple options can be selected at a time.

NOTE: Unless otherwise noted, graph view menu options are available for sample data only.

- **Sample** - Clicking this option will display raw, uncorrected sample data.

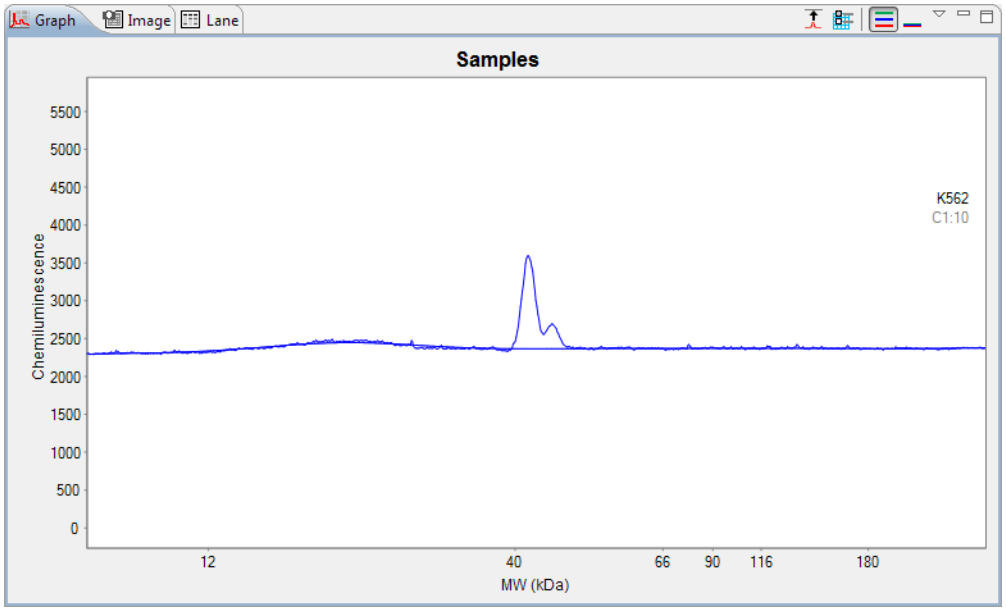


- **Sample Baseline Corrected** - Clicking this option will display sample data with the baseline subtracted (zeroed). This is the default view.



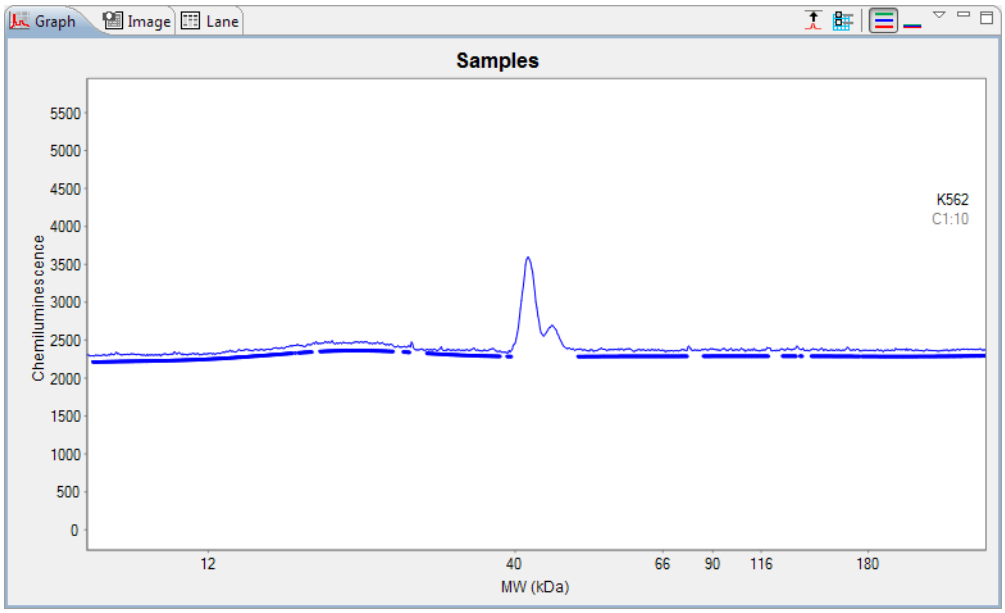
- **Baseline Fit** - Clicking this option will display the calculated baseline for the raw sample data. In the example that follows, both Baseline fit and Sample are selected.

NOTE: This option is selected automatically when Baseline Fit is selected in graph options.

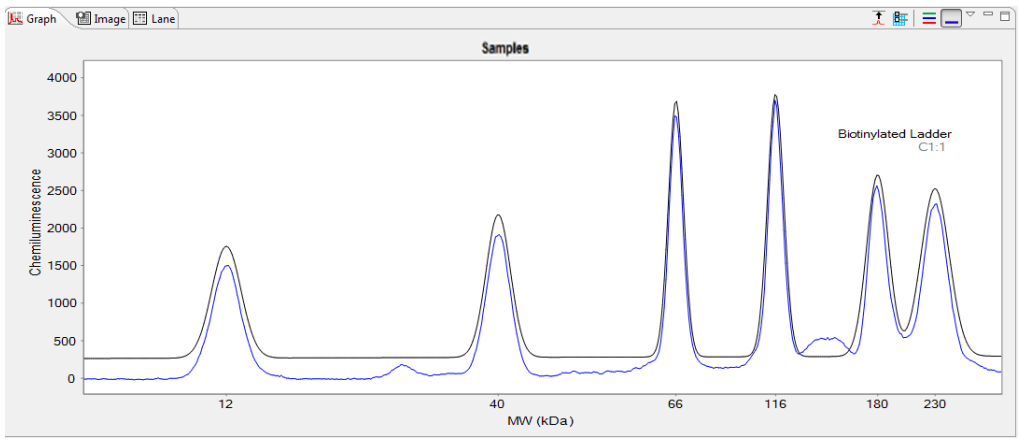


- **Baseline Points** - Clicking this option will display regions of the electropherogram considered to be at baseline. In the example that follows, both Baseline Points and Sample are selected.

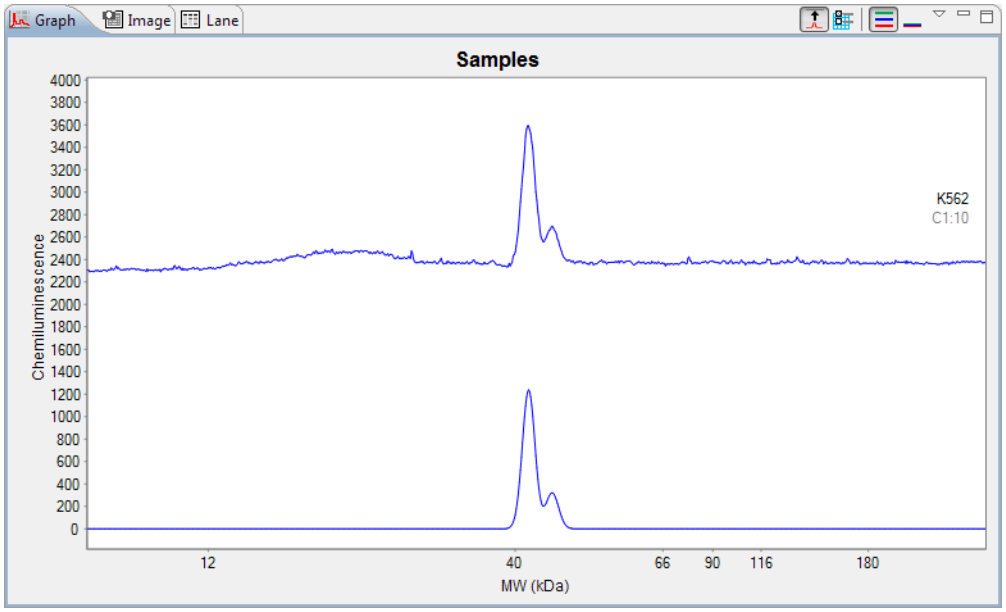
NOTE: This option is selected automatically when Baseline Fit is selected in graph options.



- **Fit** - Clicking this option will display the bounding envelope of the fitted peaks as calculated by the software for the raw sample data. In the example that follows, both Fit and Sample are selected.

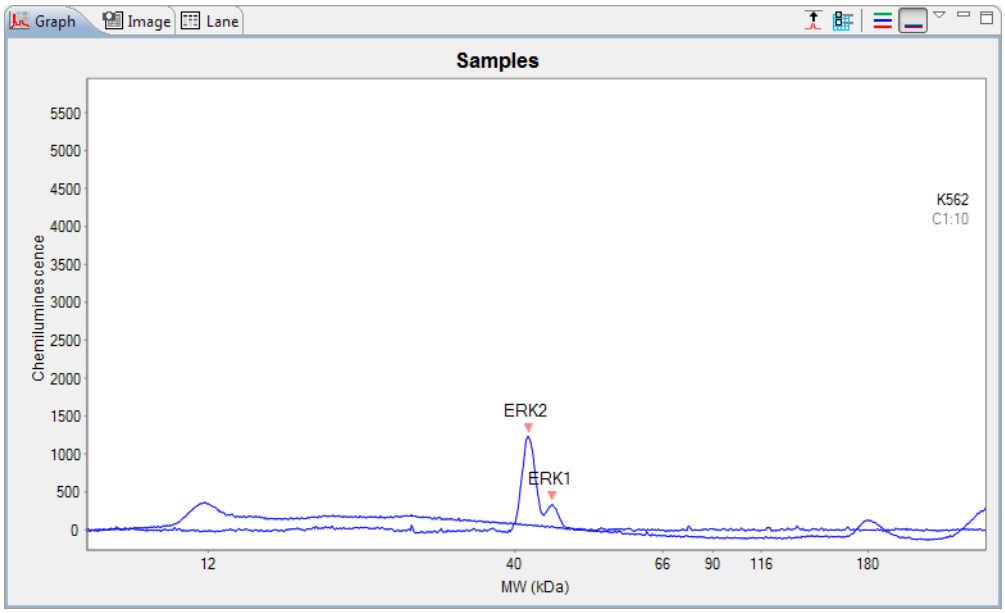


- **Fit Baseline Corrected** - Clicking this option will display the fitted peaks as calculated by the software for the sample baseline corrected data. In the example that follows, both Fit Baseline Corrected and Sample are selected, the fit plot is on the bottom.



NOTE: When viewing standards or registration data, this option is called Standards Fluorescence or Registration Fluorescence, respectively.

- **Standards** - Checking this box aligns the molecular weight of the raw standards data to the sample data and overlays both electropherograms in the graph pane.

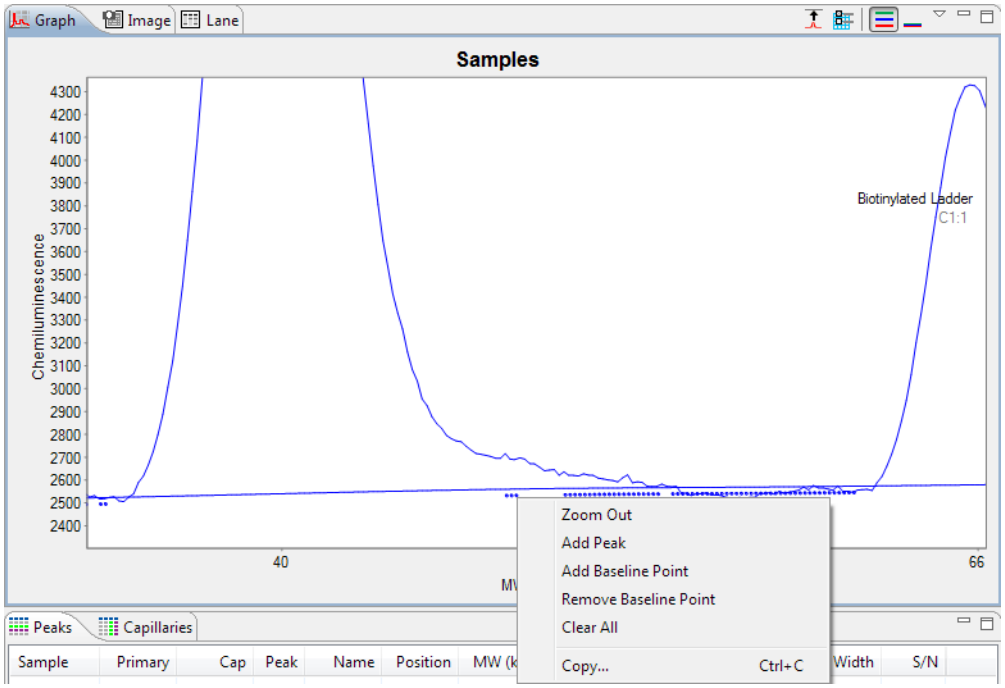


Adding and Removing Baseline Points

Points in the baseline can be added or removed as needed. To do this:

1. Click the **Graph Options** button in the graph pane toolbar and check **Baseline Fit**. This will display baseline points for the raw sample data.
2. Use the mouse to draw a box around the area you want to correct. This will zoom in on the area.

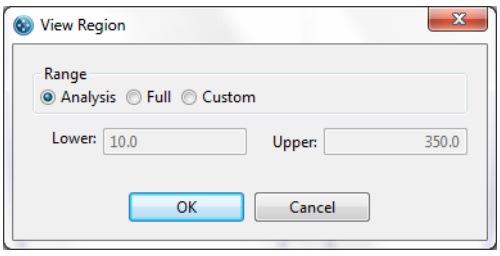
3. Right click a baseline point and click **Add Baseline Point** or **Remove Baseline Point**.



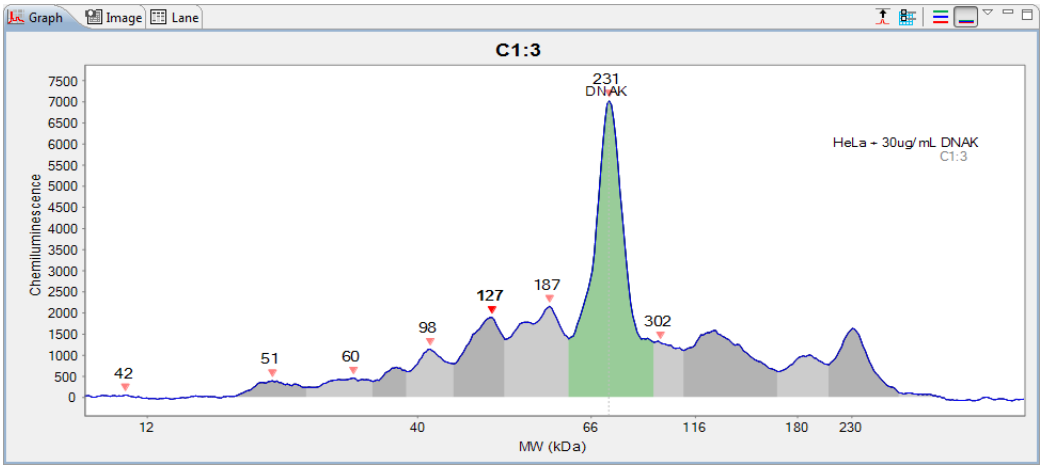
*NOTE: To clear the manual addition or removal of baseline points and go back to the original view of the data, right click in the electropherogram and click **Clear All**.*

Selecting the X-Axis Molecular Weight Range

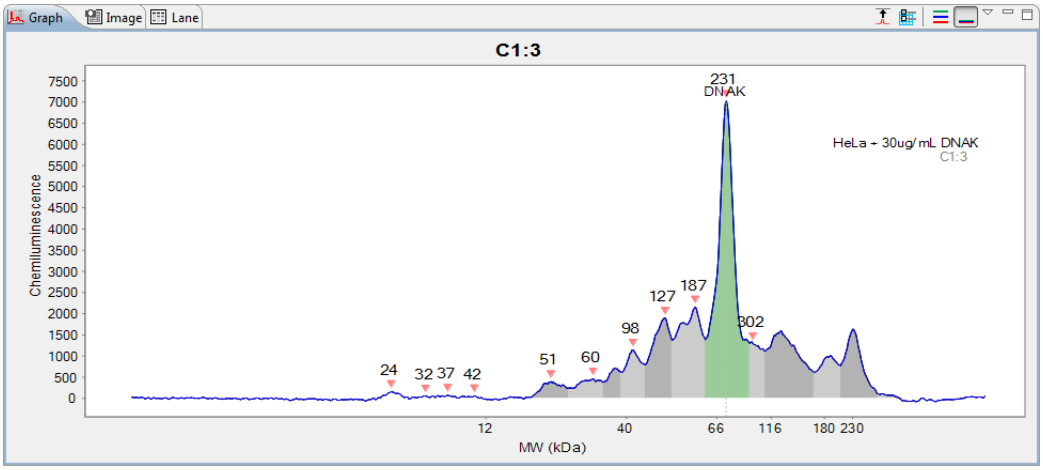
The molecular weight range used for the x-axis can be changed. To do this, Select **View** in the main menu and click **View Region**. The following pop-up window will display:



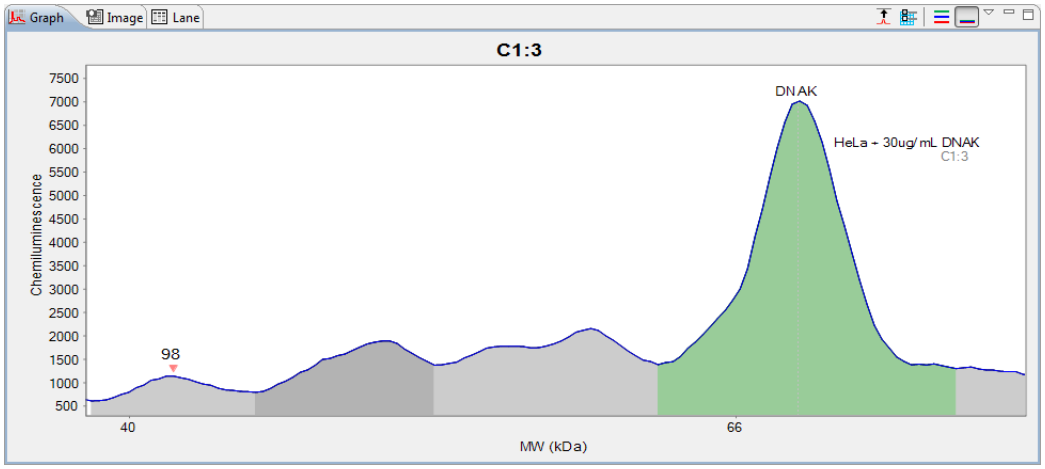
- Selecting **Analysis** will match the range of the run data that was selected in the Peak Fit Analysis Settings in both the electropherogram and virtual blot view.



- Selecting **Full** will display the range of the run data for the entire capillary in both the electropherogram and virtual blot view. (Insert View region Full)



- Selecting **Custom** allows you to manually enter the Lower and Upper ranges of the run data to be displayed in both the electropherogram and virtual blot view.(Insert View region Custom)



NOTE: You can change the default x-axis range that Compass for Simple Western uses. For more information, see "Normalization (Jess only)" on page 297.

Closing Run Files

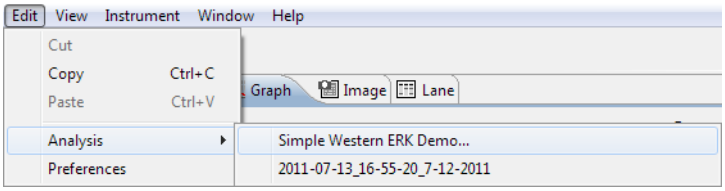
If more than one run file is open, you can close just one file or all the open files at the same time.

- **To close one of multiple open run files** - In the experiment pane, click on one of the sample rows in the file. Next click **File** from the main menu and click **Close**.
- **To close all open run files** - Select **File** from the main menu and click **Close All**.

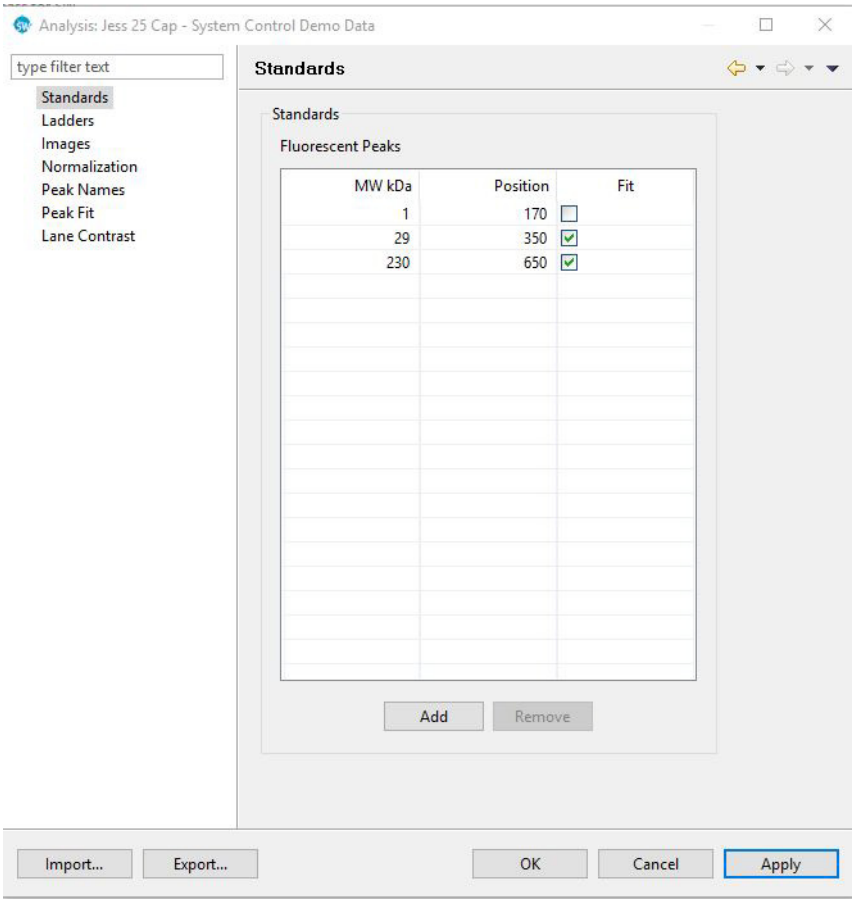
Analysis Settings Overview

Compass for Simple Western has a variety of analysis features and settings that you can modify as needed to enhance run data.

To access the analysis settings, select **Edit** in the main menu and click **Analysis**. If more than one run file is open, select the run file you want to view analysis settings for from the list:



The following screen will display:



To move between analysis pages in this window, click on an option in the list on the left. The following analysis settings can be user-customized in Compass for Simple Western:

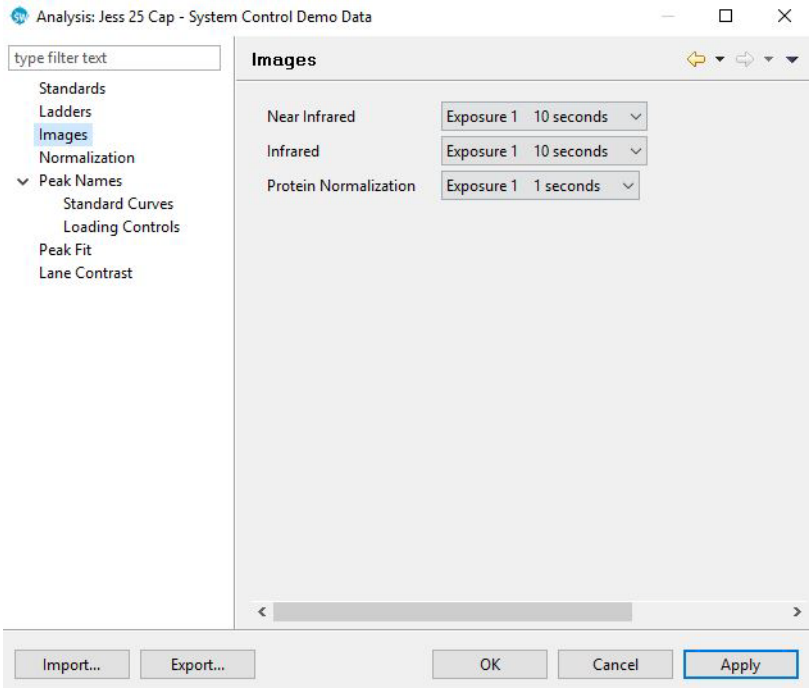
- **Standards** - Lets you customize the positions Compass for Simple Western uses to identify fluorescent standards and registration peaks.
- **Ladders** - Lets you customize the molecular weight Compass for Simple Western uses to identify molecular weight ladder(s), as well as change the capillary used.
- **Images** - Lets you view the chemiluminescent exposures taken during the run and view data for different exposures in the Analysis screen.
- **Normalization (Jess only)** - Lets you change the reference capillary used for protein normalization calculations and define region for normalization area.
- **Peak Names** - Lets you enter custom naming settings for sample proteins associated with specific blocking reagents, primary antibodies or attributes and have Compass for Simple Western automatically label the peaks in the run data.
- **Peak Fit** - Lets you customize peak fit settings for sample data.
- **Lane Contrast** - Lets you enter custom contrast settings for detection channels in Lane View.

Images Analysis Settings

The Images settings in the Analysis window lets you see what Detection Profile was selected, what exposures were taken during the run, and view data for different exposures in the Analysis screen. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Images** in the options list.

NOTES:
If you're using Compass for Simple Western v3.1 or higher, High Dynamic Range is the default setting.

If the run was performed with HDR unchecked, the data can still be analyzed with the improved HDR algorithm by selecting High Dynamic range from the drop down menu. For Jess/Wes runs, the increase in dynamic range will not be as noticeable since the substrate refresh was not performed.

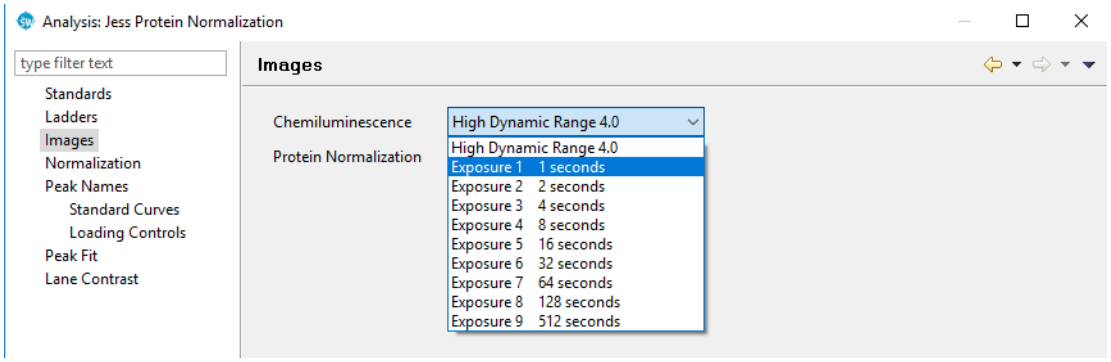


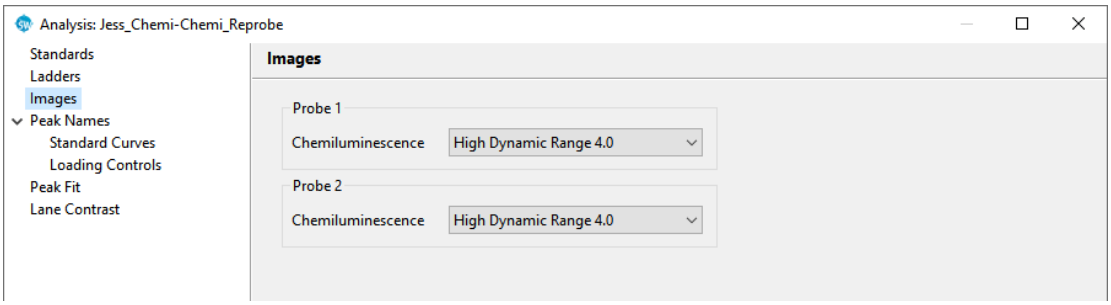
NOTE: The Images pane will only list the channels of data that are present in the run data file.

- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 331.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 331.
- Click **Apply** to see effects of changes before saving.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Exposure Settings

The exposure used for the sample data displayed in the Analysis screen is shown below for a standard chemiluminescent Immunoassay with Protein Normalization and for a RePlex Assay (Jess only) with two chemiluminescent Immunoassays:





Even when the exposures for HDR are set and can't be edited from the assay protocol, here you can choose to view each one separately including High Dynamic Range (HDR multi-image).

NOTE: Sally Sue and Peggy Sue run up to eight cycles (12 capillaries per cycle). The individual exposure setting selected for a specific cycle is applied for all 12 capillaries in that cycle unless selected for All cycles.

- **Multi-Image Analysis** - Sample data displayed in the Analysis screen is compiled from all exposures taken during the run. The Multi-Image analysis allows you to keep using exactly the same settings as before, which is calculated using the algorithm in versions of Compass for Simple Wester v3.0 or Compass v2.7 or earlier. This is provided for backward compatibility and direct comparison to older data sets, but the HDR method is preferred for all new analyses.
- **High Dynamic Range** - The HDR method uses information from multiple exposures to achieve good signal-to-noise for low protein concentration while simultaneously maintaining signal monotonicity at high protein concentration (*i.e.* avoiding "burnout").
- **High Dynamic Range 4.0** - Algorithm improvements were made to the original High Dynamic Range analysis in HDR 4.0 to handle a wider range of burnout cases. HDR 4.0 is less sensitive to the level at which burnout occurs. When burnout occurred at a low level, the classical HDR method had trouble picking the optimal exposure and interpolating between exposures.

- **Exposure #** - Sample data displayed in the Analysis screen is for this specific exposure only.

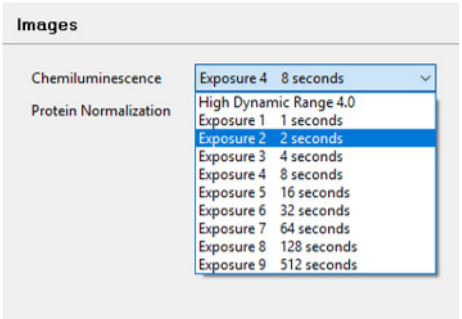
  To see the number of exposures and exposure times used for the run data, click the arrow in the drop down list next to **All**.

NOTE: The number of exposures taken and exposure times shown were specified in the assay protocol (Assay screen) prior to executing the run and cannot be modified.

Changing the Sample Data Exposure Displayed

The exposure used for the sample data displayed in the Analysis screen can be changed. To do this:

1. Select **Edit** in the main menu and click **Analysis**, then click **Images** in the options list.
2. Click the arrow in the drop down list next to **All** and select an exposure setting:



3. Click **Apply** and then click **OK** to save changes and exit. Sample data for the exposure selected will display in the Analysis screen.

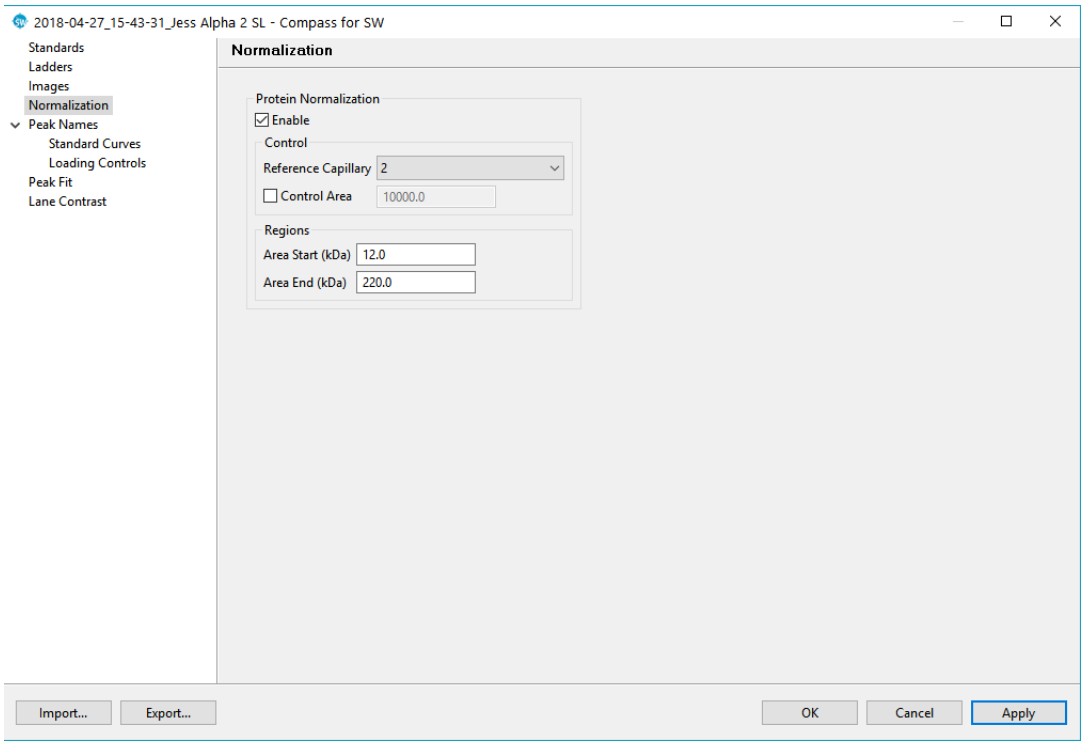
Normalization (Jess only)

The Normalization settings in the Analysis window lets you view and change the reference capillary used for protein normalization calculations, and adjust the control area and size region for determining the normalization area. Normalization settings are applicable when using the Protein Normalization Module or when enabling the Total Protein feature for RePlex Assays. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Normalization** in the options list.

NOTES:

For Jess runs that include Protein Normalization or RePlex Total Protein assays, the default Reference Capillary is set to 2.

The Enable checkbox is selected by default and must stay selected to normalize immunoassay targets.

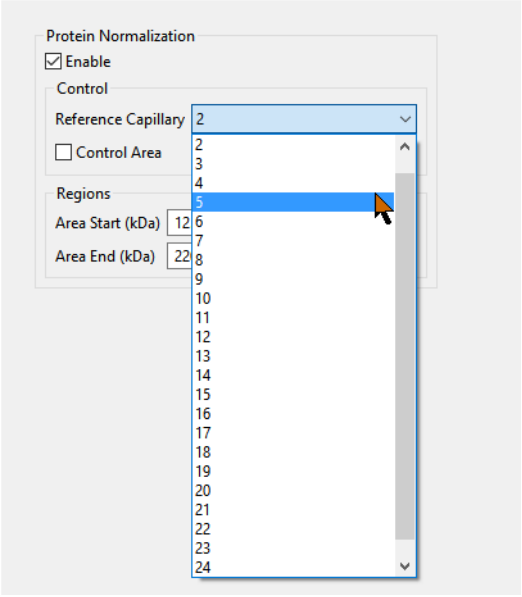


- Click **Import** to import an analysis settings file. This will be explained in more detail in “Importing Analysis Settings” on page 331.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in “Exporting Analysis Settings” on page 331.

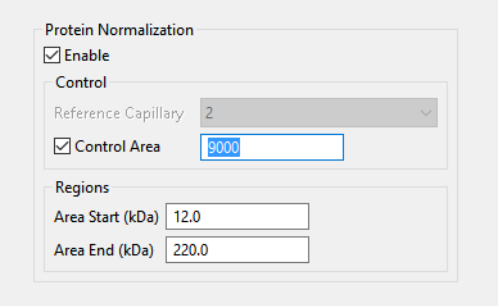
- Click **Apply** to see effects of changes before saving.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

NOTE: If you deselect the Enable checkbox in the Normalization settings, only Protein Normalization total protein values will display, immunoassay target data won't be normalized.

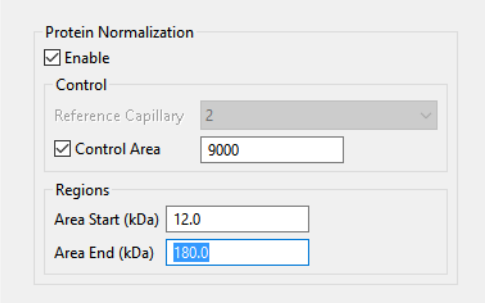
To use the Protein Normalization fluorescence signal or RePlex Total Protein chemiluminescent signal from a reference capillary for protein normalization: Click the Reference Capillary drop down menu and select the capillary you'd like to use as the normalization control to normalize all other capillaries against. ProteinSimple recommends choosing the capillary that had the highest sample concentration. The default reference capillary is 2.



To manually assign a reference signal for protein normalization: Select the **Control Area** box and enter a signal value. This setting is helpful when you want to use the average signal from more than one capillary for normalization.



To change the sizing region that the normalization total area is determined: Enter new kDa values in the **Area Start** and **Area End** boxes.

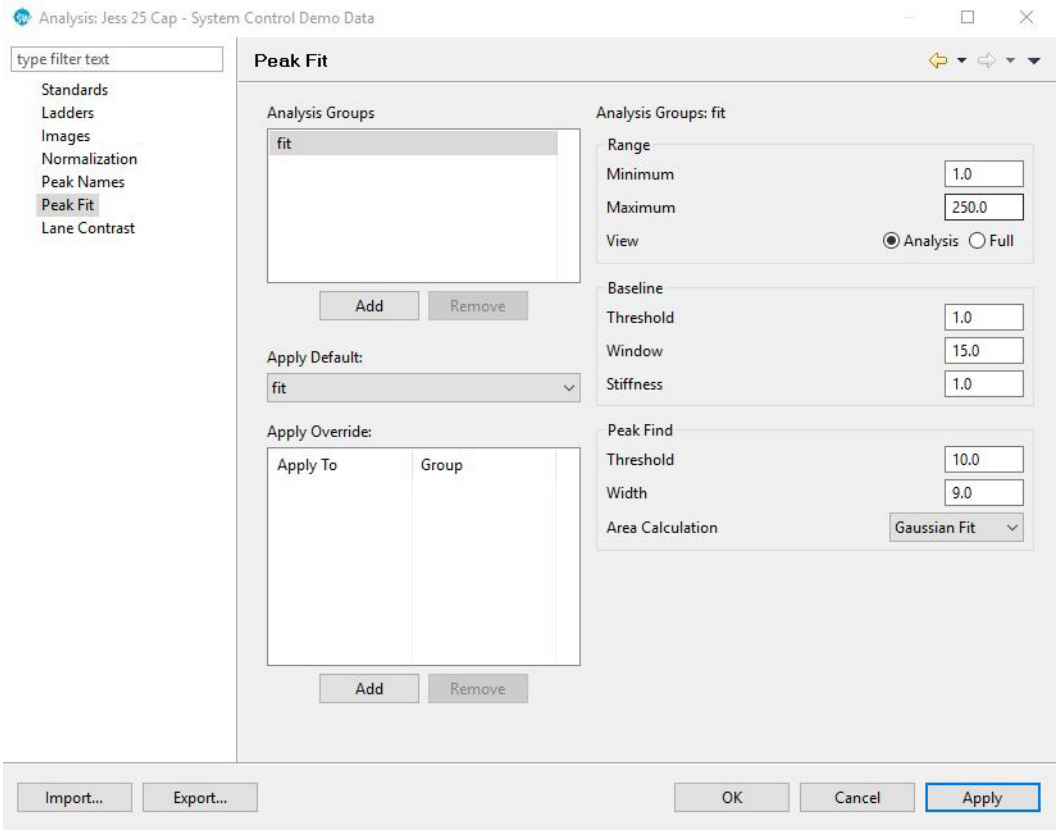


Click **Apply** and then click **OK** to save changes and exit. Updated sample data using the new normalization settings will display in the Analysis screen.

Peak Fit Analysis Settings

The peak fit analysis settings page lets you view and change peak fit settings for sample data. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 331.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 331.
- Click **Apply** and then click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Range Settings

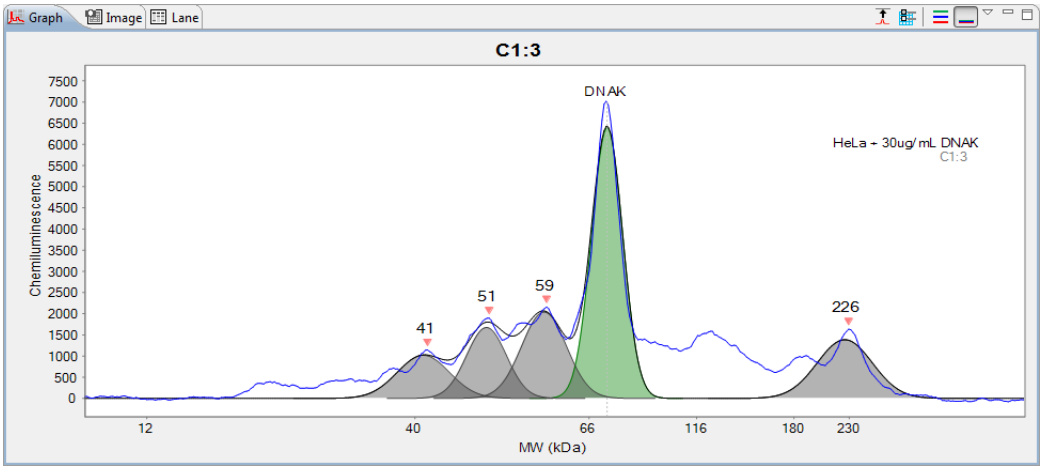
- **Minimum** - The molecular weight value (in kDa) below which peaks will not be identified. This value will also be used as the default lower MW range for the data displayed in the electropherogram and virtual blot.
- **Maximum**: The molecular weight value (in kDa) above which peaks will not be identified. This value will also be used as the default upper MW range for the data displayed in the electropherogram and virtual blot.

Baseline Settings

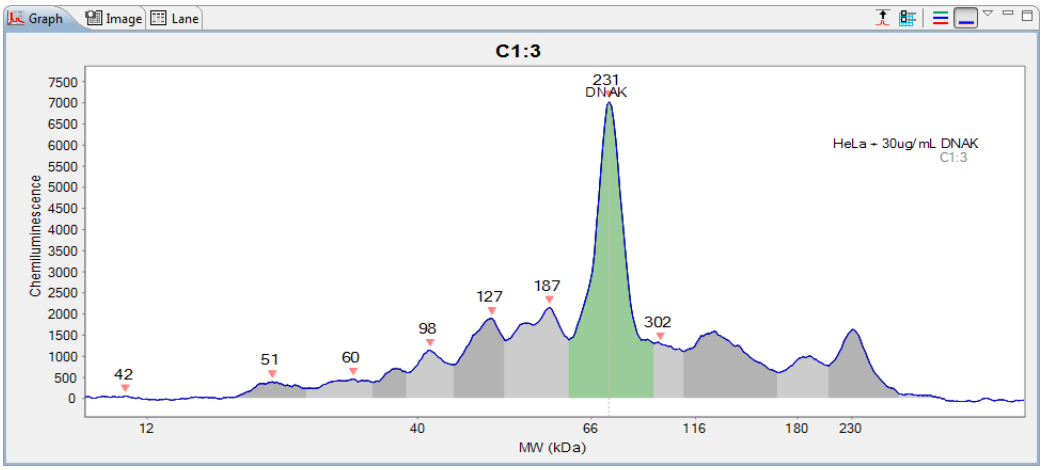
- **Threshold** - The variance, or roughness, in a baseline data segment below which a point will be called part of the baseline.
- **Window** - How long baseline data segments are expected to be in pixels. Shorter segments will allow the baseline to follow plateau sections of the signal.
- **Stiffness** - The amount the baseline is allowed to vary from a straight line. Settings between 0.1 and 1.0 make the baseline fit closer to a straight line. Settings from 1.0 to 10.0 will make the baseline fit follow the data more closely.

Peak Find Settings

- **Threshold** - The minimum signal to noise ratio required for a peak to be identified. A setting of 1.0 will detect many peaks, a setting of 10.0 will detect fewer peaks.
- **Width** - The approximate peak width (at full width half max) in pixels used to detect peaks. The minimum value for this setting is 3.0. Larger widths help to eliminate the detection of shoulder peaks and noise peaks.
- **Area Calculation** - Two fits are used, either Gaussian Fit or Dropped Lines. These settings can be changed before or after the run is finished.
    - For Immunoassays, peak area is calculated using Gaussian distribution by default:



- For Total Protein Assays, peak area is calculated using Dropped Lines. This type of area calculation is also often called the perpendicular drop method. This method is preferred when peaks overlap or are close to each other as they are in Total Protein Assays. This method draws two vertical lines from the left and right bounds of the peak down to the x-axis and then measures the total area bounded by the signal curve, the x-axis (y=0 line), and the two vertical lines.



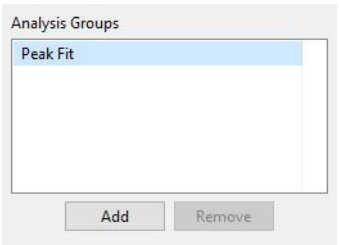
Peak Fit Analysis Settings Groups

Peak fit settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:
We recommend using the Compass for Simple Western default values for peak fit analysis settings. These settings are included in the default Peak Fit group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 331.

Peak fit groups are displayed in the analysis settings box:

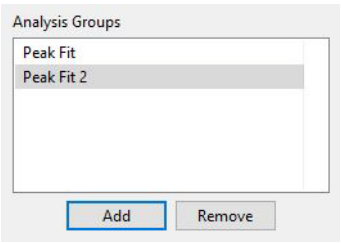


The Peak Fit group shown contains the Compass for Simple Western default analysis settings. You can make changes to this group and create new groups.

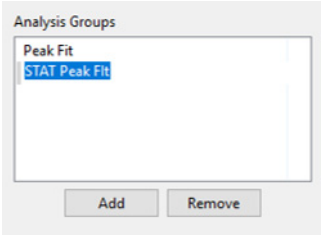
To view settings for a group, click on the group name in the analysis settings box.

Creating a New Peak Fit Group

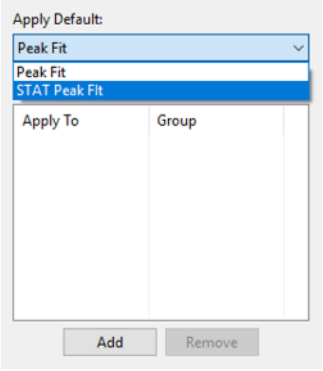
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



3. Click on the new group and enter a new name.



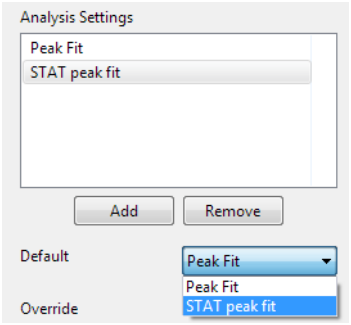
4. Modify range, baseline or peak find parameters as needed.
5. To use the new group as the default peak fit settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Peak fit settings in the new group will then be applied to the run data.



6. Click **Apply** and then click **OK** to save changes.

Changing the Default Peak Fit Group

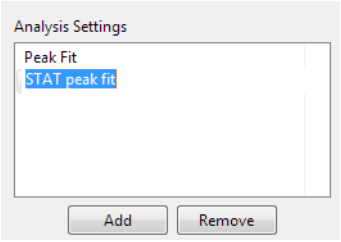
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click the arrow in the drop down list next to Default, then click a new default group from the list.



3. Click **Apply** and then click **OK** to save changes. Peak fit settings in the group selected will be applied to the run data.

Modifying a Peak Fit Group

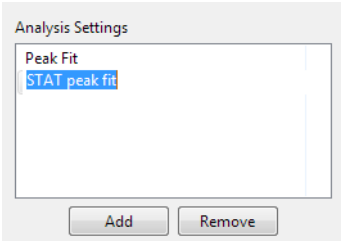
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Modify range, baseline or peak find parameters as needed.
4. Click **Apply** and then click **OK** to save changes. The new peak fit settings will be applied to the run data.

Deleting a Peak Fit Group

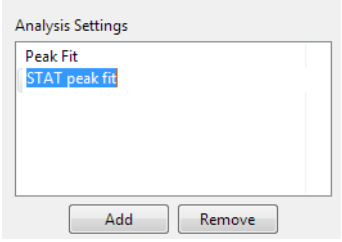
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



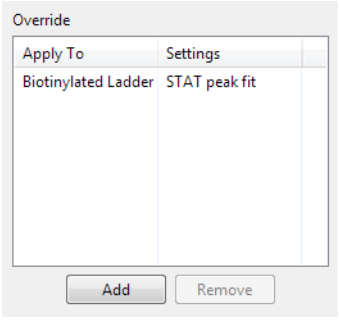
3. Click **OK** to save changes.

Applying Peak Fit Groups to Specific Run Data

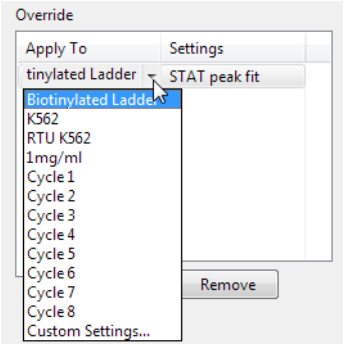
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of peak fit groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

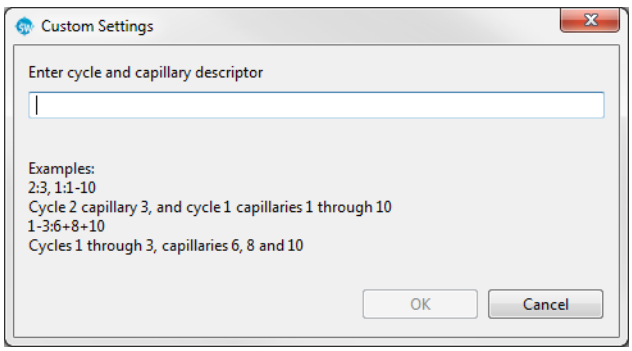


4. Click the cell in the **Apply To** column, then click the down arrow.

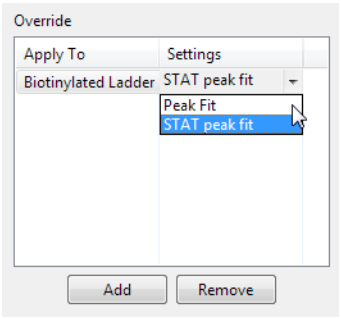


5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

   - **Sample names** - All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a sample name to apply group settings to all capillaries that use this sample name in the run file.
   - **Attributes** - All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
   - **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
   - **Probe (RePlex Assays only)** - Select a probe to apply Peak Fit group settings to all capillaries within that probe.
   - **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:



6. If you need to change the peak fit group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.

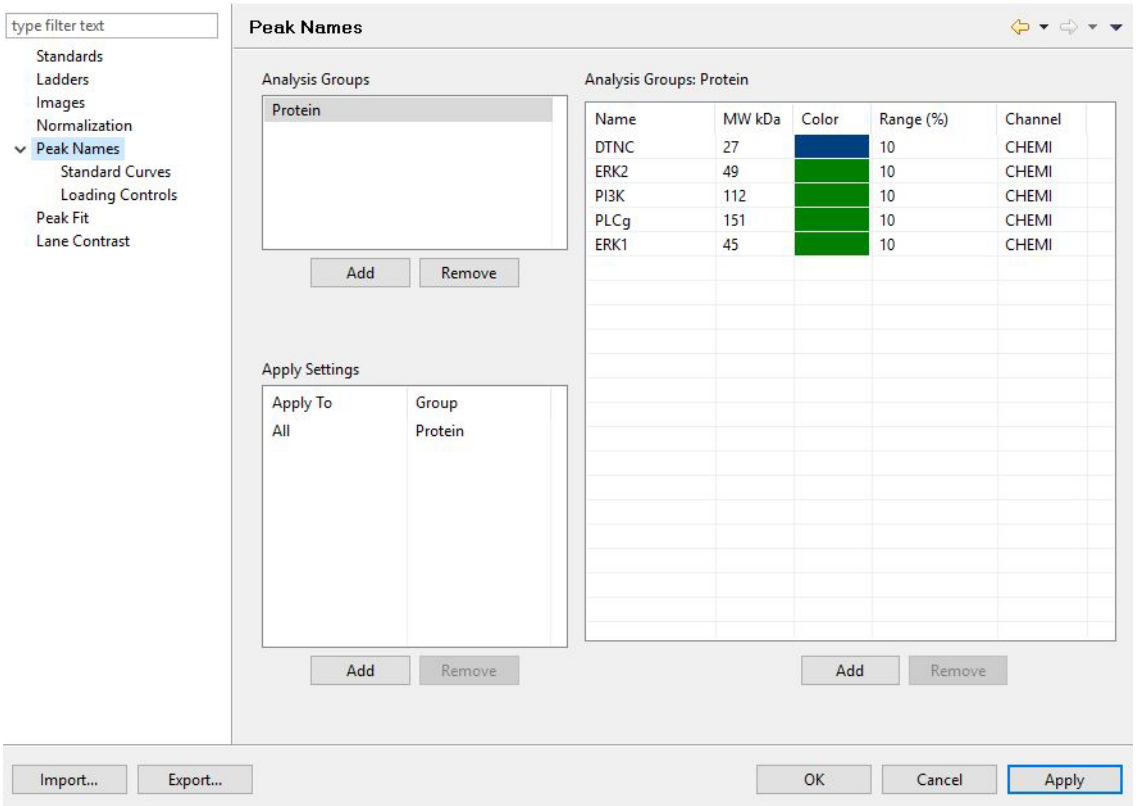


7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **Apply** and then click **OK** to save changes.

Peak Names Settings

The peak names analysis settings page lets you enter custom naming settings for sample proteins. Compass for Simple Western can automatically label sample peaks in the run data associated with specific blocking reagent names, primary antibody names or attributes. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



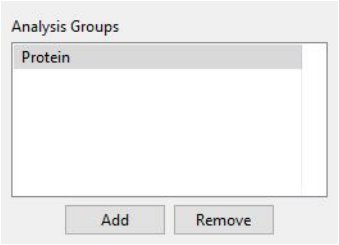
- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 331.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 331.
- Click **Apply** and then click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Peak Names Analysis Groups

Peak name settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, blocking reagent names or primary antibody names and attributes in the run data.

NOTE: Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 331.

Peak name groups are displayed in the analysis groups box:

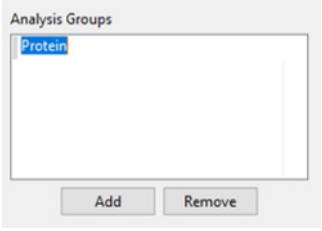


The Peak Names group shown is a Compass for Simple Western template group. You can make changes to this group and create new groups.

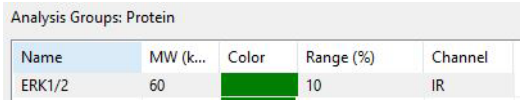
To view settings for a group, click on the group name in the analysis settings box.

Creating a Peak Names Group

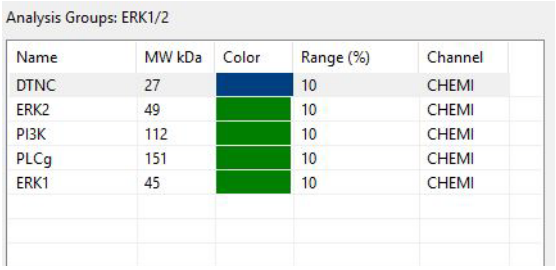
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the **Protein** template group in the analysis group box.



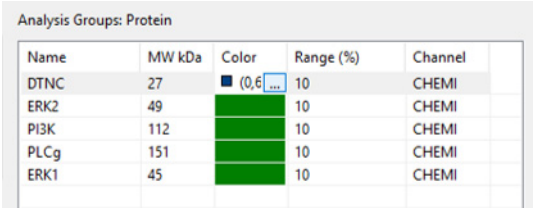
3. Enter a new name for the group.
4. Click in the first cell in the **Name** column in the analysis groups peak table.
5. Enter a sample protein name associated with the primary antibody used in the run.



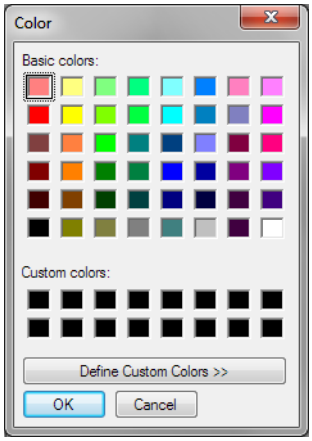
6. Click in the first cell in the **MW** column.
7. Enter the molecular weight (in kDa) for the sample protein.



8. Click in the first cell in the **Color** column, then click the button.

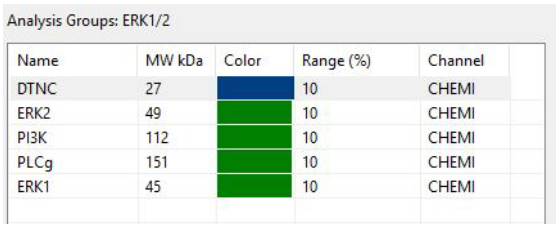


The color selection box will display:

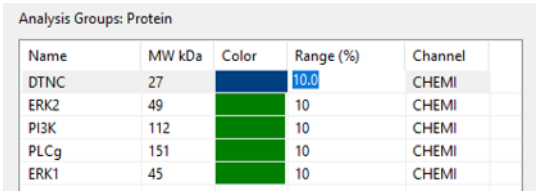


The color selected will be used to identify the sample protein peak in the peaks and capillaries table in the Analysis screen.

9. Click a color or define a custom color and click **OK**. The color selection will update in the table:



10. Click in the first cell in the **Range (%)** column.

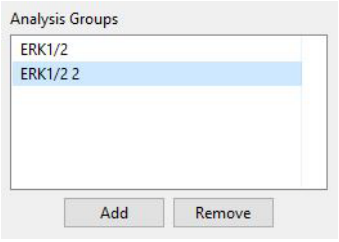


11. Enter a range window for the MW entered. Compass for Simple Western will automatically name peaks found within this percent of the molecular weight. For example, if the molecular weight entered is 40 kDa and a 10% range is used, all peaks between 36 and 44 kDa will be identified with this peak name.

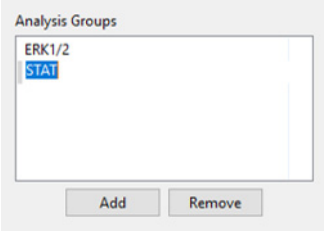
NOTE: The reported molecular weight for sample proteins detected by an Immunoassay or a Total Protein Assay in Compass for Simple Western may vary from apparent molecular weights using other techniques and based on sample and assay conditions.

Adding Peak Names Groups

1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click **Add** under the Analysis Groups box. A new group will be created:



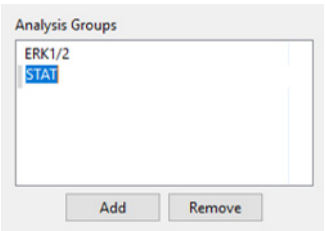
3. Click on the new group and enter a new name.



4. Enter information in the Analysis Groups peak table as described in "Creating a Peak Names Group" on page 310.
5. Click **Apply** and then click **OK** to save changes.

Modifying a Peak Names Group

1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the group in the Analysis Groups box you want to modify.



3. Change the information in the Analysis Groups peak table as described in "Creating a Peak Names Group" on page 310.
4. Click **Apply** and then click **OK** to save changes.

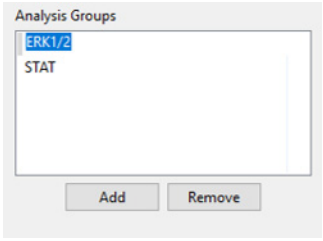
Deleting a Peak Names Group

1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the group in the Analysis Groups box you want to delete and click **Remove**.
3. Click **Apply** and then click **OK** to save changes.

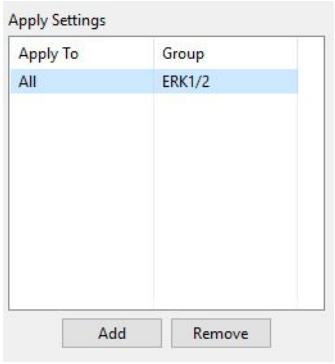
Applying Peak Names Groups to Run Data

1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.

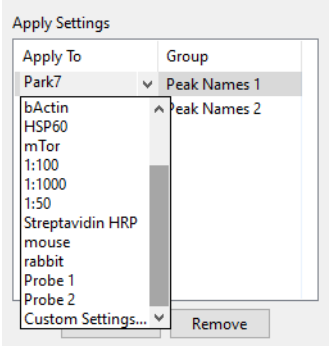
2. Click on the group in the Analysis Groups box you want to apply to specific run data.



3. Application of peak names groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created.



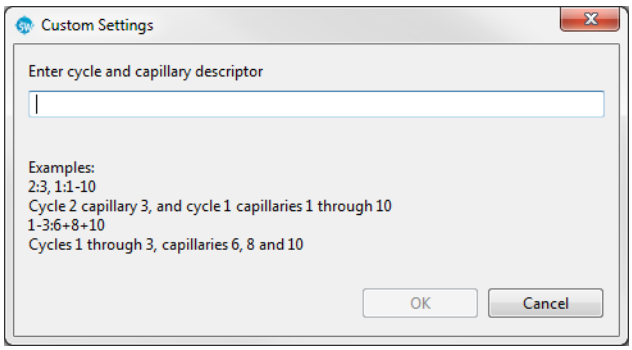
4. Click the cell in the **Apply To** column, then click the down arrow.



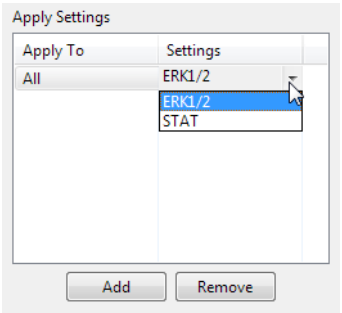
5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

   - **All** - When this option is selected, peak names group settings will be applied to all capillaries in the run file.

- **Blocking reagent** - When this option is selected, group settings will be applied to all capillaries that use this reagent name in the run file.
- **Antibody names** - All primary and secondary antibody names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Primary will be shown. Select a name to apply group settings to all capillaries that use the primary antibody name in the run file.
- **Attributes** - All primary and secondary antibody attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
- **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
- **Probe (RePlex Assays only)** - Select a probe to apply group settings to all capillaries within that probe.
- **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:

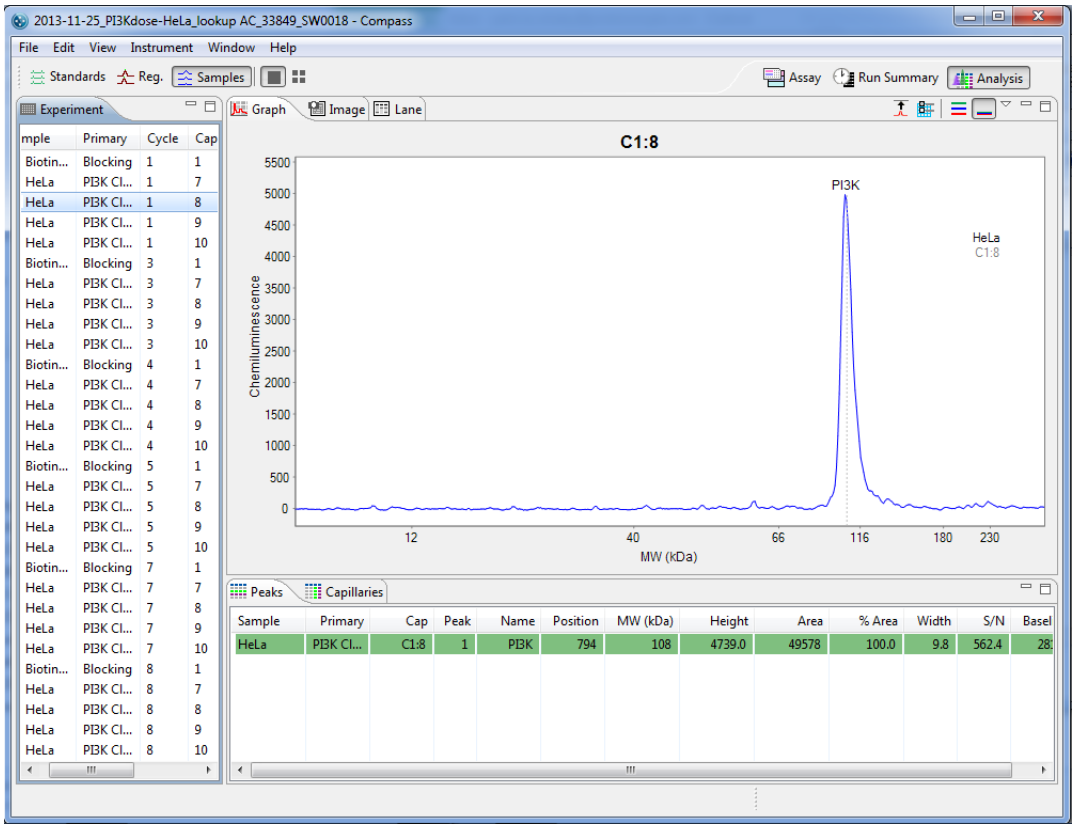


6. If you need to change the peak names group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.



7. Repeat the previous steps to apply other groups to specific run data.

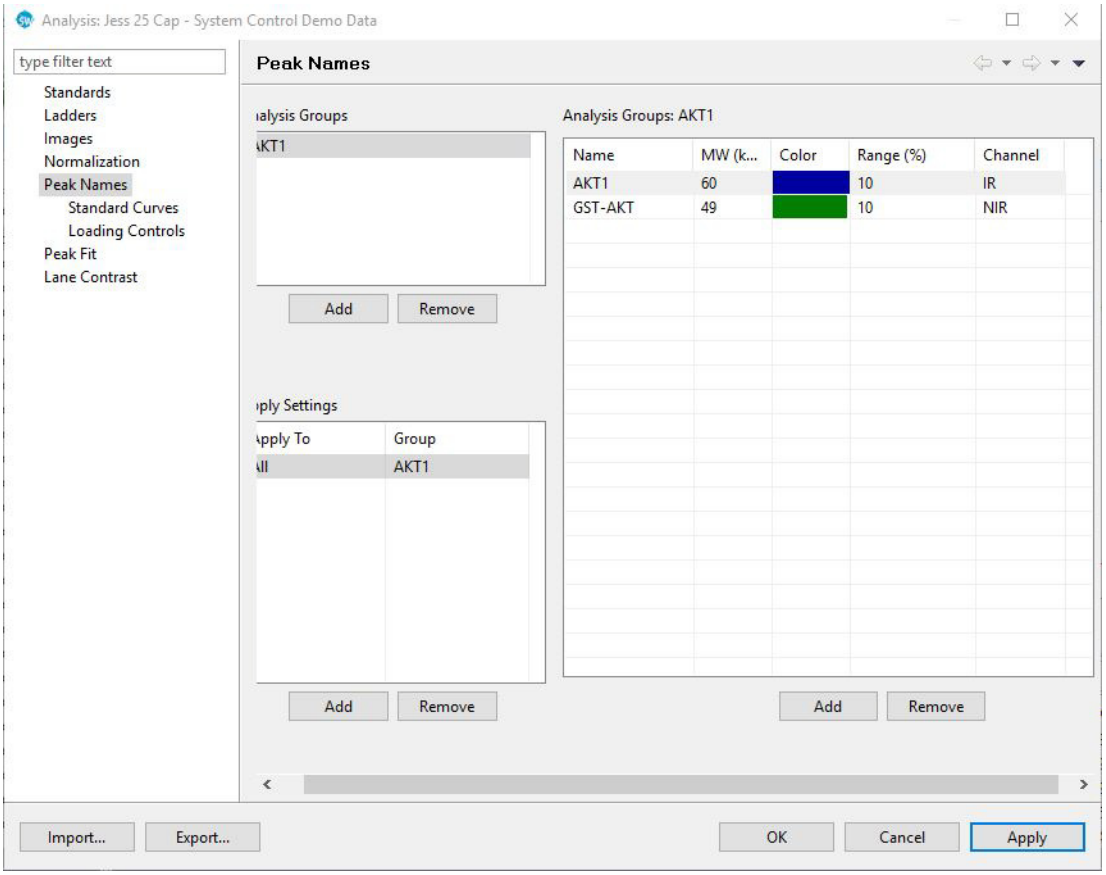
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **Apply** and then click **OK** to save changes. Named peaks will be identified with a peak name label in the electropherogram and virtual blot, and will be color coded in the peaks and capillaries tables:



Standard Curve Settings

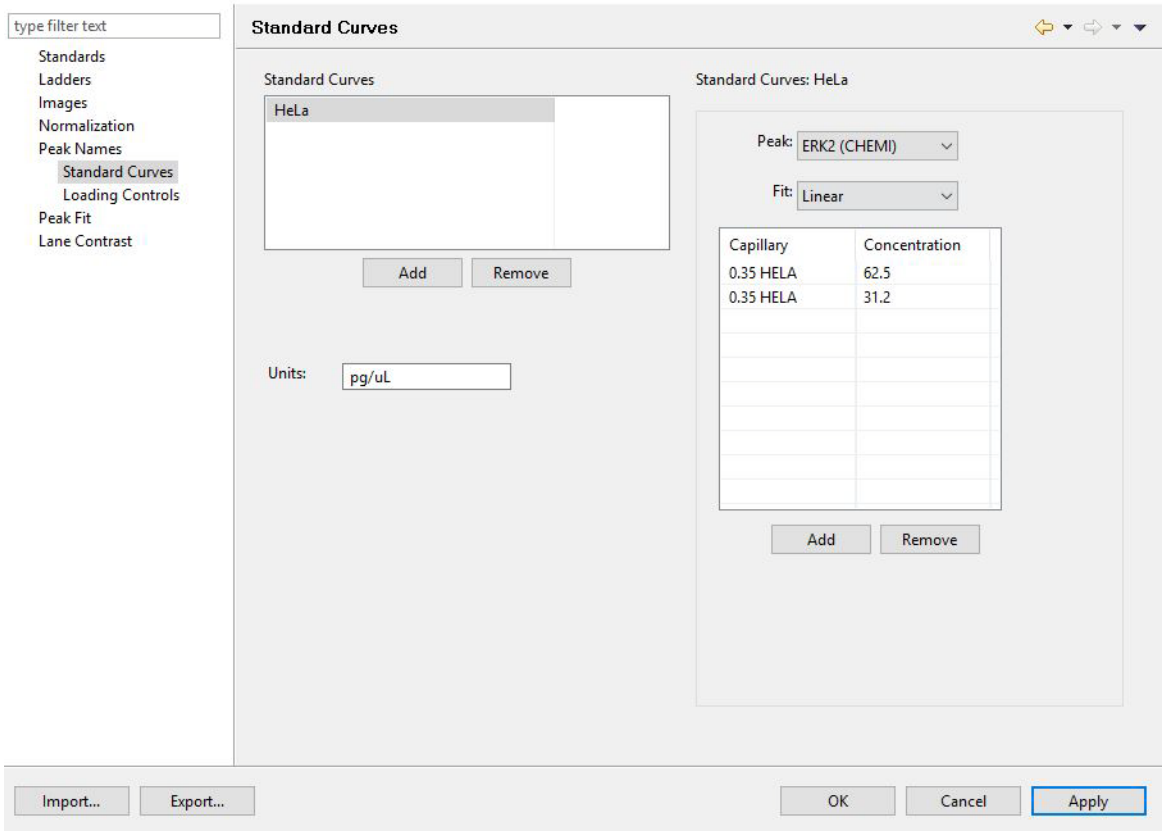
To use a standard curve to quantitate the concentration of a target protein detected either by an Immunoassay or a Total Protein Assay, first create peak names groups as described earlier for your standard curve protein and the target protein. In the example below, GST-AKT1 is the standard curve protein and AKT1 is the target protein.

NOTE: Analysis settings are run file-specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 331.

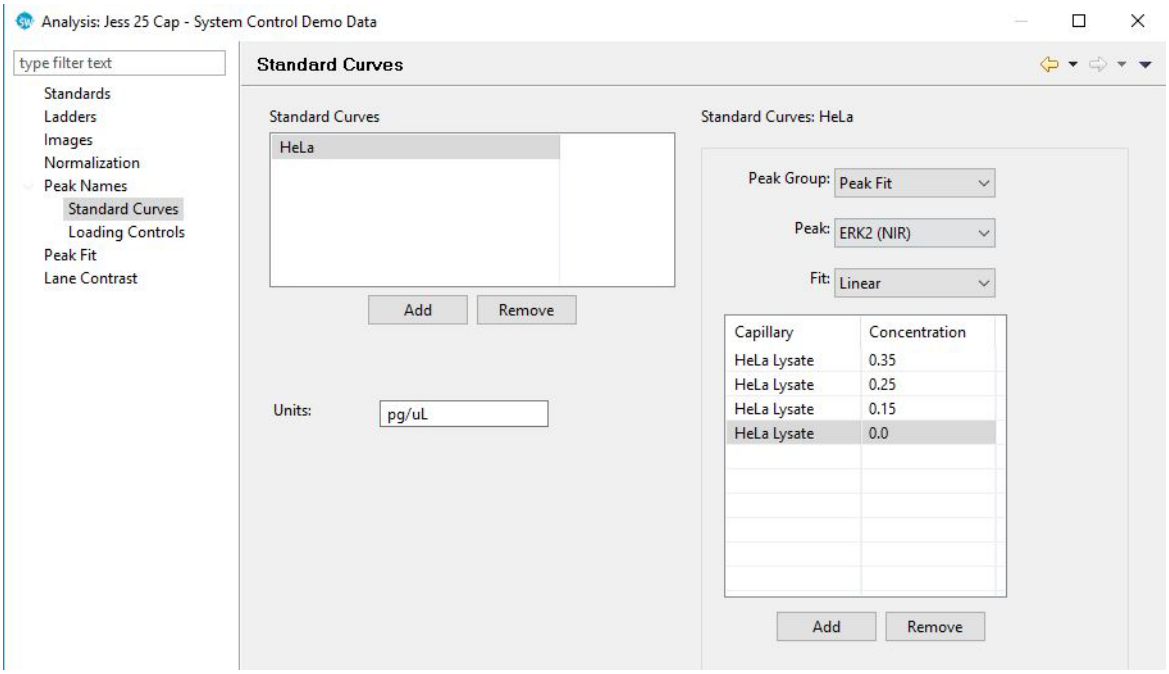


To set up a standard curve:

1. Click **Standard Curves** from the Peak Name submenu options.



2. From the Peak drop down list, select the peak name for your standard curve protein.
3. Choose either a **Linear** or **4 Parameter (4PL)** curve fit from the **Fit** drop down list.
4. To add another concentration, click **Add** under the Standard Curve table.
5. Repeat the previous steps to enter information for other concentrations. In the following example, four concentrations were entered:

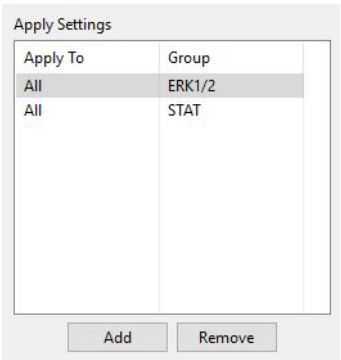


To remove a concentration, select its row and click **Remove**.

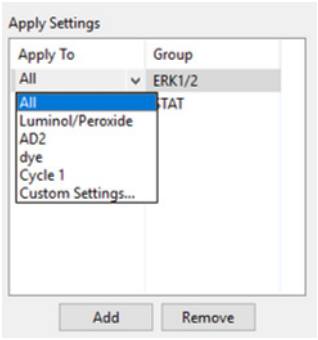
6. Enter the concentration units in the box (for example, pg/µL).
7. Click **OK** to save changes.

Applying Peak Names Groups to Run Standard Curve

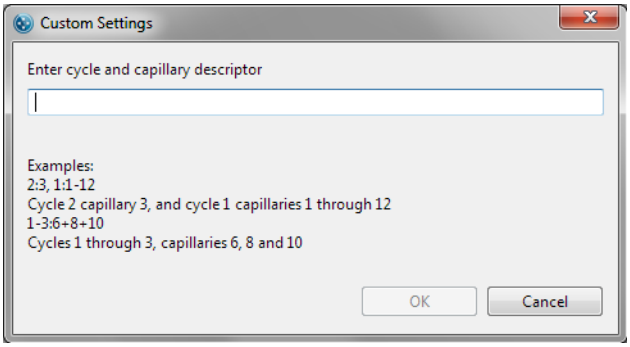
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



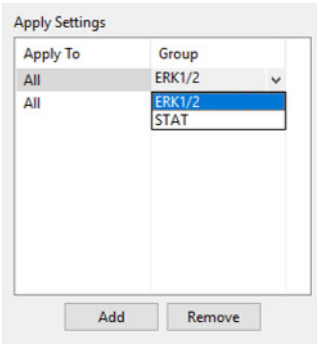
3. Application of peak names groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created.
4. Click the cell in the Apply To column, then click the down arrow.



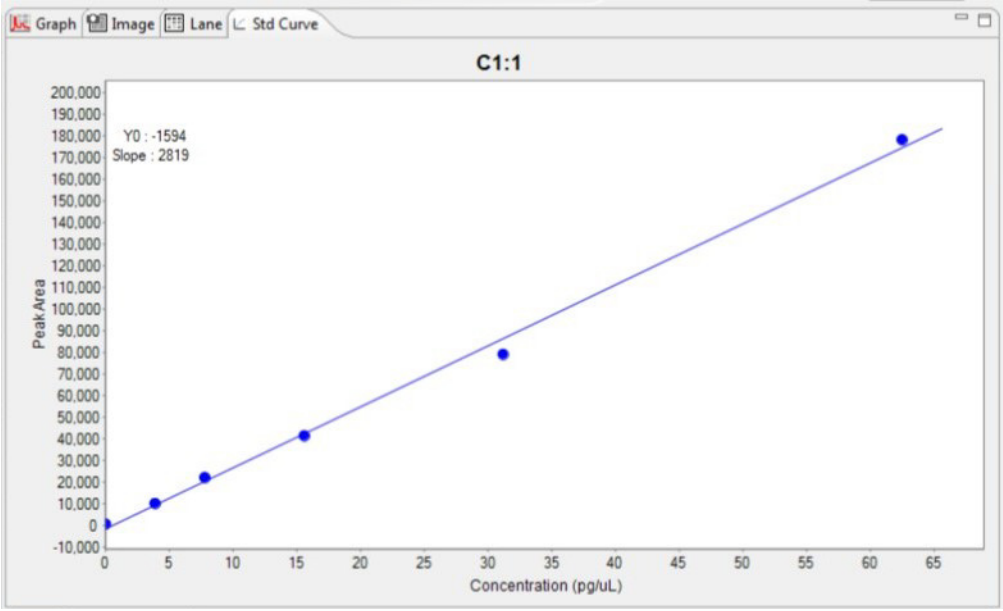
5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:
   a. **All** - When this option is selected, peak names group settings will be applied to all capillaries in the run file.
   b. **Blocking reagent** - When this option is selected, group settings will be applied to all capillaries that use this reagent name in the run file.
   c. **Primary antibody names** - All primary antibody names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Primary will be shown. Select a name to apply group settings to all capillaries that use the primary antibody name in the run file.
   d. **Attributes** - All primary antibody attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
   e. **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
   f. **Probe (RePlex Assays only)** - Select a probe to apply group settings to all capillaries within that probe.
   g. **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:



6. If you need to change the peak names group used for a data set, click the cell in the Settings column and click the down arrow. Select a group from the drop down list.



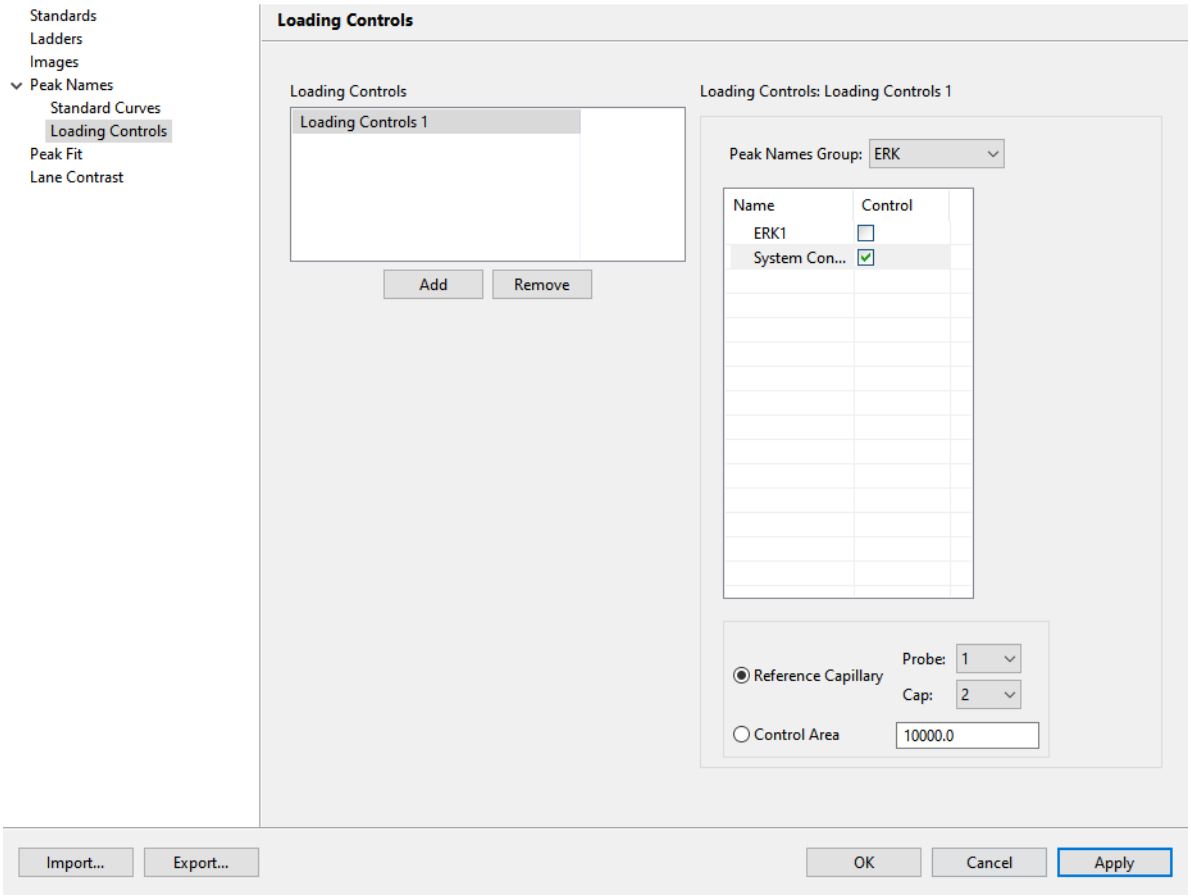
7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **Apply** and then click **OK** to save changes. The curve fit will be visible on the Std Curve tab and the concentration of the proteins will appear in the Peaks table:



System or Loading Control Settings

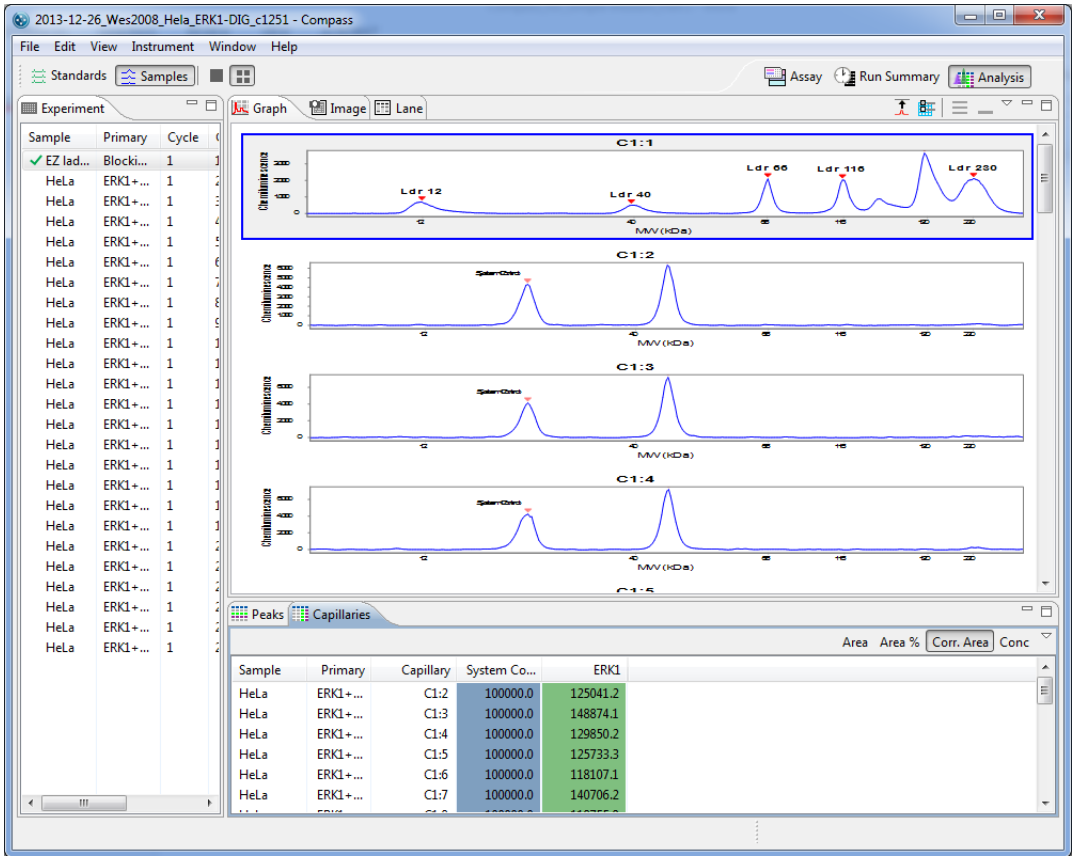
It is possible to use a system or loading control protein to normalize data between capillaries, between runs and between instruments. First create peak names groups as described earlier for your control protein and the target protein. In the example below, System Control is the control protein and ERK1 is the target protein.

NOTE: Analysis settings are run file-specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 331.



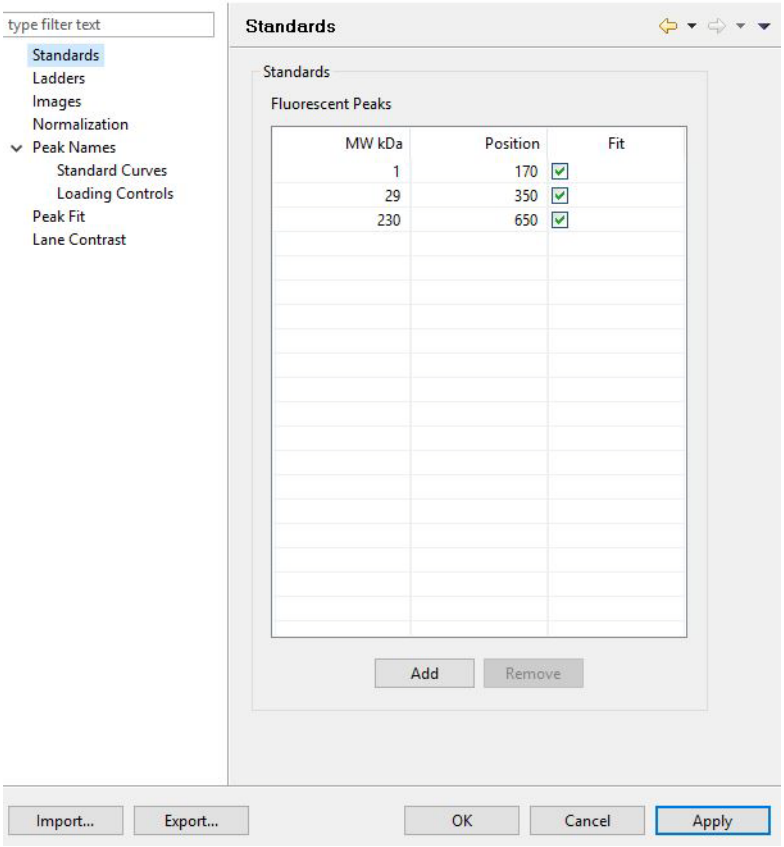
To identify the control protein:

1. Click **Peak Names** and select **Loading Controls**.
2. Select a Reference Capillary from the pull-down menu. The peak area of this capillary will be used to normalize the peak area of the named peaks. For RePlex assays (Jess only), the user can specify which probe to use as the reference, Probe 1 or Probe 2. All named peaks in both Probe 1 and Probe 2 will be normalized to the reference.
3. To manually assign the Control Area, click the box and enter the peak area of your control protein.
4. Click **Apply** and then click **OK** to save changes. Compass for Simple Western will automatically normalize the peak area of the target protein against the control protein. The corrected area appears in the Corr. Area column in the Peaks table and in the Capillaries table:



Standards Settings

The standards analysis settings page lets you view and change the position for fluorescent standards and set the registration peaks. To access this setting, select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list. The default standard settings for the 12-230 kDa, 66-440 kDa and 2-40 kDa size ranges are shown in the following example:



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 331.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 331.
- Click **Apply** and then click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Standards Analysis Settings Groups

Standards settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

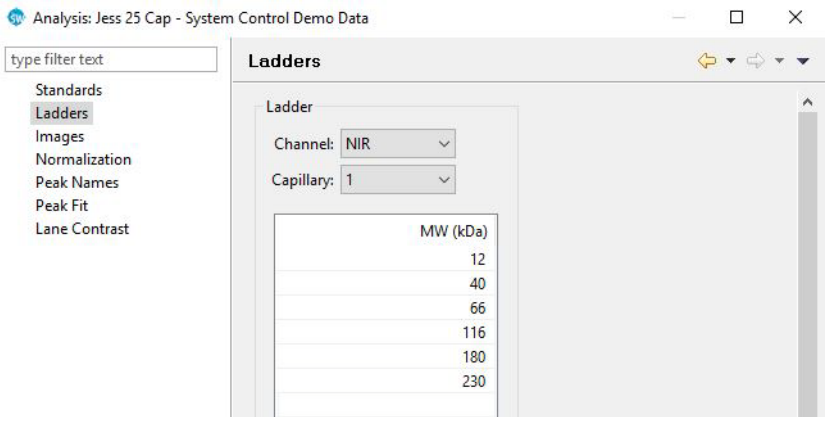
Ladders Settings

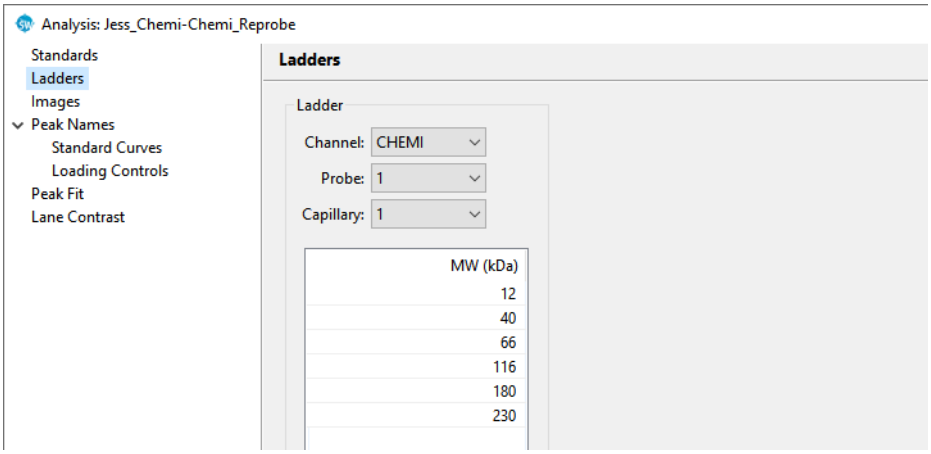
The ladders analysis settings page lets you view and change the molecular weight and position for the ladder and change the capillary and channel/probe (Jess only) used for it. To access these settings, select Edit in the main menu and click Analysis, then click Ladders in the options list.

For RePlex Assays (Jess only), select the ladder in Probe 1 or Probe 2, depending on the assay setup.

NOTE: Probe selection is not available with RePlex Assays that include Total Protein.

The default standard settings for the 12-230 kDa size range is shown in the following example for a standard Immunoassay and a RePlex Assay (Jess only):





NOTES:
Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.

We recommend using the Compass for Simple Western default values for standards and ladders analysis settings. These settings are included in the default Standards and ladders group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 331.

Changing the Capillary Used for the Ladder

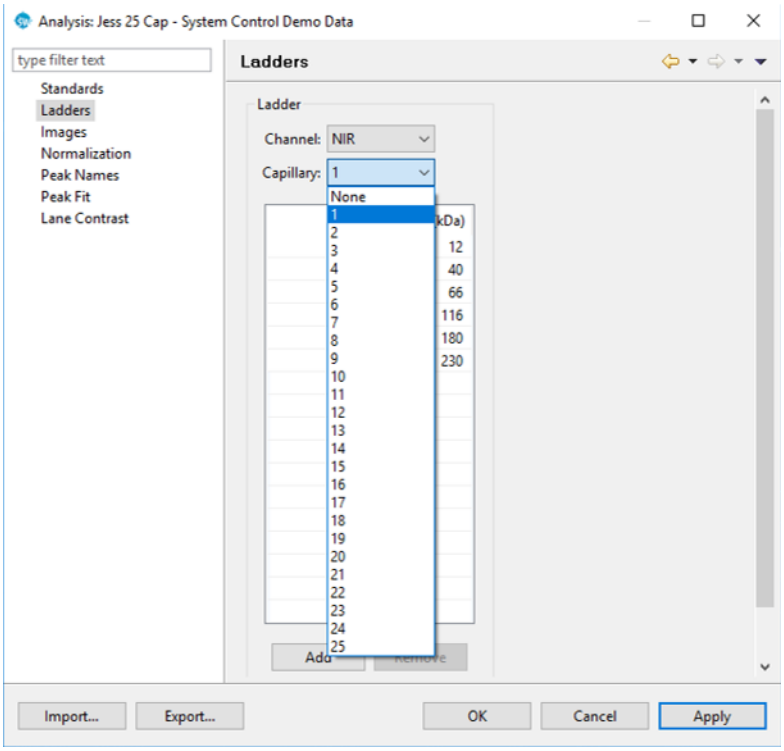
Known ladders are used to calculate the molecular weights of unknown sample proteins. As noted in each of the Master Kit Product Inserts, we strongly recommend that you use capillary 1 for the ladder. However, you can change the ladder capillary as needed, or opt to not use a ladder at all.

NOTES:
When the ladder capillary is set to none, fluorescent standards information is used to calculate sample protein molecular weight instead of the ladder.

The reported molecular weight for sample proteins detected by an Immunoassay or a Total Protein Assay in Compass for Simple Western may vary from apparent molecular weights using other techniques and based on sample and assay conditions.

To change the ladder capillary:

1. Select **Edit** in the main menu and click **Analysis**, then click **Ladders** in the options list.
2. Click the arrow in the drop down list next to Capillary, then click a capillary number or none from the list.



Compass for Simple Western will use the data in the selected capillary to recalculate molecular weights for sample proteins in the run data using the information in the ladder table. If none is selected, Compass for Simple Western will instead use the information in the fluorescent peaks table (fluorescent standards) to calculate molecular weight for sample proteins.

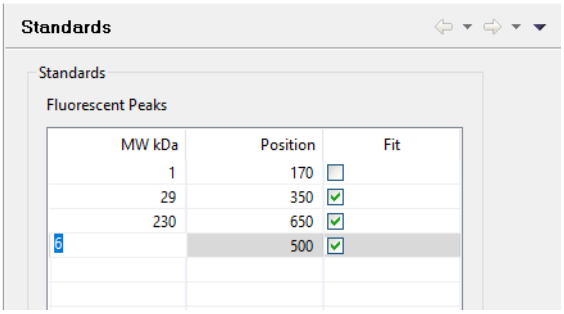
NOTES:
When the ladder capillary is set to none, the ladder table becomes inactive and cannot be modified.

The reported molecular weight for sample proteins detected by an Immunoassay or a Total Protein Assay in Compass for Simple Western may vary from apparent molecular weights based on other techniques as well as sample and assay conditions.

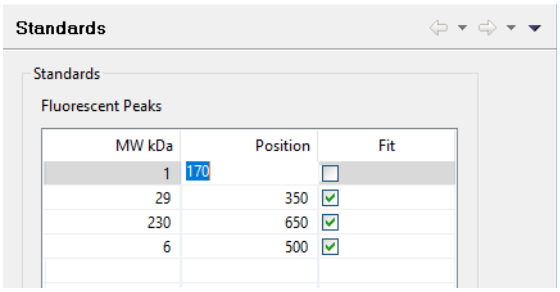
Creating a New Standard

1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click **Add** under the analysis settings box. A new MW will be added.

3. Click in the first cell in the **MW** column in the Fluorescent Peaks table.
4. Enter the molecular weight (in kDa) for the fluorescent standard.



5. Click in the first cell in the **Position** column.
6. Enter the position of the fluorescent standard peak.



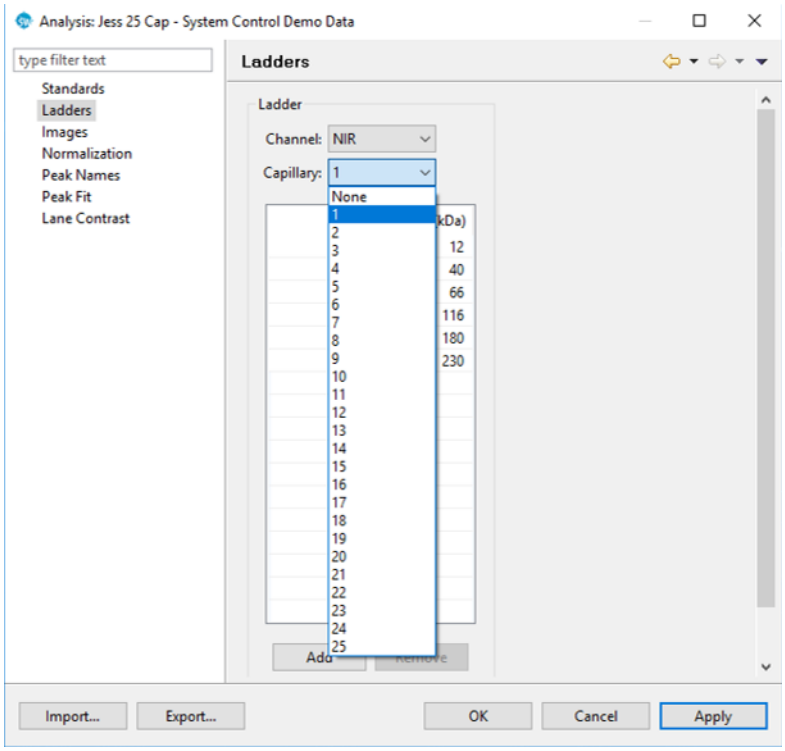
NOTE: Standards peak positions are relative to each other. Only the difference in their position is used to help identify the standard peaks. When entering standard peak information for the first time, review the standards data in the Analysis screen to find the correct peak position.

---

7. Repeat the steps above for the remaining standards in the table.
8. Select which standards should be used for molecular weight determination of sample proteins by clicking the checkbox in the **Fit** column.

Creating a New Ladder

1. Select **Edit** in the main menu and click **Analysis**, then click **Ladders** in the options list.
2. Click the arrow in the drop down list next to Capillary, then click a capillary number or none from the list. Capillary 1 is typically used for the ladder.



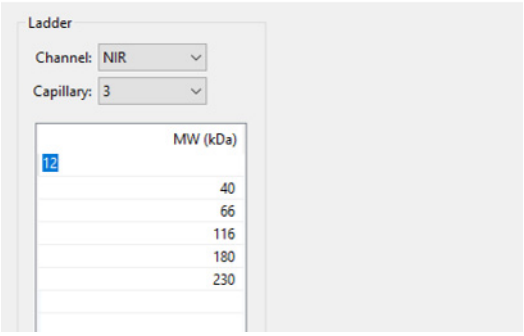
Compass for Simple Western will use the data in the selected capillary to calculate the molecular weights for sample proteins using the information in the ladder table. If none is selected, Compass for Simple Western will instead use the information in the fluorescent peaks table (fluorescent standards) to calculate molecular weight for sample proteins.

NOTES:
When the ladder capillary is set to none, the ladder table becomes inactive and cannot be modified.

The reported molecular weight for sample proteins detected by an Immunoassay or a Total Protein Assay in Compass for Simple Western may vary from apparent molecular weights using other techniques and based on sample and assay conditions.

---

3. If a ladder capillary was selected, click in the first cell in the **MW** column in the ladder table. Enter the molecular weight (in kDa) for the ladder standard.



4. Repeat the steps above for the remaining ladder MW values in the table.
5. Click **Apply** and then click **OK** to save changes.

Importing and Exporting Analysis Settings

The analysis settings in a run file can be exported as a separate file. This allows the same analysis settings to be imported into other assays or run files at a later time, rather than having to re-enter settings manually.

Importing Analysis Settings

NOTE: Importing an analysis settings file populates the settings in all Analysis pages.

---

1. Open the run file or assay you want to import analysis settings to.
2. Select **Edit** in the main menu and click **Default Analysis** (Assay screen) or **Analysis** (Analysis screen).
3. Click **Import** on any page.
4. Select a settings file (*.settings) and click **OK**. The imported settings will display in all analysis pages.

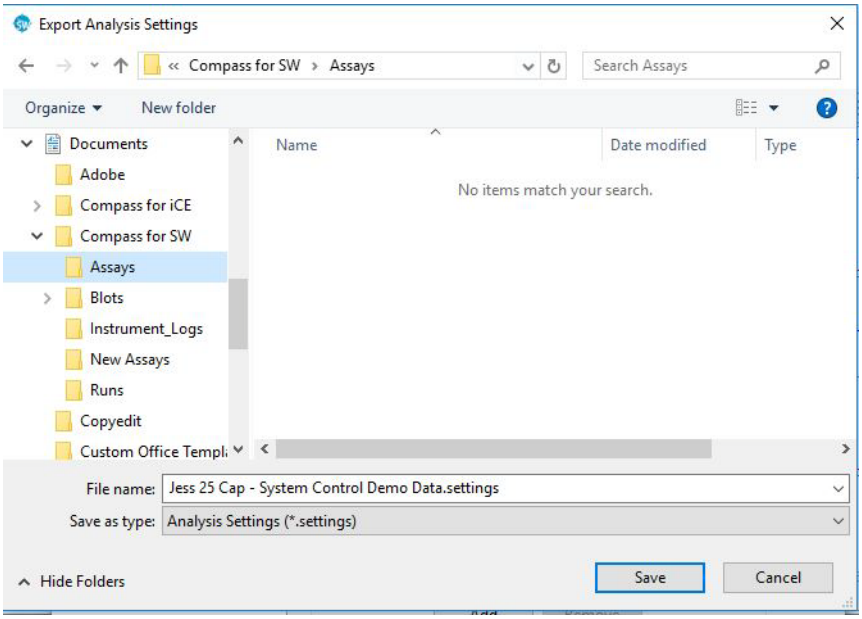
Exporting Analysis Settings

NOTE: Exporting an analysis settings file exports the settings in all Analysis pages.

---

1. Open the run file or assay you want to export analysis settings from.
2. Select **Edit** in the main menu and click **Default Analysis** (Assay screen) or **Analysis** (Analysis screen).

3. Click **Export** on any page. The following window displays:



4. The default directory is Compass/Assays. Change the directory if needed.
5. Enter a file name and click **Save**. The settings will be saved as a *.settings file.

Chapter 10:

Charge Assay Data Analysis

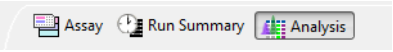
Chapter Overview

- Analysis Screen Overview
- Opening Run Files
- How Run Data is Displayed in the Analysis Screen
- Viewing Run Data
- Run Data Notifications and Warnings
- Checking Your Results
- Group Statistics
- Copying Data Views and Results Tables
- Exporting Run Files
- Running Reports
- Changing Sample Protein Identification
- Changing the Virtual Blot View
- Changing the Electropherogram View
- Closing Run Files
- Analysis Settings Overview
- Advanced Analysis Settings
- Images Analysis Settings
- Peak Fit Analysis Settings

- Peak Names Settings
- Standards Settings
- Importing and Exporting Analysis Settings

Analysis Screen Overview

The Analysis screen is used to view run data including electropherograms, capillary images, lane view data and tabulated results. Any post-run analysis is also done in this screen. To access this screen, click **Analysis** in the screen tab:



Analysis Screen Panes

The Analysis screen has six panes, each displays the following data for up to 96 capillaries per experimental run:

- **Experiment** - Lists the assay protocol steps and assay template information.
- **Graph** - Displays electropherogram data for immunodetected sample proteins, fluorescent standards or capillary registrations.
- **Image** - Displays a 12-capillary image of the separated immunodetected sample proteins, fluorescent standards or capillary registrations.
- **Lane** - Displays data for immunodetected sample proteins, fluorescent standards or capillary registrations as bands in individual lanes. This virtual blot-like image is similar to traditional blot results.
- **Peaks** - Lists the tabulated results for immunodetected sample proteins and information for identified fluorescent standards and capillary registrations.
- **Capillaries** - Displays a list of the immunodetected sample proteins Compass for Simple Western named automatically using the user-defined peak name analysis parameters.

Sample	Primary	Cycle
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1

Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N	Basel
High pho...	ERK1/2	C1:1	1	ppERK1	499	5.468	394.8	6337	58.7	0.0464	517.9	55(
High pho...	ERK1/2	C1:1	2	pERK1	583	5.735	153.9	1411	13.1	0.0265	321.1	55(
High pho...	ERK1/2	C1:1	3	ppERK2	607	5.805	711.2	5864	62.4	0.0239	1538.7	55(
High pho...	ERK1/2	C1:1	4	ERK1	639	5.904	401.0	3054	28.3	0.0220	879.5	55(
High pho...	ERK1/2	C1:1	5	pERK2	693	6.129	75.8	701	7.5	0.0469	159.2	54(
High pho...	ERK1/2	C1:1	6	ERK2	783	6.581	219.5	2831	30.1	0.0562	352.9	54(

NOTE: The reported pI for immunodetected sample proteins in Compass for Simple Western may vary slightly from predicted pIs based on sample, buffer and assay conditions.

Software Menus Active in the Analysis Screen

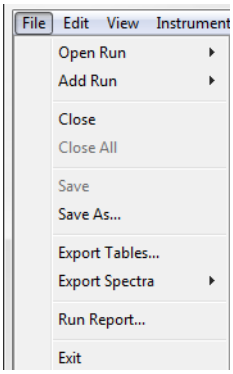
The following software menus are available:

- File
- Edit
- View
- Instrument (when Compass for Simple Western is connected to Peggy Sue)
- Window
- Help

The File, Edit and View menu options specific to the Analysis screen are described next.

File Menu

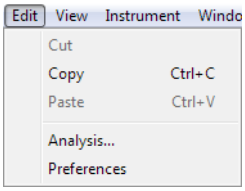
The following File menu options are active:



- **Open Run** - Opens a run file.
- **Add Run** - Opens and views other run files in addition to those that are already open.
- **Close** - Closes the run file currently being viewed.
- **Close All** - Closes all open run files.
- **Save** - Saves changes to the open run file.
- **Save As** - Saves changes to the open run file under a different file name.
- **Export Tables** - Exports the results for all capillaries in the run in .txt format.
- **Export Spectra** - Exports the raw data traces for each capillary in the run in .txt or .cdf format.
- **Run Report** - Creates a report of the run in .pdf format.
- **Exit** - Closes Compass for Simple Western.

Edit Menu

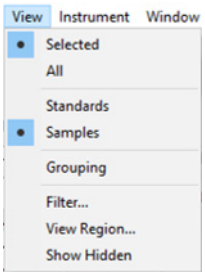
The following Edit menu options are active:



- **Copy** - Lets you copy data shown in the graph, lane, peaks or capillaries panes. See "Copying Data Views and Results Tables" on page 381 for more information.
- **Analysis** - Displays the analysis settings used to analyze the run data and lets you change them as needed. See "Analysis Settings Overview" on page 422 for more information.
- **Preferences** - Lets you set and save custom preferences for data export, plot colors in the graph and Peggy Sue's Twitter settings. See Chapter 11, *"Setting Your Preferences"* for more information.

View Menu

The following View menu options are active:



- **Selected View** - Displays data in a per capillary (single) view format.
- **All View** - Displays data in a per 12-capillary (multiple) view format.
- **Standards** - Lets you change the data view to show only the fluorescent standards.
- **Registration** - Lets you change the data view to show only the capillary registrations.
- **Samples** - Lets you change the data view to show only immunodetected sample proteins.
- **Grouping** - Lets you analyze replicates by calculating the mean, standard deviation and CV of named proteins.
- **Filter** - Lets you display data only for specific capillaries or named proteins.

- **View Region** - Lets you change the pI (x-axis) range of the data displayed.
- **Show Hidden**- Shows capillaries that are hidden from the data view.

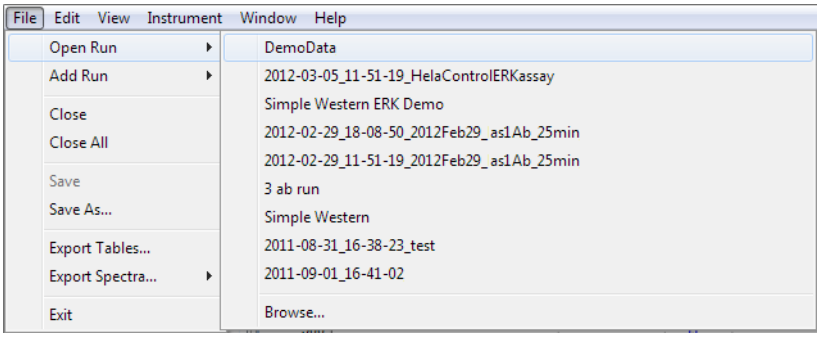
Opening Run Files

You can open one or multiple run files at a time to compare data between runs.

Opening One Run File

NOTE: If you need to open a run file when another run is executing, launch another instance of Compass for Simple Western first, then open the file.

1. Select **File** in the main menu and click **Open Run**.

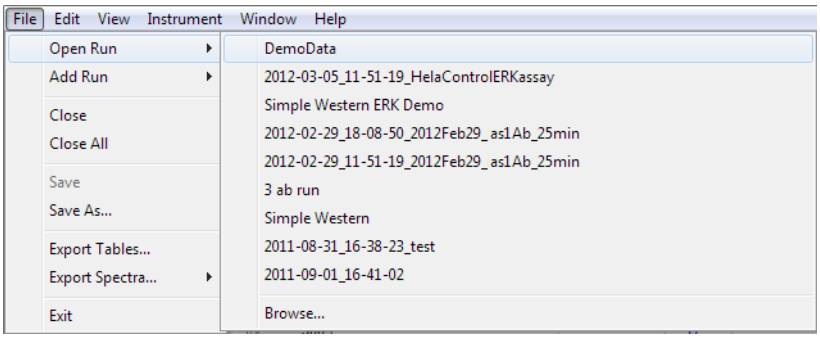


2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.

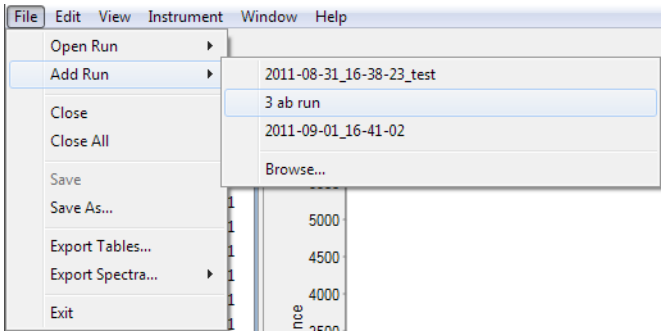
Opening Multiple Run Files

NOTE: If you need to open a run file when another run is executing, launch another instance of Compass for Simple Western first, then open the file.

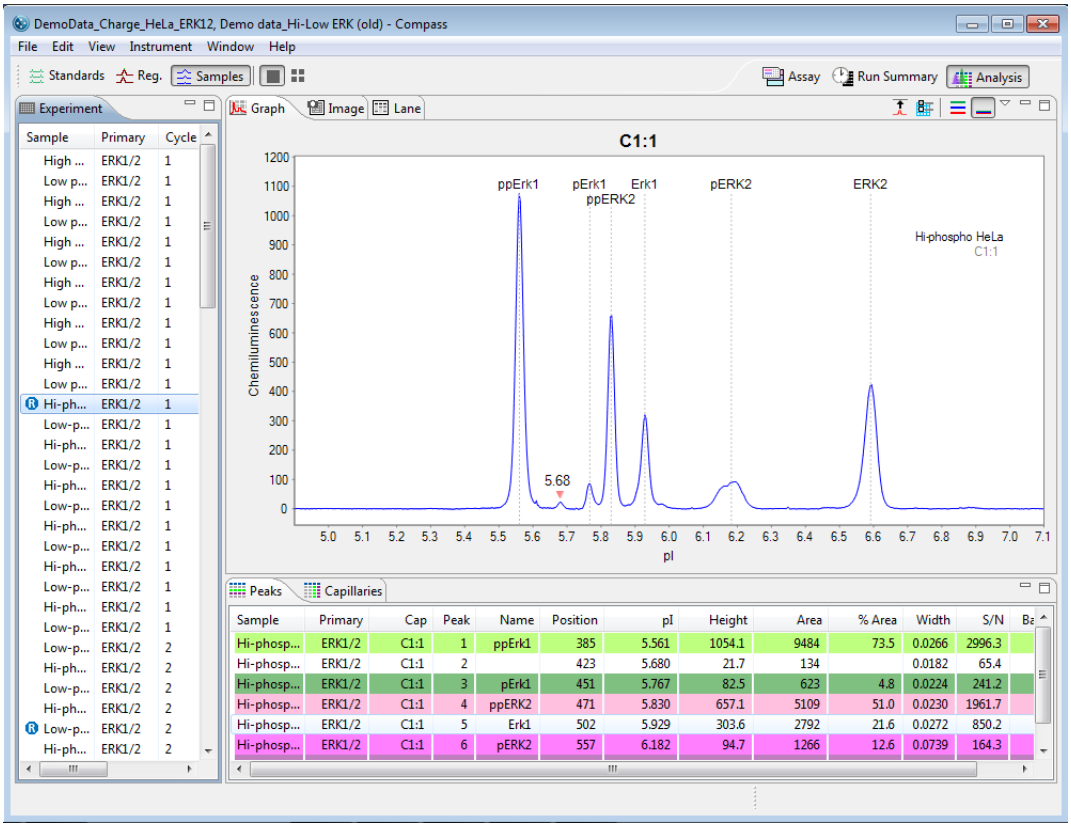
1. To open the first run file, select **File** in the main menu and click **Open Run**.



2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.
3. To open another run file, select **File** in the main menu and click **Add Run**.



4. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file. When a run is added, its data will append to the open run file, and will display as a second set of up to 96 capillaries in all screen panes. The second run file name will also appear in the Compass title bar:



Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
Hi-phosp...	ERK1/2	C1:1	1	ppErk1	385	5.561	1054.1	9484	73.5	0.0266	2996.3
Hi-phosp...	ERK1/2	C1:1	2		423	5.680	21.7	134		0.0182	65.4
Hi-phosp...	ERK1/2	C1:1	3	pErk1	451	5.767	82.5	623	4.8	0.0224	241.2
Hi-phosp...	ERK1/2	C1:1	4	ppERK2	471	5.830	657.1	5109	51.0	0.0230	1961.7
Hi-phosp...	ERK1/2	C1:1	5	Erk1	502	5.929	303.6	2792	21.6	0.0272	850.2
Hi-phosp...	ERK1/2	C1:1	6	pERK2	557	6.182	94.7	1266	12.6	0.0739	164.3

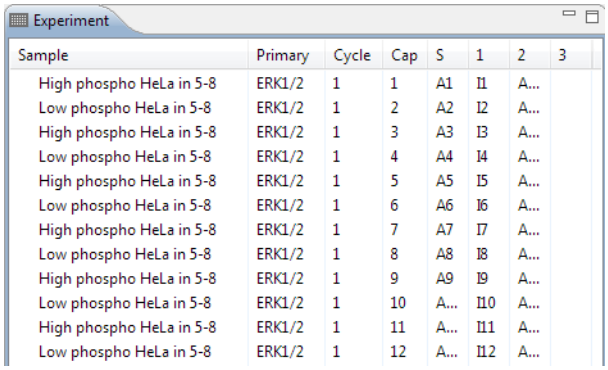
5. Repeat the last two steps to open additional runs.

How Run Data is Displayed in the Analysis Screen

Data in the run file is compartmentalized for easy review in one of six panes in the Analysis screen.

Experiment Pane: Assay and Capillary Information

The experiment pane displays assay and capillary information for each of the 96 capillaries in the run. A maximized view of the experiment pane is shown below.



Sample	Primary	Cycle	Cap	S	1	2	3
High phospho HeLa in 5-8	ERK1/2	1	1	A1	I1	A...	
Low phospho HeLa in 5-8	ERK1/2	1	2	A2	I2	A...	
High phospho HeLa in 5-8	ERK1/2	1	3	A3	I3	A...	
Low phospho HeLa in 5-8	ERK1/2	1	4	A4	I4	A...	
High phospho HeLa in 5-8	ERK1/2	1	5	A5	I5	A...	
Low phospho HeLa in 5-8	ERK1/2	1	6	A6	I6	A...	
High phospho HeLa in 5-8	ERK1/2	1	7	A7	I7	A...	
Low phospho HeLa in 5-8	ERK1/2	1	8	A8	I8	A...	
High phospho HeLa in 5-8	ERK1/2	1	9	A9	I9	A...	
Low phospho HeLa in 5-8	ERK1/2	1	10	A...	I10	A...	
High phospho HeLa in 5-8	ERK1/2	1	11	A...	I11	A...	
Low phospho HeLa in 5-8	ERK1/2	1	12	A...	I12	A...	

- **To view all columns** - Click the **Experiment** tab, then use the scroll bar or click **Maximize** in the upper right corner.
- **To resize columns** - Click the **Experiment** tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Experiment pane column descriptions for the Peggy Sue Charge default assay are as follows:

- **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.

NOTE: Data notification icons will display in the sample column if Compass for Simple Western detected a potential analysis issue or data was manually modified by the user. For more information see "Run Data Notifications and Warnings" on page 365.

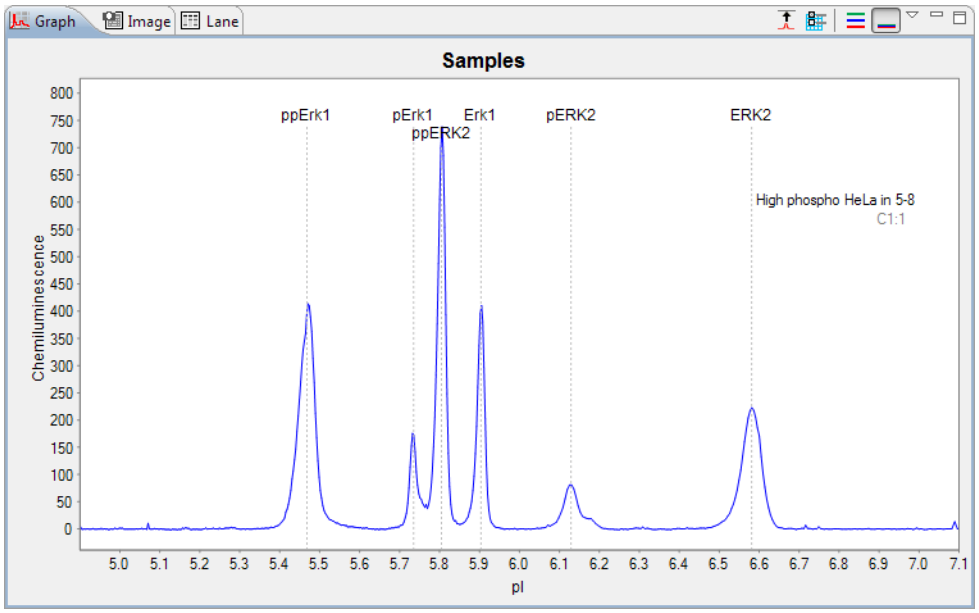
- **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Cycle** - Run cycle number. There are 12 capillaries in one cycle.

NOTE: Peggy Sue can run up to eight cycles (12 capillaries per cycle) in an experiment. Each cycle is designated in the Experiment summary.

- **Cap** - Capillary number.
- **S** - Well on the assay plate used for sample.
- **1** - Well on the assay plate used for primary antibody.
- **2** - Well on the assay plate used for secondary antibody.
- **3** - Well on the assay plate used for tertiary antibody (if used).

Graph Pane: Electropherogram Data

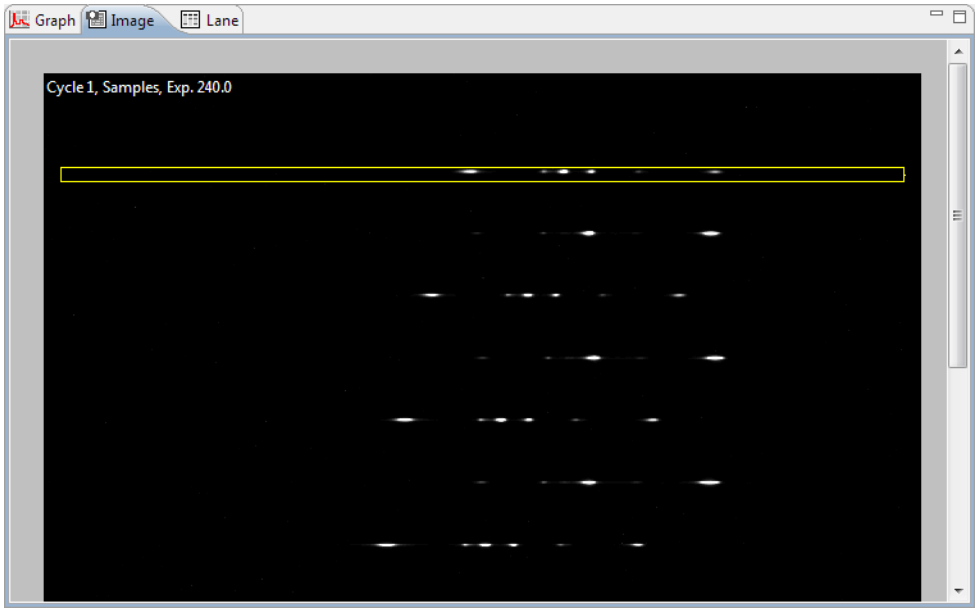
Click the **Graph** tab to view data for immunodetected sample proteins, fluorescent standards or capillary registrations. Data for samples is shown in the following example, and immunodetected proteins are displayed as peaks:



More Graph view options will be described in more detail in “Changing the Electropherogram View” on page 400.

Image Pane: Capillary Separation Image Data

Click the **Image** tab to view final separation images of immunodetected sample proteins, fluorescent standards or capillary registrations for up to 96 capillaries per experimental run. Image data for samples is shown in the following example:



Lane Pane: Virtual Blot-Like Image Data

Click the **Lane** tab to view data for immunodetected sample proteins, fluorescent standards or capillary registrations as bands in individual lanes. This virtual blot-like image is similar to traditional blot results. Data for samples in the lane view is shown in the following example, and immunodetected proteins are displayed as bands.

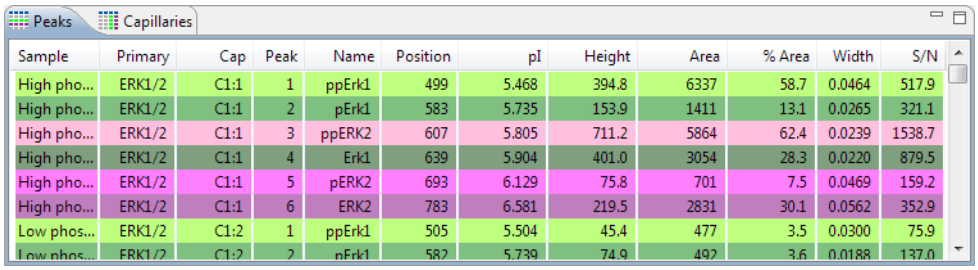
To view information for a band, roll the mouse over a band until the info box appears.

NOTE: The reported pI for immunodetected sample proteins in Compass for Simple Western may vary slightly from predicted pIs based on sample, buffer and assay conditions.

Lane data displayed in the virtual blot is automatically aligned by Compass for Simple Western. To view raw, unaligned lane data and learn more about virtual blot viewing options, see "Changing the Virtual Blot View" on page 394.

Peaks Pane: Calculated Results

Click the **Peaks** tab to view tabulated results for immunodetected sample proteins, fluorescent standards or capillary registrations. Each row in the table shows the individual results for each peak detected in each capillary. Data for samples in the peaks table is shown in the following example:



Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
High pho...	ERK1/2	C1:1	1	ppErk1	499	5.468	394.8	6337	58.7	0.0464	517.9
High pho...	ERK1/2	C1:1	2	pErk1	583	5.735	153.9	1411	13.1	0.0265	321.1
High pho...	ERK1/2	C1:1	3	ppERK2	607	5.805	711.2	5864	62.4	0.0239	1538.7
High pho...	ERK1/2	C1:1	4	Erk1	639	5.904	401.0	3054	28.3	0.0220	879.5
High pho...	ERK1/2	C1:1	5	pERK2	693	6.129	75.8	701	7.5	0.0469	159.2
High pho...	ERK1/2	C1:1	6	ERK2	783	6.581	219.5	2831	30.1	0.0562	352.9
Low phos...	ERK1/2	C1:2	1	ppErk1	505	5.504	45.4	477	3.5	0.0300	75.9
Low phos...	ERK1/2	C1:2	2	pErk1	582	5.739	74.9	492	3.6	0.0188	137.0

NOTES:
Peaks that Compass for Simple Western names automatically using the user-defined peak name analysis parameters are color-coded.

The reported pI for immunodetected sample proteins in Compass for Simple Western may vary slightly from predicted pIs based on sample, buffer and assay conditions.

- **To view all rows** - Click the **Peaks** tab, then use the scroll bar or click **Maximize** in the upper right corner.
- **To resize columns** - Click the **Peaks** tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Peak table column descriptions are as follows:

- **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Cap** - Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.

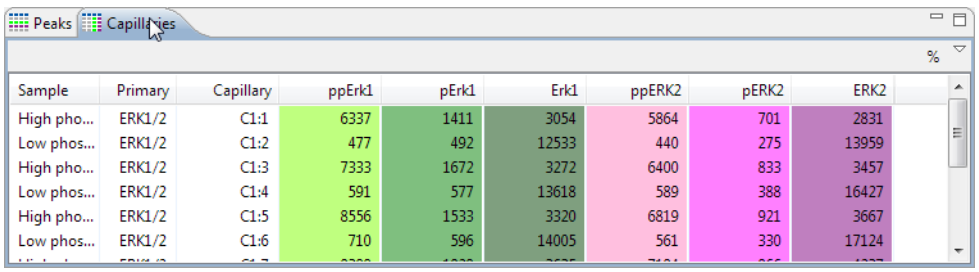
NOTE: Peggy Sue runs up to eight cycles in an experiment, 12 capillaries at a time. Capillary and cycle numbers are displayed in the Experiment tab.

- **Peak** - Peak number. Peaks are numbered in order of detection.

- **Name** - Peak name. Displays peaks that Compass for Simple Western named automatically using the user-defined peak name analysis parameters. Cells will be blank if Compass for Simple Western was not able to name the peak or if naming parameters were not entered.
- **Position** - Displays the pixel position of a peak in the image.
- **pI** - Displays the calculated pI for the peak.
- **Height** - Displays the calculated peak height.
- **Area** - Displays the calculated peak area.
- **% Area** - Displays the calculated percent area for the named peak compared to all named peaks. This value results from dividing the individual peak area by the sum of all named peak areas for the capillary and multiplying by 100 (shown for named peak sample data only).
- **Width** - Displays the calculated peak width (shown for sample data only).
- **S/N** - Displays the calculated signal to noise ratio for the peak. Please note this calculation is based on a peak finding algorithm to provide a relative ratio comparing all peaks within the electropherogram in Compass.

Capillaries Pane: User-Specified Peak Names

Click the **Capillaries** tab to view tabulated results for sample proteins associated with specific primary antibodies in the run data. Compass for Simple Western labels these sample peaks automatically using user-defined peak name settings. Each row in the table shows the individual results for the named peaks detected in each capillary. Data for samples in the capillaries table is shown in the following example.



Sample	Primary	Capillary	ppErk1	pErk1	Erk1	ppERK2	pERK2	ERK2
High pho...	ERK1/2	C1:1	6337	1411	3054	5864	701	2831
Low phos...	ERK1/2	C1:2	477	492	12533	440	275	13959
High pho...	ERK1/2	C1:3	7333	1672	3272	6400	833	3457
Low phos...	ERK1/2	C1:4	591	577	13618	589	388	16427
High pho...	ERK1/2	C1:5	8556	1533	3320	6819	921	3667
Low phos...	ERK1/2	C1:6	710	596	14005	561	330	17124

NOTES:
Peaks that Compass for Simple Western names automatically with user-defined peak name settings are color-coded.

Information displayed for fluorescent standards and capillary registration data will be for identified standards or registration peaks.

- **To view all rows** - Click the **Capillaries** tab, then use the scroll bar or click **Maximize** in the upper right corner.
- **To resize columns** - Click the **Capillaries** tab. Roll the mouse over a column border until the sizing arrow appears, then click and drag to resize.

Capillaries table column descriptions are as follows:

- **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Capillary** - Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.

NOTE: Peggy Sue runs12 capillaries at a time in a cycle and is able to run up to eight cycles in an experiment. The Information on cycle and capillary number is displayed in the Experiment tab.

- **Peak Name Columns** - An individual column per peak name will display for every peak identified by name in the run data. Cells for capillaries in these columns will be blank if Compass for Simple Western did not find peaks automatically using the user-defined peak name analysis parameters (or none were entered).
  - **To view % area in the peak name columns** - Select **%** in the upper right corner of the pane. This displays the calculated percent area for the named peak compared to all named peaks. This value results from dividing the individual peak area by the sum of all named peak areas for the capillary and multiplying by 100.

Sample	Primary	Capillary	ppErk1	pErk1	Erk1	ppERK2	pERK2	ERK2
High pho...	ERK1/2	C1:1	58.7	13.1	28.3	62.4	7.5	30.1
Low phos...	ERK1/2	C1:2	3.5	3.6	92.8	3.0	1.9	95.1
High pho...	ERK1/2	C1:3	59.7	13.6	26.7	59.9	7.8	32.3
Low phos...	ERK1/2	C1:4	4.0	3.9	92.1	3.4	2.2	94.4
High pho...	ERK1/2	C1:5	63.8	11.4	24.8	59.8	8.1	32.1
Low phos...	ERK1/2	C1:6	4.6	3.9	91.5	3.1	1.8	95.0

  - **To view peak area in the peak name columns (default)** - Deselect **%** in the upper right corner of the pane. This displays calculated peak area for the individual peak only.

Viewing Run Data

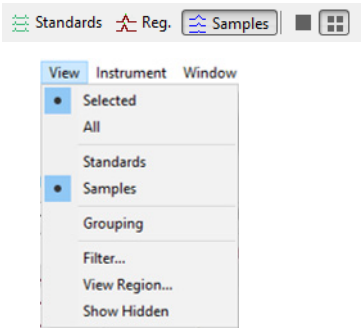
Each run file contains the following data for up to 96 capillaries:

- **Sample data** - For the immunodetected proteins in the sample.
- **Standards data** - For the fluorescent standards run with each sample.
- **Registration data** - For tracking capillaries as they are moved for various assay steps.

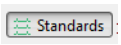
Data can be viewed for one, all, or individually selected capillaries.

Switching Between Sample, Standards and Registration Data Views

You can switch between viewing sample, standards and registration data in a run file using the View bar or View menu:

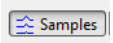


Data buttons in the View bar:



Show Standards

Show Registrations



Show Samples

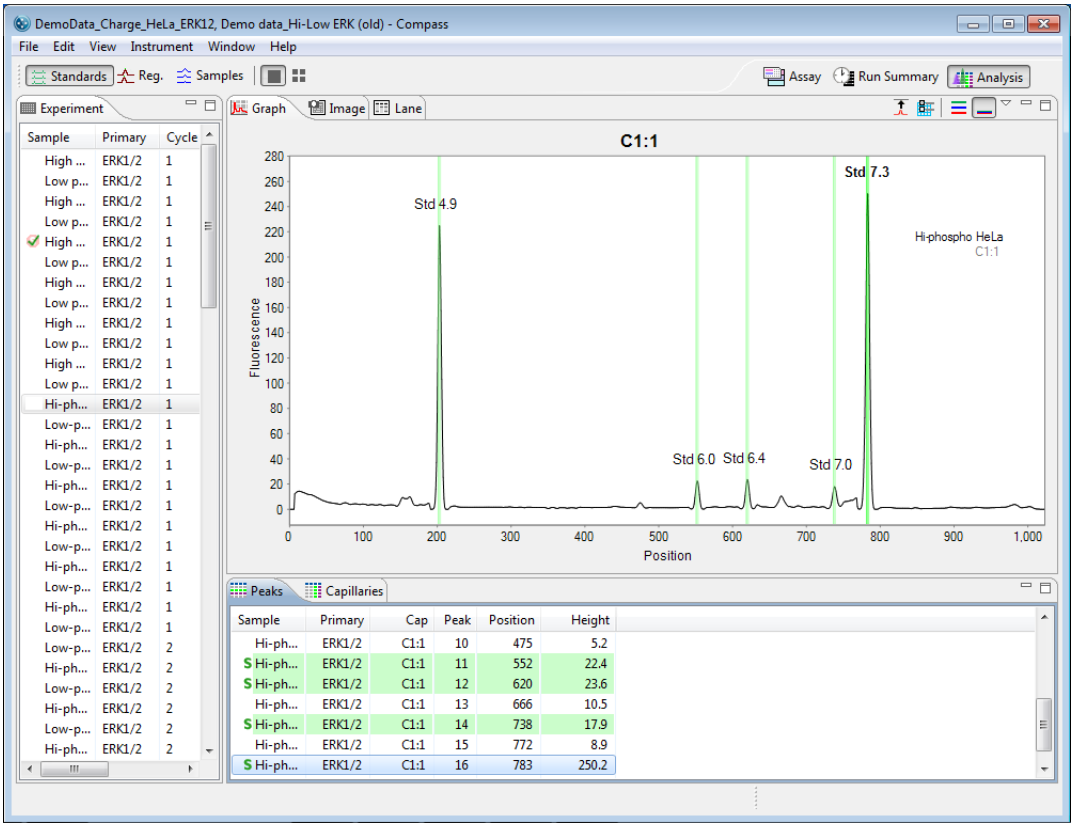
- **To view sample data** - Click **Show Samples** in the View bar or select **View** in the main menu and click **Samples**:

- Data in this view is for immunodetected sample proteins only.
- Graph view data displays electropherograms in chemiluminescence units (y-axis) and pI (x-axis).
- Lane view data displays immunodetected sample proteins only.
- Image view data displays immunodetected sample proteins only.
- Results for each immunodetected protein are shown in the peaks and capillaries tables.

NOTE: The reported pI for immunodetected sample proteins in Compass for Simple Western may vary slightly from predicted pIs based on sample, buffer and assay conditions.

For information on checking and identifying sample peaks, see "Step 4 – Checking Samples" on page 372.

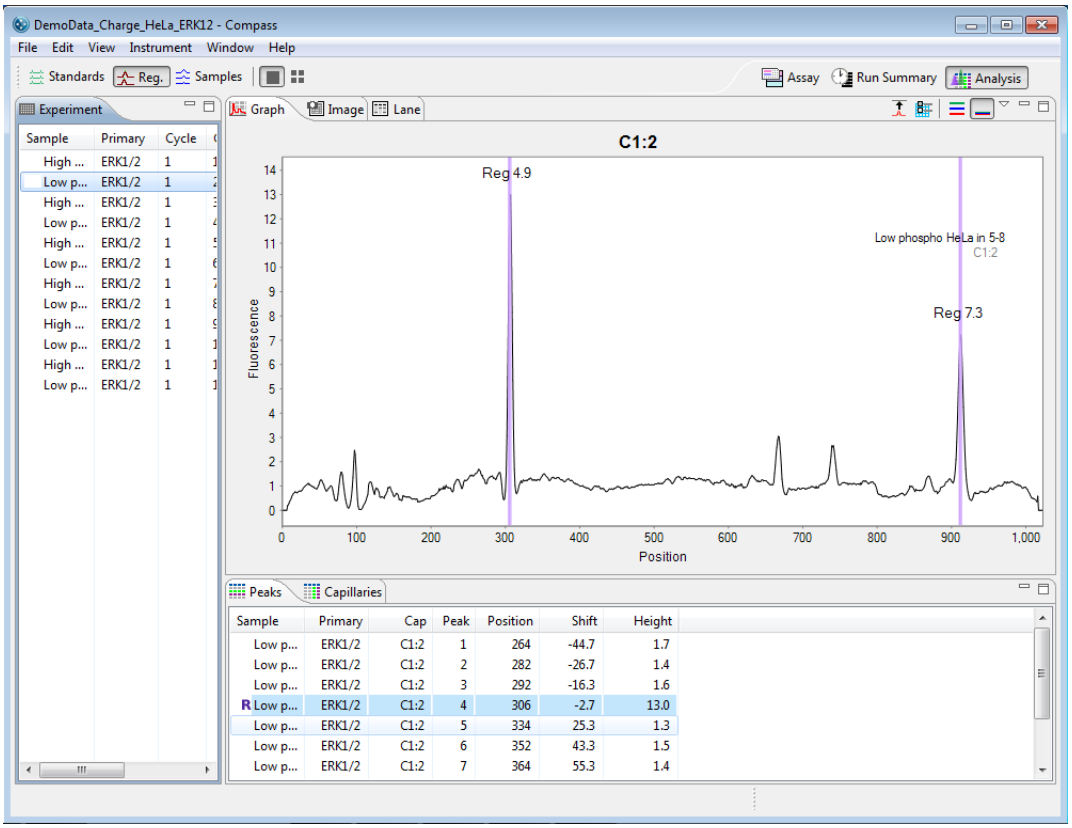
- **To view standards data** - Click **Show Standards** in the View bar or select **View** in the main menu and click **Standards**:



- Data in this view is for the fluorescent standards only. These standards are premixed with each sample prior to analysis.
- Graph view data displays electropherograms in fluorescence (y-axis) and position (x-axis).
- Lane view data displays fluorescent standards only.
- Image view data displays fluorescent standards only.
- Standards are highlighted in both the peaks and capillaries tables and marked with an **S**.

For information on checking and identifying standards peaks, see "Step 2 – Checking Fluorescent Sizing Standards" on page 367.

- **To view registration data** - Click **Show Registrations** in the View bar or select **View** in the main menu and click **Registration**:



- Data in this view shows capillary registration only.
- Graph view data displays electropherograms in fluorescence (y-axis) and position (x-axis).
- Lane view data displays capillary registration only.
- Image view data displays capillary registration only.
- Registrations are highlighted in both the peaks and capillaries tables and marked with an R.

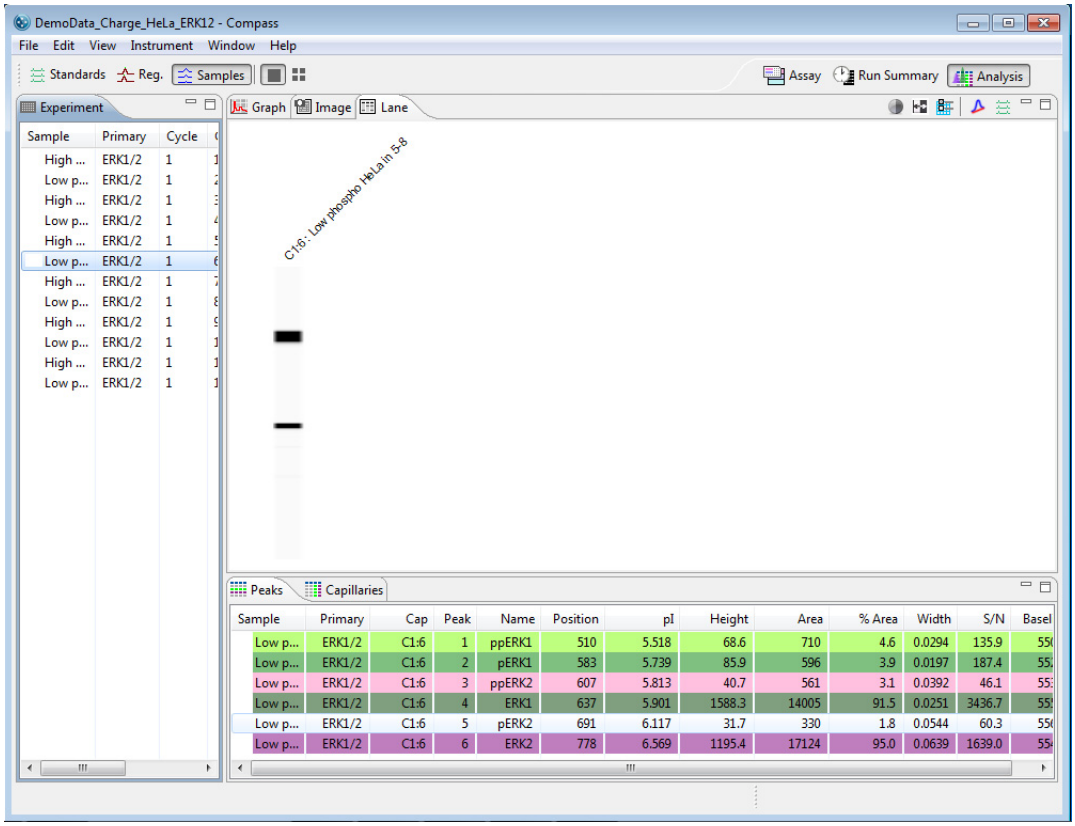
Because capillaries must be moved multiple times during the run, their positions are tracked to account for potential changes in imaging position as they are moved to and from the separation block. To do this, one of the standards is used as a capillary registration. An image of the standard used for registration is taken at the end of the separation step prior to chemiluminescent imaging. The position of this peak in the registration image is then compared to the position of the same standard peak in the standards image. The shift value listed in the peaks table is the change in pixel position between the two images. Compass for Simple Western data is then aligned to account for any changes in capillary position.

For information on checking and identifying registration peaks, see "Step 3 – Checking Capillary Registrations" on page 371.

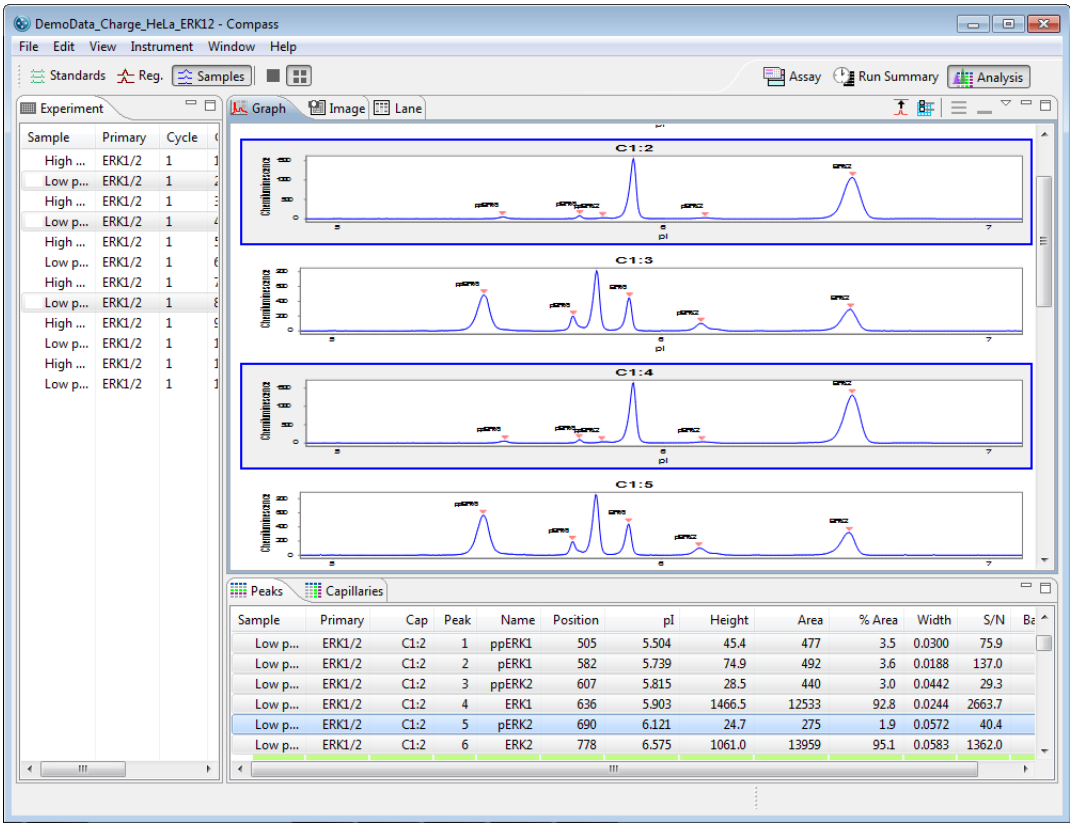
Selecting and Displaying Capillary Data

You can choose to view data from one, multiple, or all capillaries at once.

- **To look at data for one capillary** - Click a row in the experiment pane. Data for just the row selected will display in all data views and tables. The following example shows sample data displayed in the lane and peaks panes when one sample is selected:



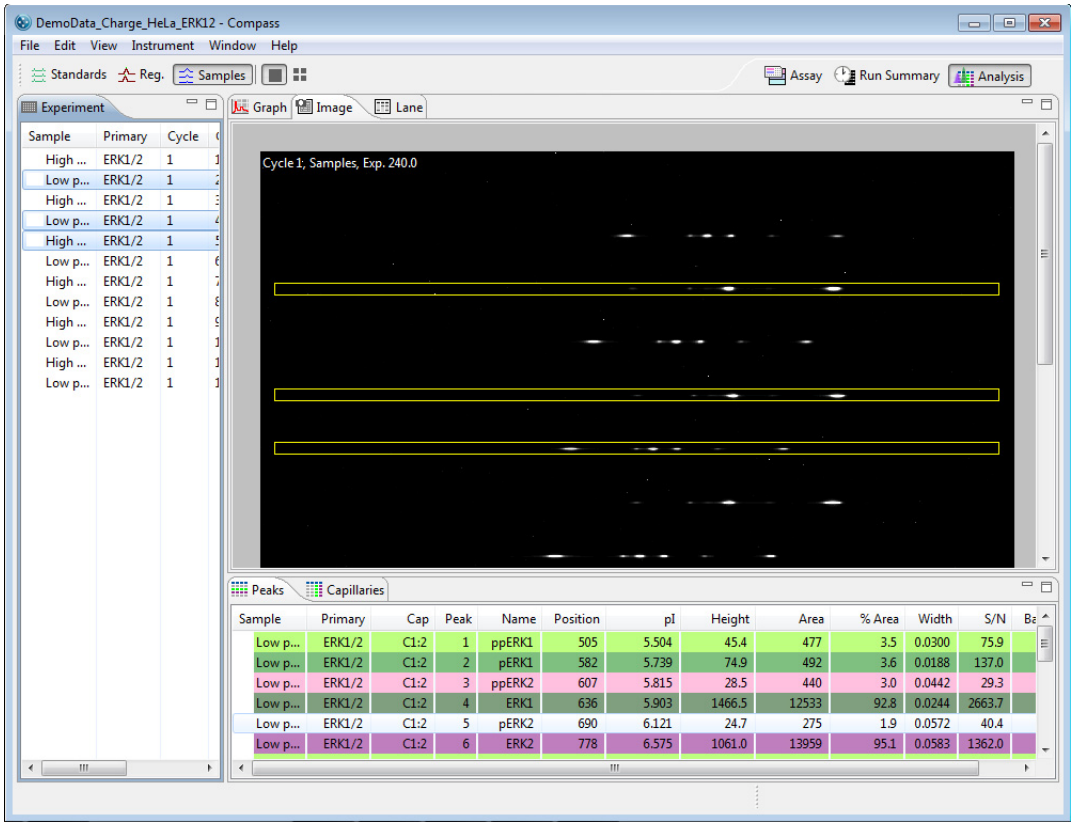
- **To look at data for multiple non-sequential capillaries** - Hold the **Ctrl** key and select the rows you want to view in the experiment pane. Data for only the rows selected will display in all data views and results tables. The following example shows sample data displayed in the graph (in multiple view) and capillaries panes when multiple rows are selected:



Sample	Primary	Cycle
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1

Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
Low p...	ERK1/2	C1:2	1	ppERK1	505	5.504	45.4	477	3.5	0.0300	75.9
Low p...	ERK1/2	C1:2	2	pERK1	582	5.739	74.9	492	3.6	0.0188	137.0
Low p...	ERK1/2	C1:2	3	ppERK2	607	5.815	28.5	440	3.0	0.0442	29.3
Low p...	ERK1/2	C1:2	4	ERK1	636	5.903	1466.5	12533	92.8	0.0244	2663.7
Low p...	ERK1/2	C1:2	5	pERK2	690	6.121	24.7	275	1.9	0.0572	40.4
Low p...	ERK1/2	C1:2	6	ERK2	778	6.575	1061.0	13959	95.1	0.0583	1362.0

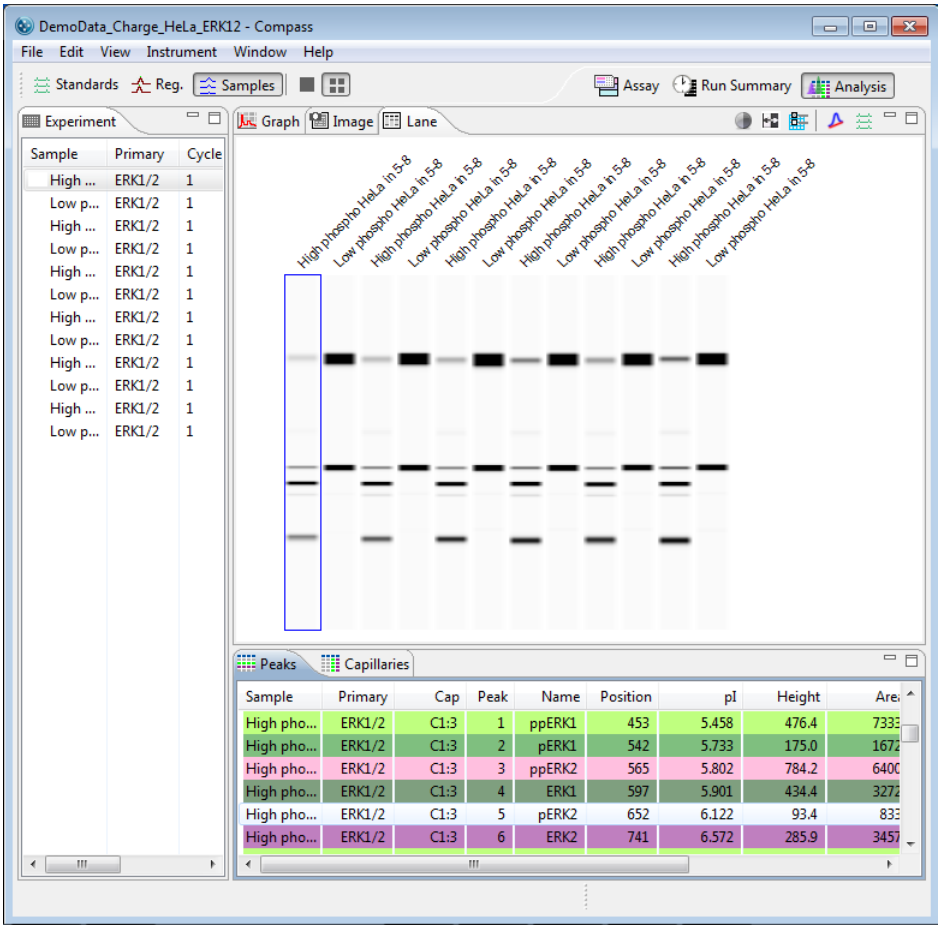
- **To look at data for multiple sequential capillaries** - Hold the **Shift** key and select the first and last rows you want to view in the experiment pane. Data for only the selected rows will display in all data views and results tables. The following example shows standards data displayed in the image and peaks panes when multiple sequential rows are selected:



Sample	Primary	Cycle
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1
High ...	ERK1/2	1
Low p...	ERK1/2	1

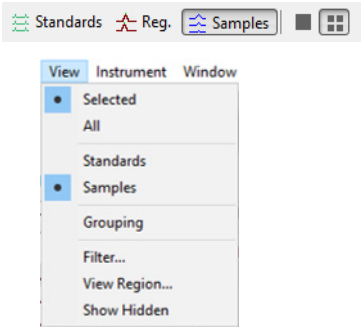
Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
Low p...	ERK1/2	C1:2	1	ppERK1	505	5.504	45.4	477	3.5	0.0300	75.9
Low p...	ERK1/2	C1:2	2	pERK1	582	5.739	74.9	492	3.6	0.0188	137.0
Low p...	ERK1/2	C1:2	3	ppERK2	607	5.815	28.5	440	3.0	0.0442	29.3
Low p...	ERK1/2	C1:2	4	ERK1	636	5.903	1466.5	12533	92.8	0.0244	2663.7
Low p...	ERK1/2	C1:2	5	pERK2	690	6.121	24.7	275	1.9	0.0572	40.4
Low p...	ERK1/2	C1:2	6	ERK2	778	6.575	1061.0	13959	95.1	0.0583	1362.0

- **To look at data for all capillaries** - Hold the **Shift** key and select the first and last rows in the experiment pane. Data for all rows will display in all data views and results tables. The following example shows sample data displayed in the lane and peaks panes when all rows are selected. The pane scroll bars can be used to view all 8 cycles:



Switching Between Single and Multiple Views of the Capillaries

You can switch between displaying run data in the graph, image and lane panes in a per capillary (single) format or up to 96 capillary format. This view is selected in the View bar or the View menu.

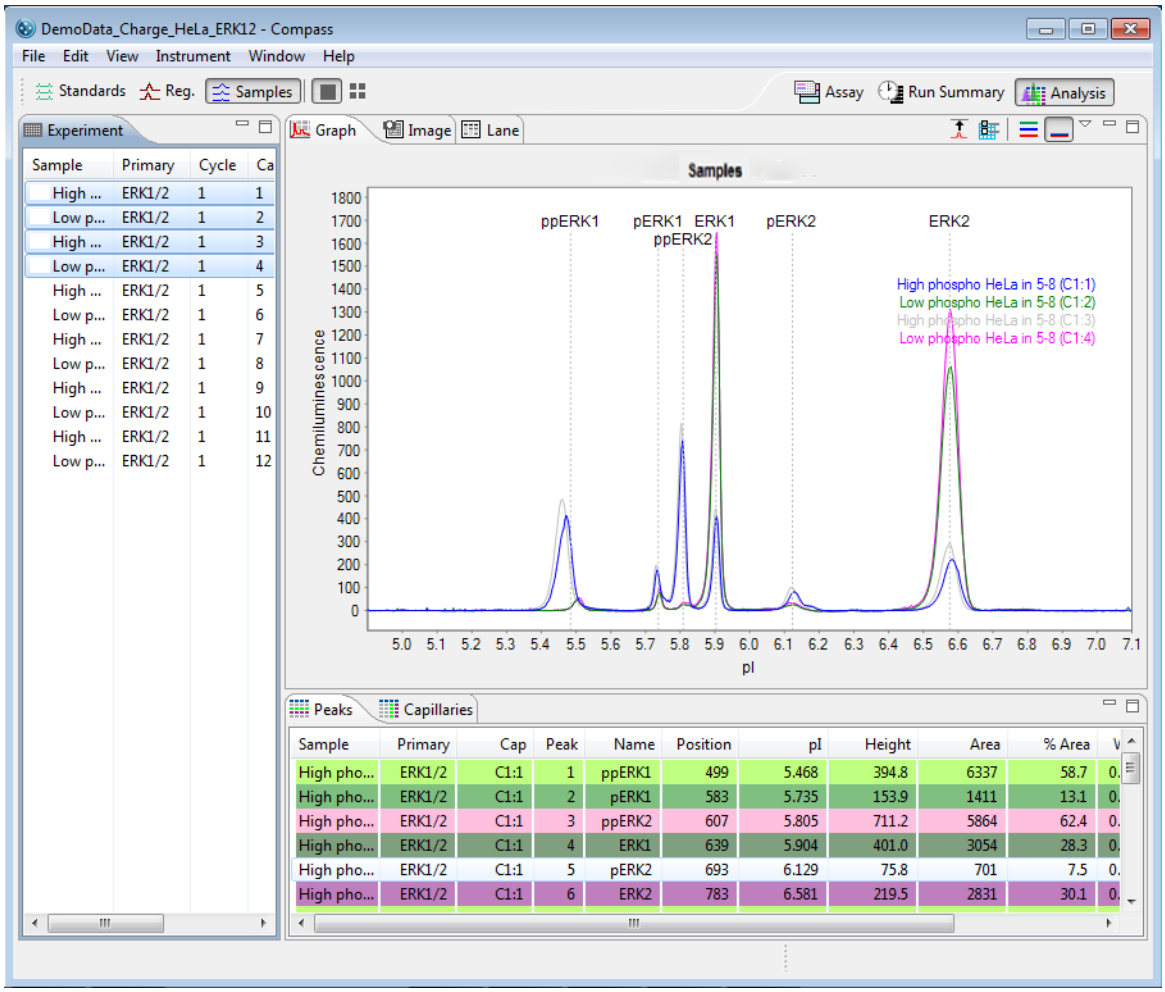


Capillary view buttons in the View bar:

Selected View

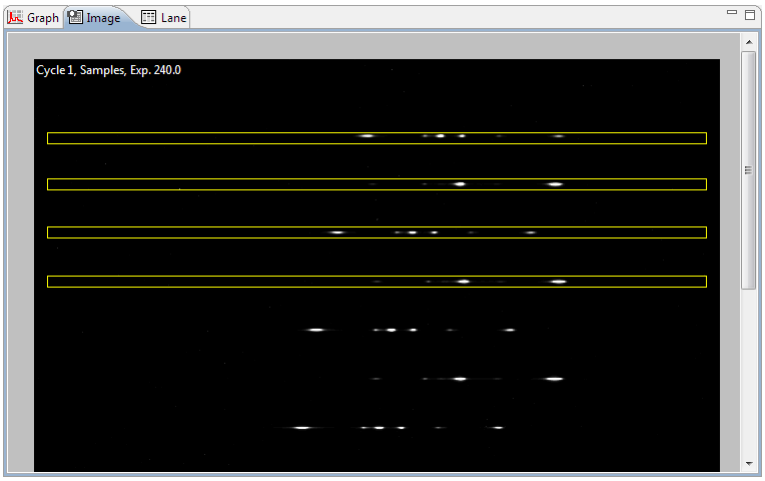
All View

- **To view data in a per capillary format** - Click **Single View** in the View bar or select **View** in the main menu and click **Single View**:

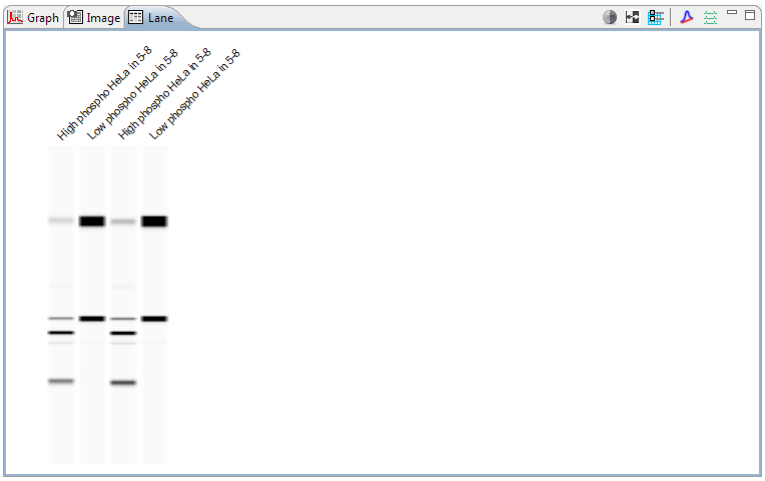


Data for the row(s) selected in the experiment pane will display as follows:

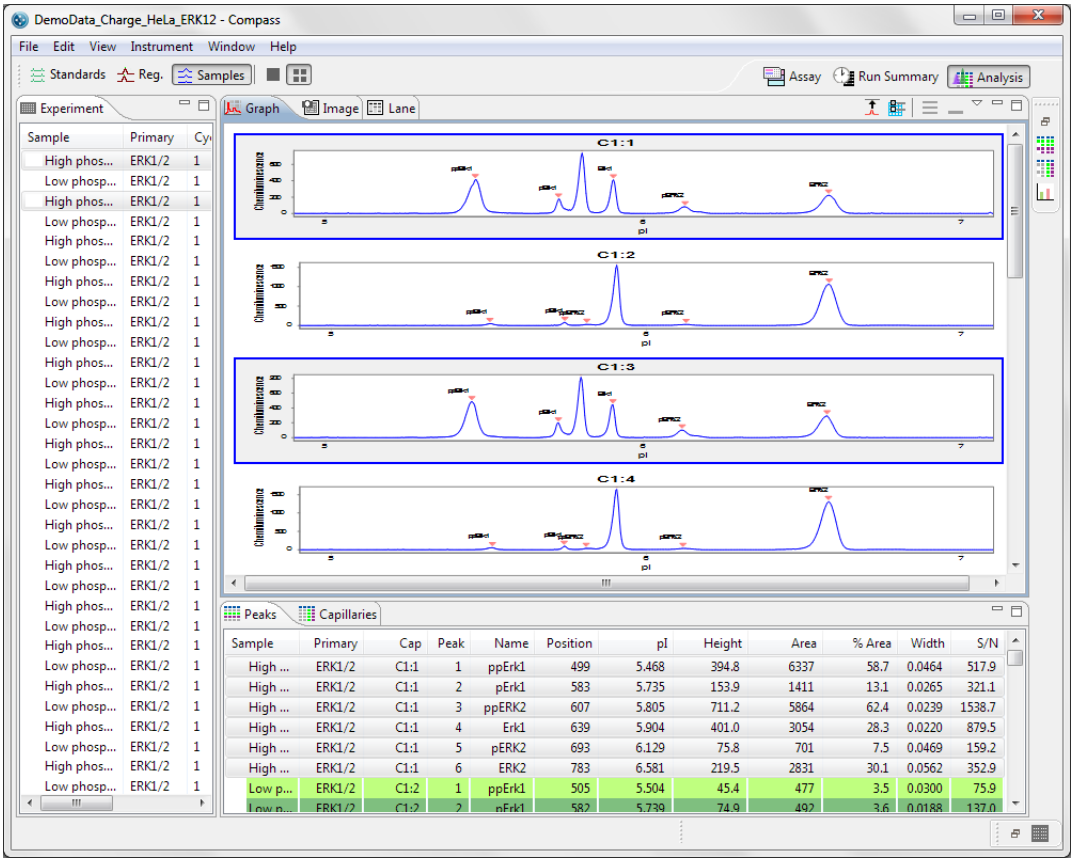
- Electropherograms in the graph pane display overlaid or stacked (depending on the graph option chosen).
- Results for only the selected row(s) will display in the peaks and capillaries tables.
- The selected row(s) are highlighted in the image pane:



- Lanes for only the selected row(s) are displayed in the lane pane:

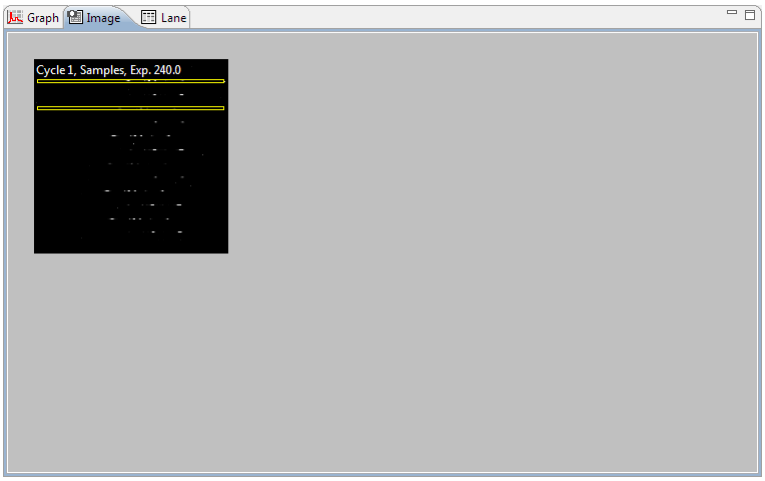


- **To view data up to 96-capillary format** - Click **Multiple View** in the View bar or select **View** in the main menu and click **Multiple View**:

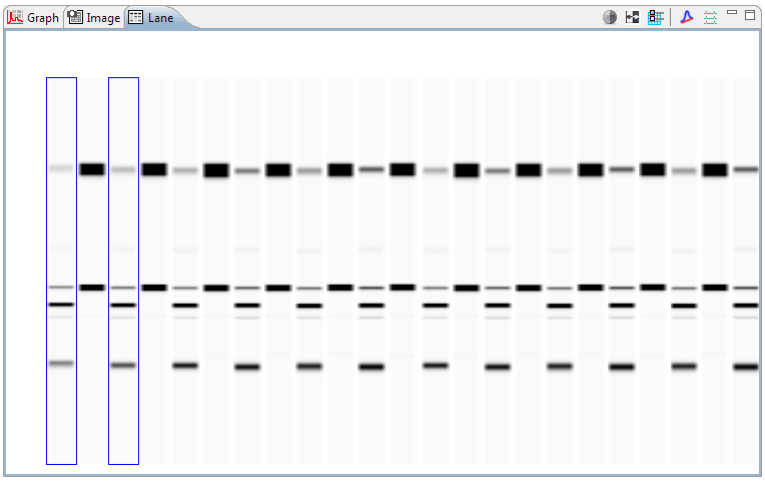


Data for the row(s) selected in the experiment pane will display as follows:

- Electropherograms corresponding to the selected row(s) are highlighted and displayed in the graph pane.
- Results for selected capillaries display in the peaks and capillaries tables and are highlighted.
- The selected row(s) are highlighted in the image pane:



- All selected lanes display in the lane pane, and lanes corresponding to the selected row(s) are highlighted.

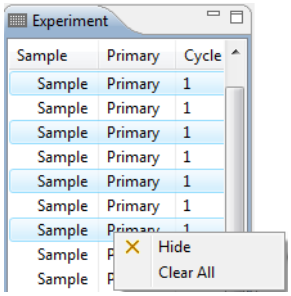


Hiding Capillary Data

Capillary data can be hidden from view if needed. To do this:

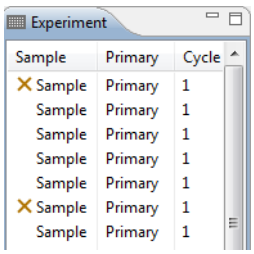
1. Click the **Experiment** tab.

2. Select the rows you want to hide, then right click on one of the selected rows and click **Hide**:



Data for selected rows will be hidden in all data views and results tables (except for the image pane).

- **To view hidden rows** - Select **View** in the main menu and click **Show Hidden**. Hidden rows will become visible again in all panes, and hidden rows will be marked with an X in the experiment pane:



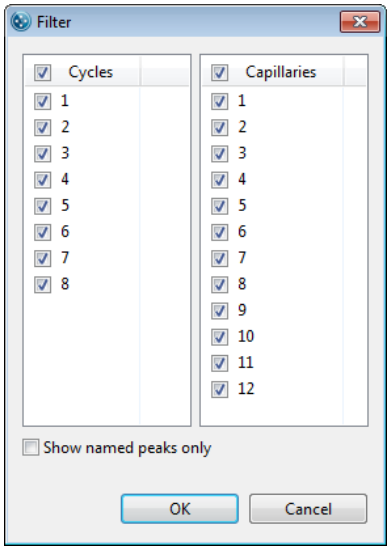
- **To unhide rows** - Select the hidden row(s). Right click on one of the selected rows and click **Unhide.**

Setting Run Data Display Filters

The filter lets you auto-hide specific capillaries in the run file data, or only show data for peaks that Compass for Simple Western identified automatically using the user-defined peak name analysis parameters.

NOTE: When more than one run file is open, filter settings will be applied to all files.

- **To filter data to show specific capillaries only** - Select **View** in the main menu and click **Filter**. Uncheck the boxes for the capillaries you do not want shown, then click **OK**.



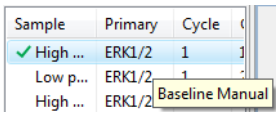
Only data for the checked capillaries will display in data views and results tables, unchecked capillaries will be hidden.

- **To filter data to show named peaks only** - Select **View** in the main menu and click **Filter**. Select **Show named peaks only** then click **OK**. Only data for peaks that Compass for Simple Western identified automatically using the user-defined peak name analysis parameters will display in data views and results tables, all other peak data will be hidden.

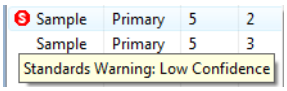
Run Data Notifications and Warnings

If Compass for Simple Western detects a potential data issue, a notification or warning will display next to the row in the experiment pane. A list of warnings, notifications and corrective actions are as follows:

- **Manual correction of sample data notification** - Indicates the user modified sample data manually, such as adding or removing a sample peak. Rolling the mouse over the icon displays the type of data modification made.

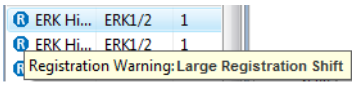


- **Standards warning -** Indicates that one of the standards may not be identified properly. This can be resolved by manually identifying the standards. Please refer to "Step 2 – Checking Fluorescent Sizing Standards" on page 367 for details. Rolling the mouse over the icon displays warning details.



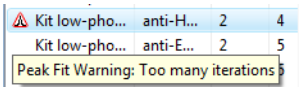
- **Manual correction of standards data notification** - Indicates the user modified the standards data manually. Rolling the mouse over the icon displays the type of data modification made.

- **Registrations warning -** Indicates that a capillary registration was not identified properly. This can be resolved by manually identifying the registration peak. Please refer to "Step 3 – Checking Capillary Registrations" on page 371 for details. Rolling the mouse over the icon displays warning details.



- **Manual correction of registrations notification -** Indicates the user modified the capillary registration data manually. Rolling the mouse over the icon displays the type of data modification made.

- **Peak fit warning -** Indicates that a peak cannot be fit properly. This is typically caused either by a very small peak in the electropherogram or a peak that is very close to the end of the pI range. This can be resolved by removing the peak. Please refer to "Step 4 – Checking Samples" on page 372 or "Step 4 – Checking Samples" on page 372 for details. Rolling the mouse over the icon displays warning details.



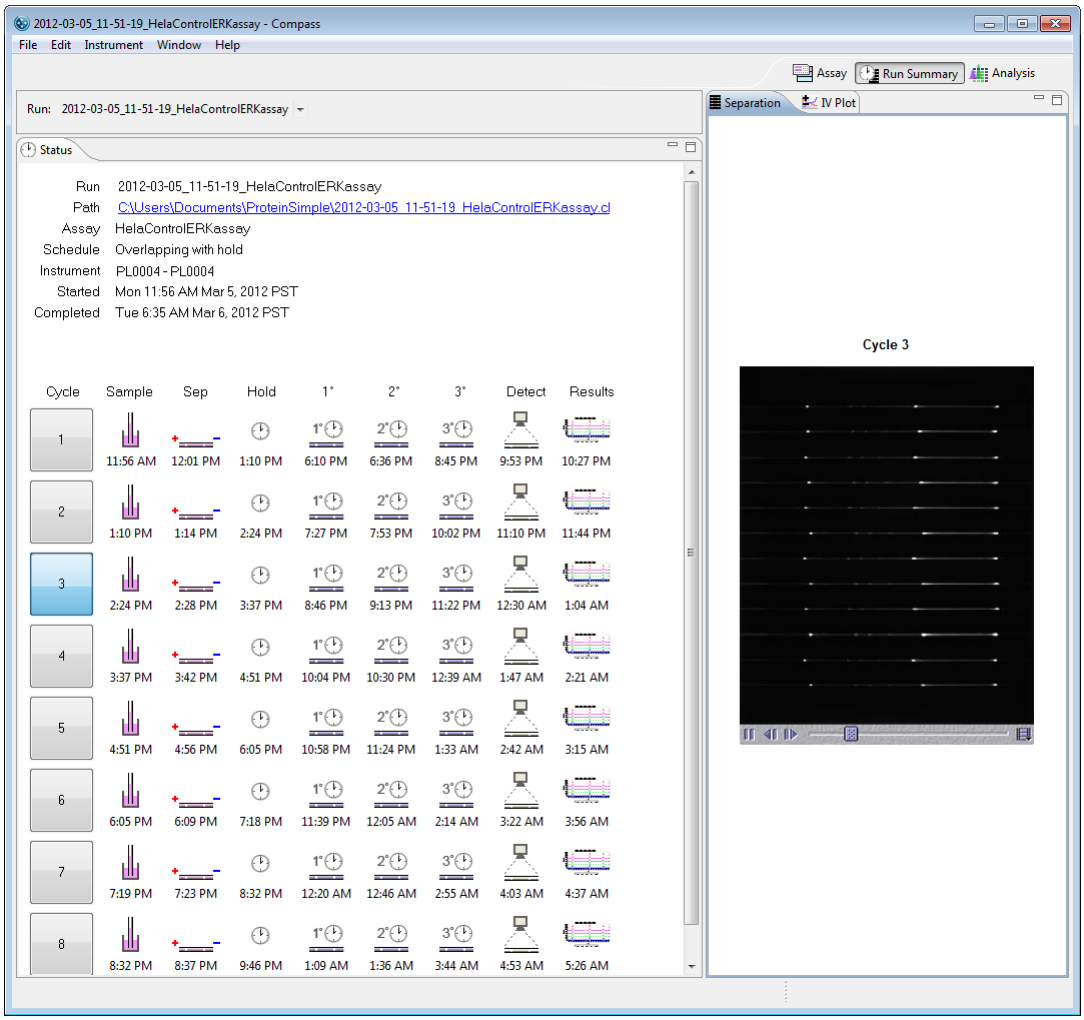
Checking Your Results

Compass for Simple Western detects immunodetected proteins, fluorescent standards and capillary registrations and reports results automatically with no user-interaction. However, we recommend you review their data as outlined in this section as good general practice to ensure accurate results. If a data warning is presented in the experiment pane, the following steps will also help you identify and correct any issues.

Step 1 – Review the Fluorescent Sizing Standards Movie

All capillaries should have three fluorescent sizing standards. To verify the standards separated in the capillary correctly:

1. When the run has completed, click the **Run Summary** screen tab.
2. Click the **Separation** tab and play the movie (this will be the fluorescent standards separation).



3. For each capillary, verify that the standards visibly separated. Each fluorescent point in the capillary represents one of the three sizing standards.

Step 2 – Checking Fluorescent Sizing Standards

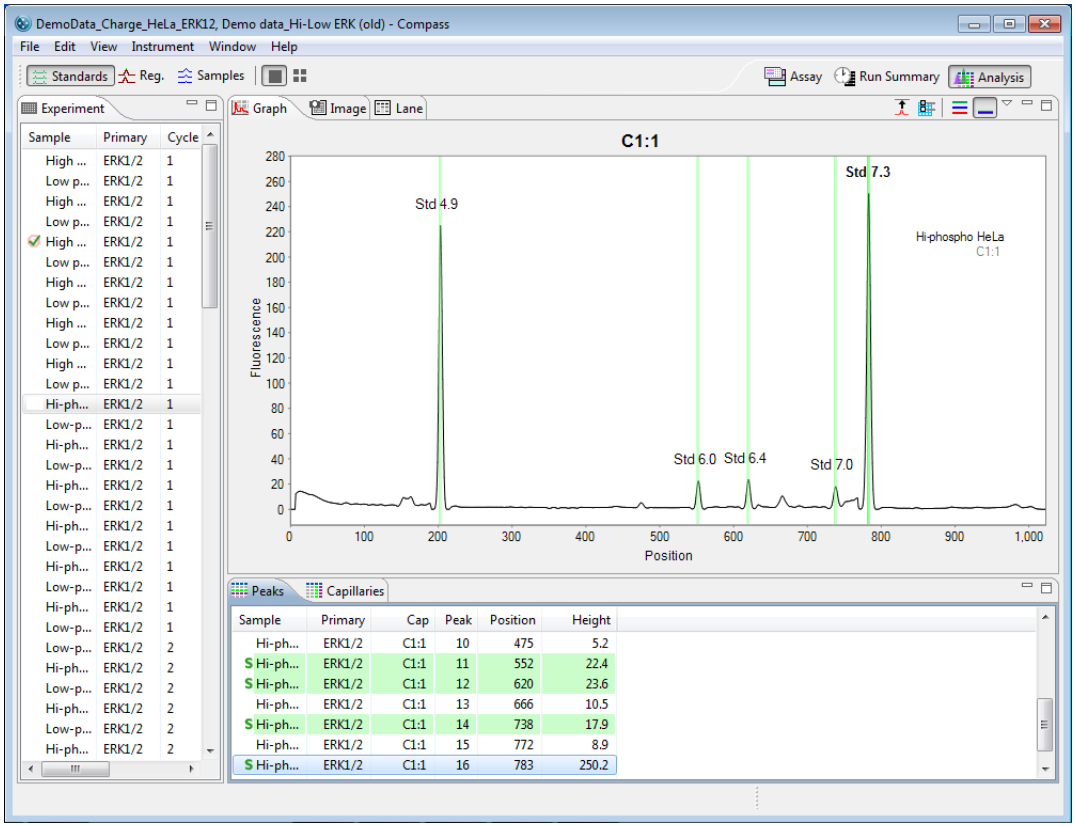
To verify the standards are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Standards** in the View bar. Verification that the standards have been correctly identified can be done in either the graph or lane panes.

Graph Pane:

a. Click **Single View** in the View bar.

b. Click on the first row in the experiment pane, then click the **Graph** tab. Check that the electropherogram has the appropriate number of fluorescent pI standard peaks for the pI Standard Ladder you are using. They will also be identified with a green **S** in the peaks table. The pI standards at the low and high end of the pI range in the electropherogram are at higher concentrations as they are also used for capillary registration. In the following example, the pI standards shown are those for pI Standard Ladder 3 (P/N 040-646).



If standards are not identified correctly, they can be manually corrected as follows:

- **If an incorrect peak is identified as a standard** - Right click the peak in the electropherogram or peaks table and select **Not a Standard**. Compass for Simple Western should correctly reassign the remaining peaks as standards, and update the peaks table.

- **To set an unidentified peak as a standard** - Right click the peak in the electropherogram or peaks table and select **Force Standard**. Compass for Simple Western will assign the peak as a standard, and correctly reassign the remaining standard peaks. A lock icon indicating the standard was set manually will display next to the peak in the peaks table.

---

*NOTE: To remove standards peak assignments that were made manually, right click on the peak in the electropherogram or peaks table and click **Clear**.*

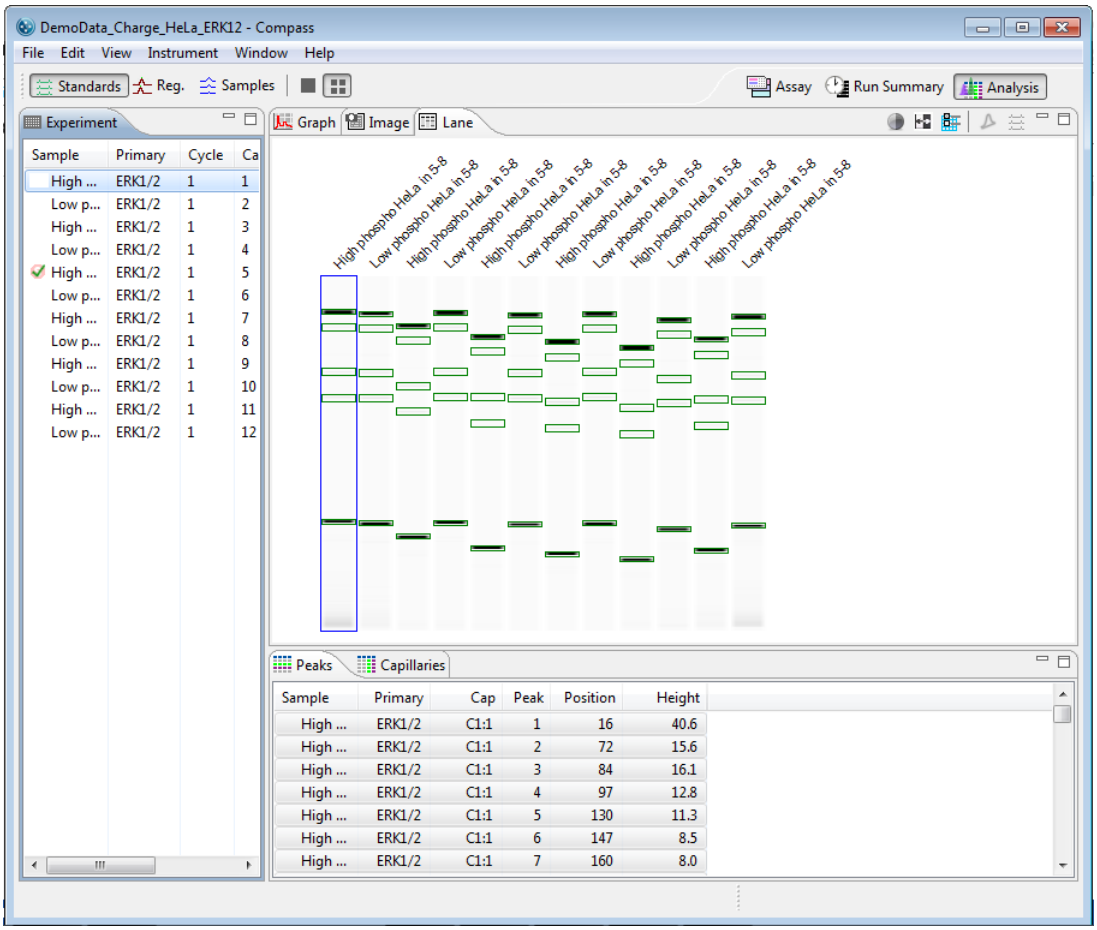
---

c. Repeat the previous steps for the remaining rows in the experiment pane to make sure all standards are identified correctly.

Lane Pane:

a. Click **Multiple View** in the View bar.

b. Click on the first row in the experiment pane, then click the **Lane** tab. Standards will be bands and identified with a green outline. Check that each lane has the appropriate number of fluorescent pI standard bands for the pI Standard Ladder you are using. They will also be identified with a green **S** in the peaks table. The pI standard bands at the low and high end of the pI range in each lane will display greater intensity as they are at higher concentrations and are also used as registration standards. In the following example, the pI standards shown are those for pI Standard Ladder 3 (P/N 040-646). To view band labels, roll the mouse over the individual bands.



If standards are not identified correctly, they can be manually corrected as follows:

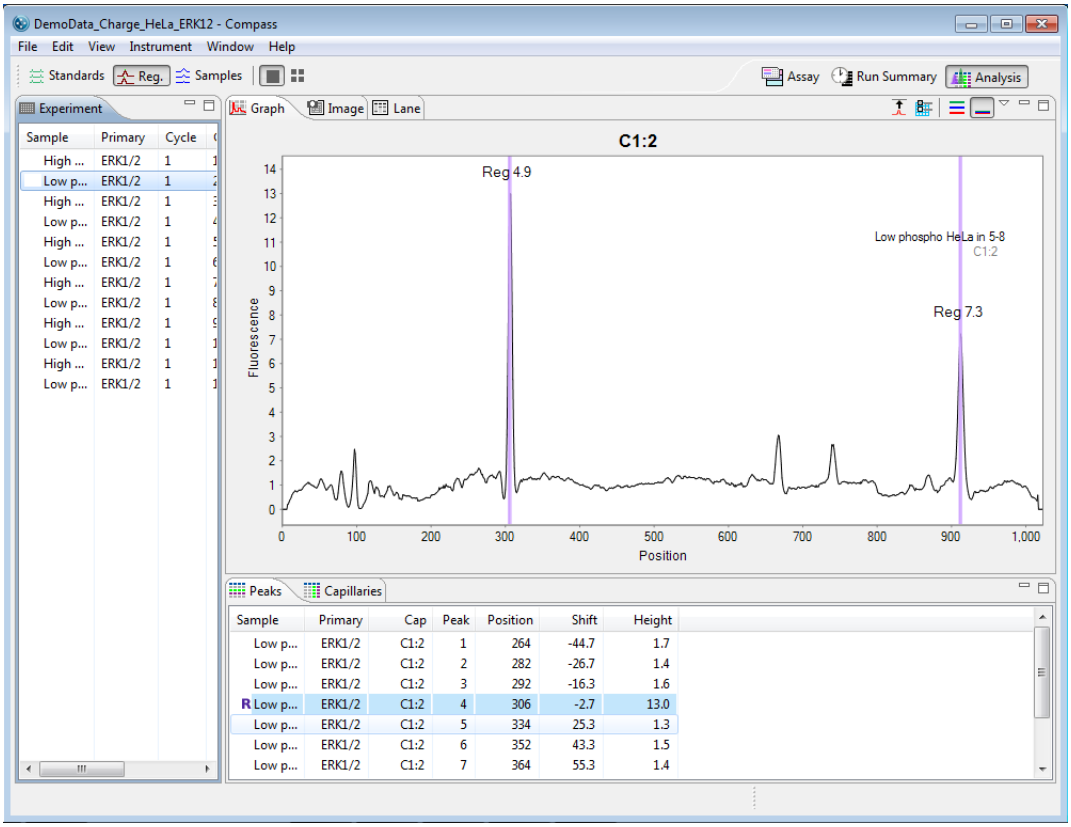
- **If an incorrect band is identified as a standard** - Right click the band in the lane or peaks table and select **Not a Standard**. Compass for Simple Western should correctly reassign the remaining bands as standards.
- **To set an unidentified band as a standard** - Right click the band in the lane or peaks table and select **Force Standard**. Compass for Simple Western will assign the band as a standard, and correctly reassign the remaining standard bands.

c. Repeat the previous steps for the remaining rows in the experiment pane to make sure all standards are identified correctly.

Step 3 – Checking Capillary Registrations

All capillaries should have a single capillary registration peak. To verify the registrations are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Registrations** and **Single View** in the View bar.
3. Click the **Graph** tab.
4. Click on the first row in the experiment pane. The registration peak will be the first and largest peak in the electropherogram. Check that the two registration peaks are identified and labeled Reg 1 and Reg 2 in the electropherogram. They will also be identified with a purple **R** in the peaks table.



Sample	Primary	Cap	Peak	Position	Shift	Height
Low p...	ERK1/2	C1:2	1	264	-44.7	1.7
Low p...	ERK1/2	C1:2	2	282	-26.7	1.4
Low p...	ERK1/2	C1:2	3	292	-16.3	1.6
R Low p...	ERK1/2	C1:2	4	306	-2.7	13.0
Low p...	ERK1/2	C1:2	5	334	25.3	1.3
Low p...	ERK1/2	C1:2	6	352	43.3	1.5
Low p...	ERK1/2	C1:2	7	364	55.3	1.4

5. If the registration peak is not identified correctly, right click the correct registration peak in the electropherogram or peaks table and select **Registration Force**. Compass for Simple Western will assign the new peak as the capillary registration. A lock icon indicating the registration was set manually will display next to the peak in the peaks table.

---

*NOTE: To remove registration peak assignments that were made manually, right click on the peak in the electropherogram or peaks table and click **Registration Clear**.*

---

6. Repeat the previous steps for the remaining rows in the experiment pane to make sure all registrations are identified correctly.

Step 4 – Checking Samples

All immunodetected sample proteins in the graph and lane panes will be labeled automatically with the calculated protein pI.

NOTE: The reported pI for immunodetected sample proteins in Compass for Simple Western may vary slightly from predicted pIs based on sample, buffer and assay conditions.

To verify that sample proteins are identified correctly:

1. Click the **Analysis** screen tab.
2. Click **Show Samples** in the View bar. Verification that sample proteins have been correctly identified can be done in either the graph or lane panes, but manual corrections must be done in the graph pane.

   Graph Pane:

   a. Click **Single View** in the View bar.

   b. Click on the row in the experiment pane that contains the sample you wish to check, then click the **Graph** tab.

If sample peaks are not identified correctly, they can be manually corrected as follows:

- **If an incorrect peak is identified as a sample peak** - Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass for Simple Western will no longer identify it as a sample peak in the electropherogram and the peak data will be removed in the results tables.
- **To set an unidentified peak as a sample peak** - Right click the peak in the electropherogram or peaks table and select **Add Peak**. Compass for Simple Western will calculate and display the results for the peak in the results tables and identify the peak in the electropherogram.

---

*NOTE: To remove sample peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click **Clear All**.*

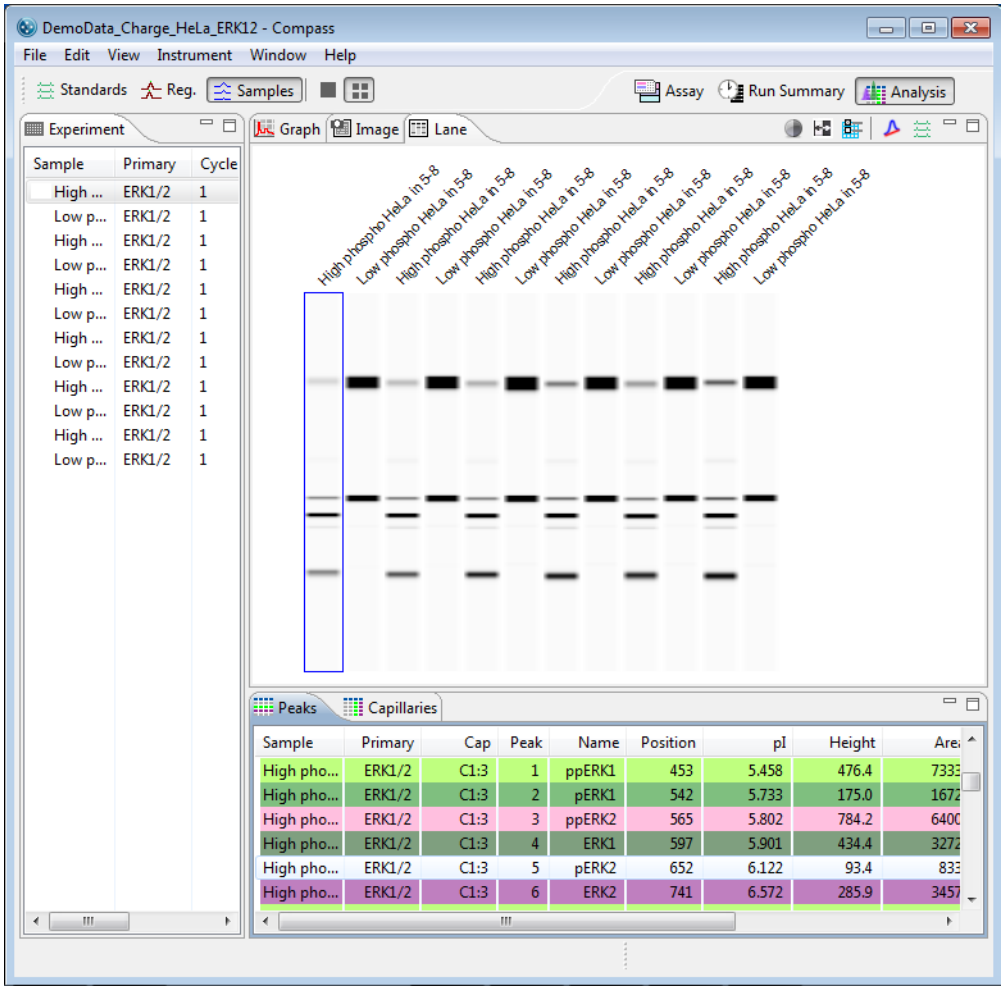
---

c. Repeat the previous steps for the remaining sample rows in the experiment pane to make sure all sample proteins are identified correctly.

Lane Pane:

a. Click either **Single View** or **Multiple View** in the View bar.

b. Click on the row in the experiment pane that contains the sample you wish to check, then click the **Lane** tab. To view sample protein band labels, roll the cursor over the individual bands. If sample bands are not identified correctly, they must be corrected in the graph pane as described in the previous section.



Step 5 – Assigning Peak Names (Optional)

Compass for Simple Western can identify and automatically name sample proteins associated with specific primary antibodies in the run data using user-specified peak name settings. For more information on how to do this see "Peak Names Settings" on page 441.

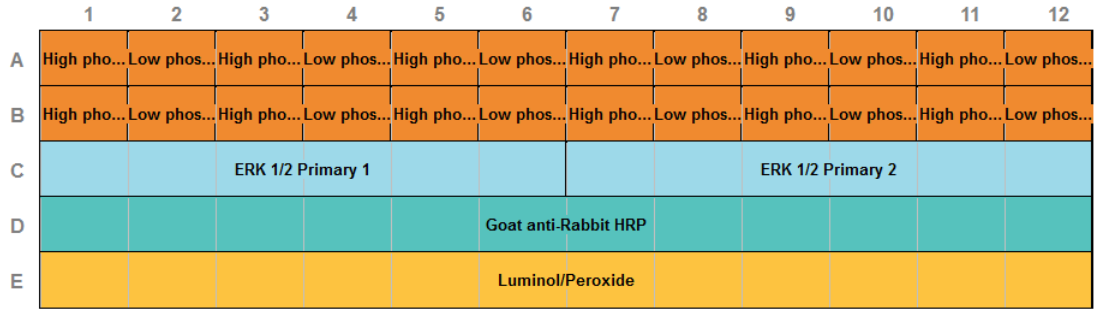
Group Statistics

The Grouping View is used to analyze replicates by calculating the mean, standard deviation, %CV and SEM for named proteins (see "Peak Names Settings" on page 441 for detailed information on entering named proteins). Statistics for each protein are also plotted for easy comparison between samples, antibodies, and proteins.

Using Groups

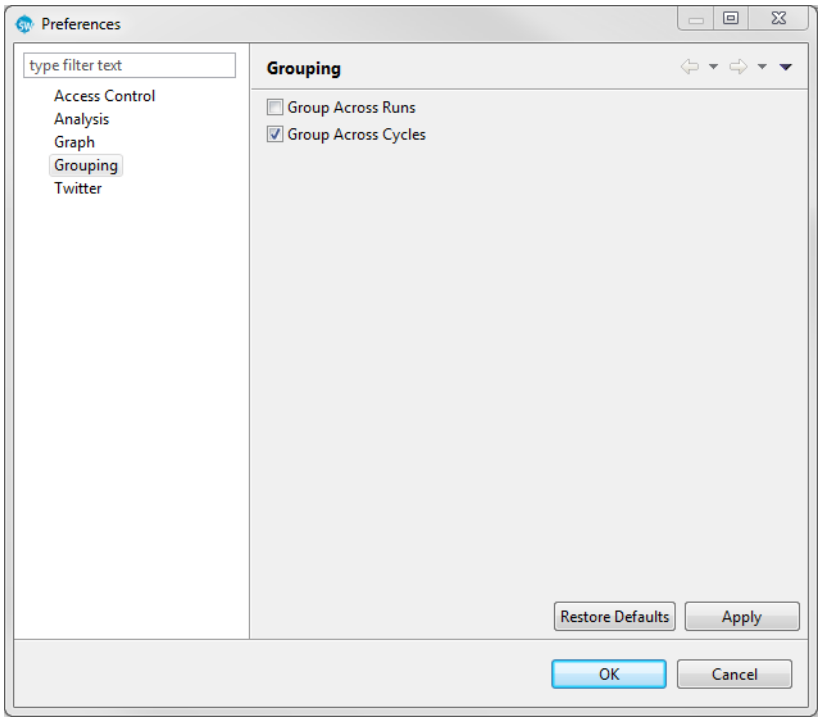
1. A group is automatically created for capillaries with the same sample and primary antibody name, so to use this feature, this information must first be entered into the Assay template. To do this:
   a. Click the **Assay** tab and go to the Template pane.
   b. Enter sample names and primary antibody names as described in "Step 6 - Add Assay Plate Annotations (Optional)" on page 122. Be sure to enter the same sample and/or primary antibody names for the groups of samples you want to calculate statistics for.

      In this example there are two sample types, **High phospho HeLa** and **Low phospho HeLa** which were run with two different antibodies, **ERK 1/2 Primary 1** and **ERK 1/2 Primary 2**.



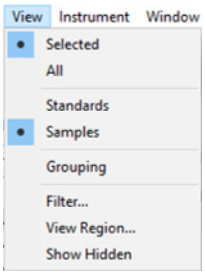
      Each of the two samples were run with the two antibodies in every cycle, and the ERK1/2 antibody generates 6 named peaks.

2. To set a grouping option, go to **Edit** >**Preferences** and select the **Grouping** page. Then check the option you want to use. These options allow you to group capillaries in multiple ways:



- **Group across runs** - Groups capillaries from multiple runs. This option creates fewer groups and generates statistics across multiple runs.
- **Group across cycles** - Groups capillaries run in different cycles. You can use this for Wes runs too.
- **No option selected** - When only one run is open, groups will only contain capillaries from the same cycle. When more than one run is open, groups are created for each run.

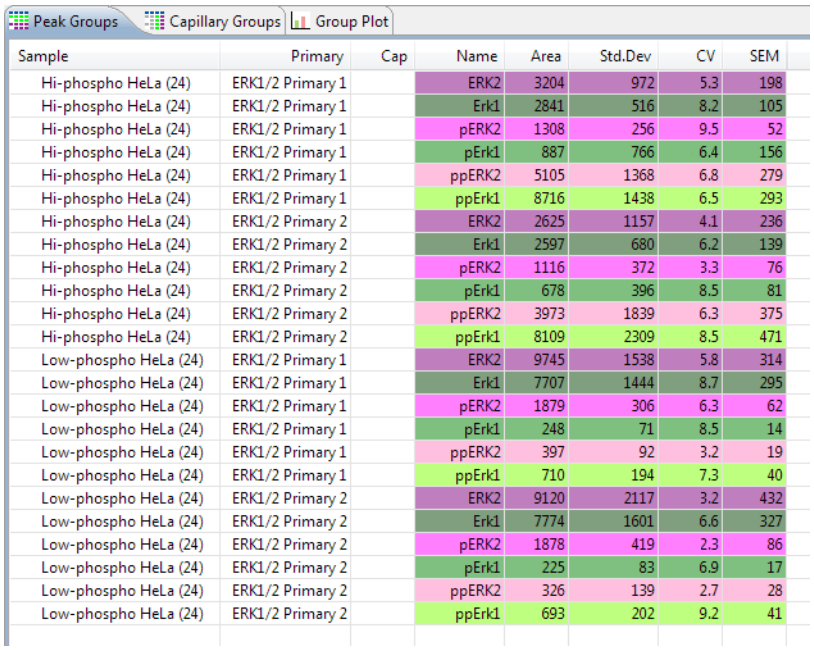
3. Click **Apply** and then select **OK**.
4. Group your results and view the associated statistics by selecting **View** and clicking on **Grouping**.



Viewing Statistics

Peak and Capillary Groups

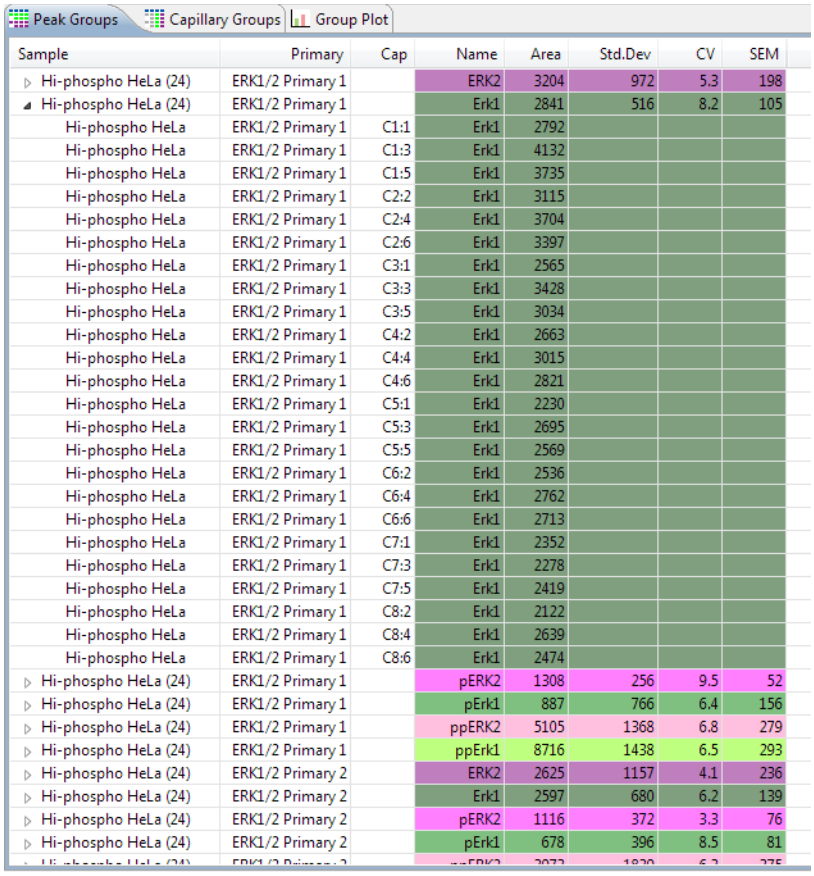
The Peak Groups pane reports statistics for each named protein in every group. Each group shows the statistics for named proteins which includes average area, standard deviation, %CV and SEM. The number in parenthesis after the sample name indicates the number of capillaries in the group.



Peak Groups | Capillary Groups | Group Plot

Sample	Primary	Cap	Name	Area	Std.Dev	CV	SEM
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ERK2	3204	972	5.3	198
Hi-phospho HeLa (24)	ERK1/2 Primary 1		Erk1	2841	516	8.2	105
Hi-phospho HeLa (24)	ERK1/2 Primary 1		pERK2	1308	256	9.5	52
Hi-phospho HeLa (24)	ERK1/2 Primary 1		pErk1	887	766	6.4	156
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ppERK2	5105	1368	6.8	279
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ppErk1	8716	1438	6.5	293
Hi-phospho HeLa (24)	ERK1/2 Primary 2		ERK2	2625	1157	4.1	236
Hi-phospho HeLa (24)	ERK1/2 Primary 2		Erk1	2597	680	6.2	139
Hi-phospho HeLa (24)	ERK1/2 Primary 2		pERK2	1116	372	3.3	76
Hi-phospho HeLa (24)	ERK1/2 Primary 2		pErk1	678	396	8.5	81
Hi-phospho HeLa (24)	ERK1/2 Primary 2		ppERK2	3973	1839	6.3	375
Hi-phospho HeLa (24)	ERK1/2 Primary 2		ppErk1	8109	2309	8.5	471
Low-phospho HeLa (24)	ERK1/2 Primary 1		ERK2	9745	1538	5.8	314
Low-phospho HeLa (24)	ERK1/2 Primary 1		Erk1	7707	1444	8.7	295
Low-phospho HeLa (24)	ERK1/2 Primary 1		pERK2	1879	306	6.3	62
Low-phospho HeLa (24)	ERK1/2 Primary 1		pErk1	248	71	8.5	14
Low-phospho HeLa (24)	ERK1/2 Primary 1		ppERK2	397	92	3.2	19
Low-phospho HeLa (24)	ERK1/2 Primary 1		ppErk1	710	194	7.3	40
Low-phospho HeLa (24)	ERK1/2 Primary 2		ERK2	9120	2117	3.2	432
Low-phospho HeLa (24)	ERK1/2 Primary 2		Erk1	7774	1601	6.6	327
Low-phospho HeLa (24)	ERK1/2 Primary 2		pERK2	1878	419	2.3	86
Low-phospho HeLa (24)	ERK1/2 Primary 2		pErk1	225	83	6.9	17
Low-phospho HeLa (24)	ERK1/2 Primary 2		ppERK2	326	139	2.7	28
Low-phospho HeLa (24)	ERK1/2 Primary 2		ppErk1	693	202	9.2	41

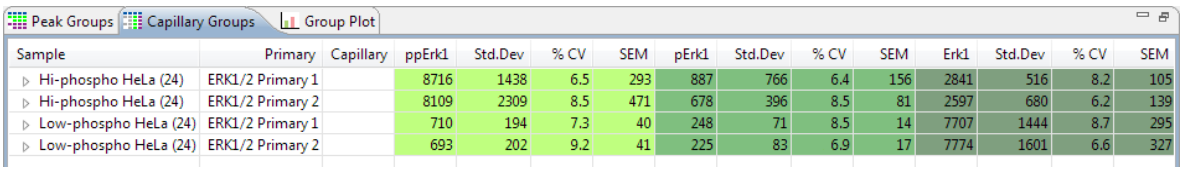
Clicking the arrow next to a group lists the individual capillaries in the group and reported data for each capillary:



Peak Groups | Capillary Groups | Group Plot

Sample	Primary	Cap	Name	Area	Std.Dev	CV	SEM
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ERK2	3204	972	5.3	198
Hi-phospho HeLa (24)	ERK1/2 Primary 1		Erk1	2841	516	8.2	105
Hi-phospho HeLa	ERK1/2 Primary 1	C1:1	Erk1	2792			
Hi-phospho HeLa	ERK1/2 Primary 1	C1:3	Erk1	4132			
Hi-phospho HeLa	ERK1/2 Primary 1	C1:5	Erk1	3735			
Hi-phospho HeLa	ERK1/2 Primary 1	C2:2	Erk1	3115			
Hi-phospho HeLa	ERK1/2 Primary 1	C2:4	Erk1	3704			
Hi-phospho HeLa	ERK1/2 Primary 1	C2:6	Erk1	3397			
Hi-phospho HeLa	ERK1/2 Primary 1	C3:1	Erk1	2565			
Hi-phospho HeLa	ERK1/2 Primary 1	C3:3	Erk1	3428			
Hi-phospho HeLa	ERK1/2 Primary 1	C3:5	Erk1	3034			
Hi-phospho HeLa	ERK1/2 Primary 1	C4:2	Erk1	2663			
Hi-phospho HeLa	ERK1/2 Primary 1	C4:4	Erk1	3015			
Hi-phospho HeLa	ERK1/2 Primary 1	C4:6	Erk1	2821			
Hi-phospho HeLa	ERK1/2 Primary 1	C5:1	Erk1	2230			
Hi-phospho HeLa	ERK1/2 Primary 1	C5:3	Erk1	2695			
Hi-phospho HeLa	ERK1/2 Primary 1	C5:5	Erk1	2569			
Hi-phospho HeLa	ERK1/2 Primary 1	C6:2	Erk1	2536			
Hi-phospho HeLa	ERK1/2 Primary 1	C6:4	Erk1	2762			
Hi-phospho HeLa	ERK1/2 Primary 1	C6:6	Erk1	2713			
Hi-phospho HeLa	ERK1/2 Primary 1	C7:1	Erk1	2352			
Hi-phospho HeLa	ERK1/2 Primary 1	C7:3	Erk1	2278			
Hi-phospho HeLa	ERK1/2 Primary 1	C7:5	Erk1	2419			
Hi-phospho HeLa	ERK1/2 Primary 1	C8:2	Erk1	2122			
Hi-phospho HeLa	ERK1/2 Primary 1	C8:4	Erk1	2639			
Hi-phospho HeLa	ERK1/2 Primary 1	C8:6	Erk1	2474			
Hi-phospho HeLa (24)	ERK1/2 Primary 1		pERK2	1308	256	9.5	52
Hi-phospho HeLa (24)	ERK1/2 Primary 1		pErk1	887	766	6.4	156
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ppERK2	5105	1368	6.8	279
Hi-phospho HeLa (24)	ERK1/2 Primary 1		ppErk1	8716	1438	6.5	293
Hi-phospho HeLa (24)	ERK1/2 Primary 2		ERK2	2625	1157	4.1	236
Hi-phospho HeLa (24)	ERK1/2 Primary 2		Erk1	2597	680	6.2	139
Hi-phospho HeLa (24)	ERK1/2 Primary 2		pERK2	1116	372	3.3	76
Hi-phospho HeLa (24)	ERK1/2 Primary 2		pErk1	678	396	8.5	81

The **Capillary Groups** pane pivots the **Peak Groups** results to show statistics for named protein peaks in individual columns.

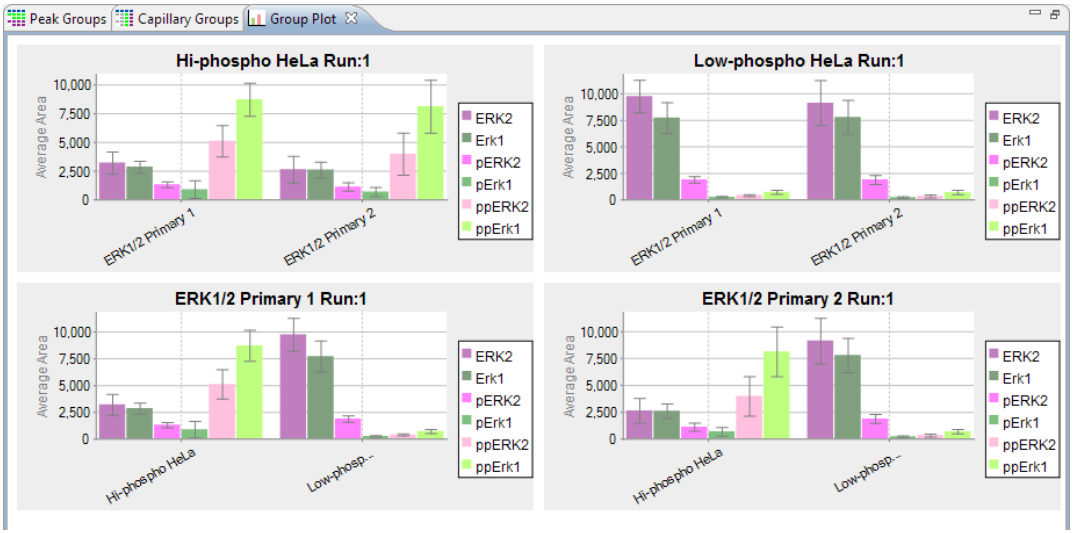


Peak Groups | Capillary Groups | Group Plot

Sample	Primary	Capillary	ppErk1	Std.Dev	% CV	SEM	pErk1	Std.Dev	% CV	SEM	Erk1	Std.Dev	% CV	SEM
Hi-phospho HeLa (24)	ERK1/2 Primary 1		8716	1438	6.5	293	887	766	6.4	156	2841	516	8.2	105
Hi-phospho HeLa (24)	ERK1/2 Primary 2		8109	2309	8.5	471	678	396	8.5	81	2597	680	6.2	139
Low-phospho HeLa (24)	ERK1/2 Primary 1		710	194	7.3	40	248	71	8.5	14	7707	1444	8.7	295
Low-phospho HeLa (24)	ERK1/2 Primary 2		693	202	9.2	41	225	83	6.9	17	7774	1601	6.6	327

Group Plots

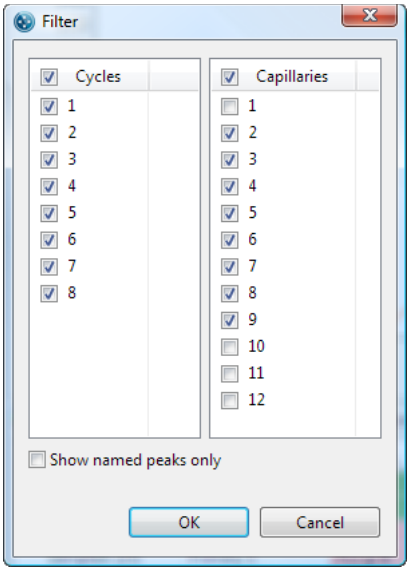
The mean values for named peaks in each group are plotted in bar graphs with error bars showing the standard deviation. The plots compare different antibodies for the same sample and different samples for the same antibody to allow a choice of presentation.



Hiding or Removing Capillaries in Group Analysis

Hidden capillaries are not included in groups. However, hiding capillaries provides an easy way to reject individual capillaries from the statistical analysis. See “Hiding Capillary Data” on page 362 for details on how to do this.

Larger groups of capillaries can also be excluded from analysis. To do this, select **View** and click **Filter**.



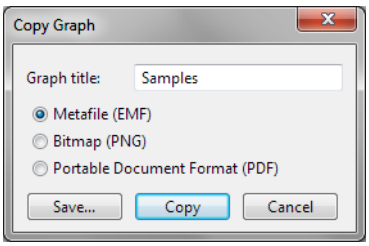
Uncheck the box next to the cycles or capillaries you wish to remove and click **OK**. This data will now be removed from the grouped view statistics.

Copying Data Views and Results Tables

You can copy and paste data and results tables into other documents, or save a data view as a graphic file.

Copying Data Views

1. Click in the graph or lane pane.
2. Select **Edit** in the main menu and click **Copy**, or right click and select **Copy**.
3. If you selected copy from the graph pane the following window will display, click **Copy**. If you selected copy from the lane pane skip to the next step.



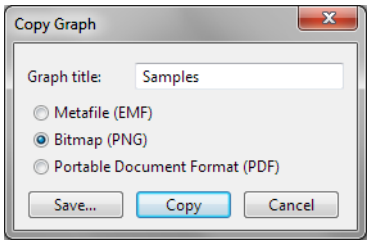
4. Open a document (Microsoft® Word®, Excel®, PowerPoint®, etc.). Right click in the document and select **Paste**. A graphic of the copied data view will be pasted into the document.

Copying Results Tables

1. Click in the peaks or capillaries pane.
2. Select one or multiple rows.
3. Select **Edit** in the main menu and click **Copy**, or right click and select **Copy**.
4. Open a document (Microsoft® Word®, Excel®, PowerPoint®, etc.). Right click in the document and select **Paste**. Data for the rows selected will be pasted into the document.

Saving the Graph View as an Image File

1. Click in the graph pane.
2. Select **Edit** in the main menu and click **Copy**, or right click and select **Copy**.
3. Select an image option (EMF, PNG or PDF) in the pop-up window, then click **Save**.



4. Select a directory to save the file to and enter a file name, then click **OK**.

Exporting Run Files

Results tables and raw plot data can be exported for use in other applications.

Exporting Results Tables

To export the information in the peaks and capillaries tables:

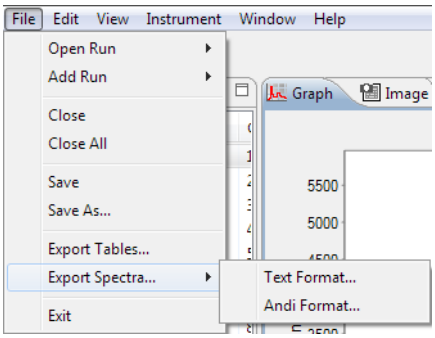
1. Click **File** in the main menu and click **Export Tables**.
2. Select a directory to save the files to and click **OK**. Data will be exported in .txt format.

NOTE: To exclude export of standards data or export results table data in .csv format, see "Setting Data Export Options" on page 465.

Exporting Raw Sample Electropherogram Data

To export raw sample plot data:

1. Click **File** in the main menu and click **Export Spectra**.



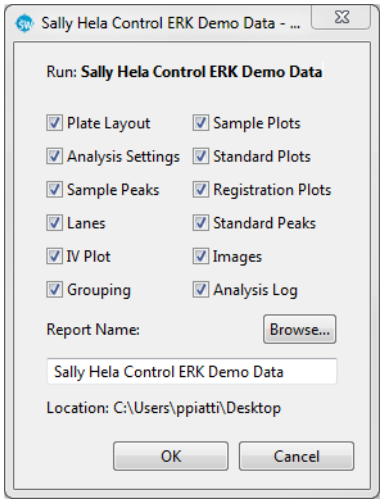
- **To export data in .txt format** - Select **Text Format**. Plots will be exported in one file for all capillaries.
- **To export data in .cdf format** - Select **Andi Format**. Plots will be exported in one file per capillary.

2. Select a directory to save the files to and click **OK**. Data will be exported in the selected format.

Running Reports

Compass for Simple Western v3.0 and higher can create and export run reports in .pdf format.

1. Open your run file, then select **File** > **Run Report**. The Run Report window displays.



2. Select or deselect the information you want included in your report.
3. Change the file name for the report if you don't want to use the default run file name. The report file is saved in the same location as the run file by default.

4. Click **OK**. When the report is done, the directory containing the report will display so you can open the report.

A full report includes the following:

- **Run information**

Run

Run	2012-03-05_11-51-19_HelaControlERKassay
Path	C:\Users\Desktop
Assay	HelaControlERKassay
Schedule	Overlapping with hold
Kit Info	Regular: 12-230 kDa
Instrument	Sally : Sally PL0004 - PL0004
Firmware Version	0.1.15673
Started	Mon 11:56 AM Mar 5, 2012 PST
Completed	Tue 6:35 AM Mar 6, 2012 PST
Error	None

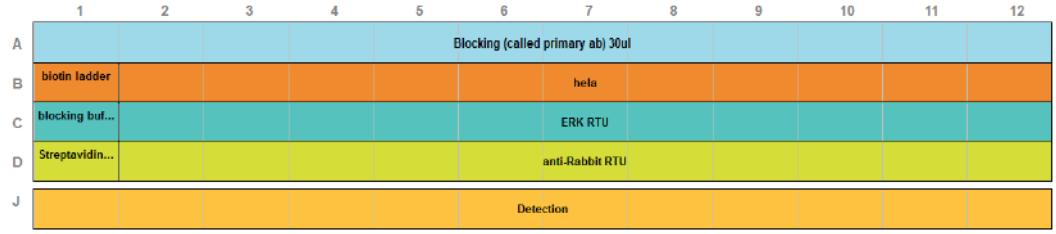
- **Protocol details**

Protocol

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
Separation Matrix								
Well Row	N7	N7	N7	N7	N7	N7	N7	N7
Load Time (sec)	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Stacking Matrix								
Well Row	O7	O7	O7	O7	O7	O7	O7	O7
Load Time (sec)	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Sample								
Well Row	B1	B1	B1	B1	B1	B1	B1	B1
Load Time (sec)	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Separation Time (min)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Separation Voltage (volts)	250	250	250	250	250	250	250	250
Standards Exposure (sec)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Immobilization Time (sec)	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Matrix Removal								
Matrix Removal Time (sec)	140.0	140.0	140.0	140.0	140.0	140.0	140.0	140.0
Matrix Washes	3	3	3	3	3	3	3	3
Matrix Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Wash Soak Time (sec)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0

- **Plate layout**

Plate Layout

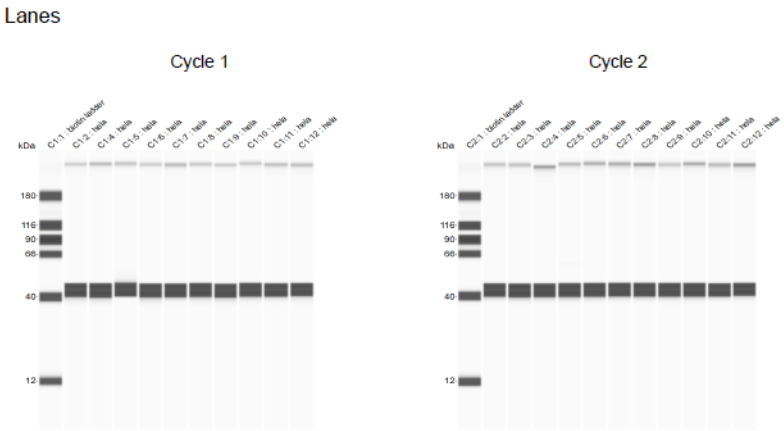


- **Analysis settings**

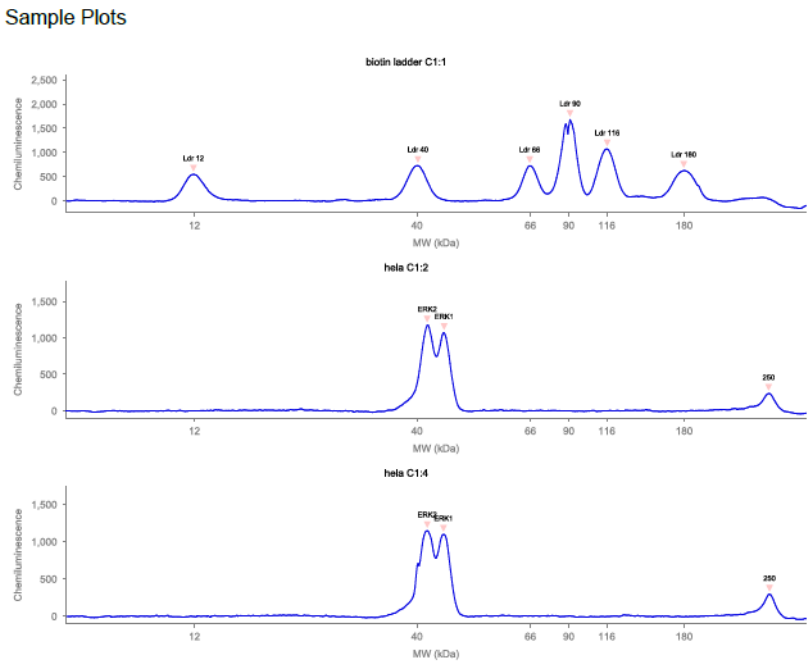
Analysis Settings

Hidden Capillaries	C1:3
Images	
All Cycles Luminescence (sec)	960.0
Peak Fit	fit
Apply To	Default
Range Min (MW kDa)	1.0
Range Max (MW kDa)	250.0
Range View	Analysis
Baseline Threshold	1.0
Baseline Window (pixels)	15.0
Baseline Stiffness	1.0
Peak Find Threshold	20.0
Peak Find Width (pixels)	13.0
Peak Find Area Calculation	Gaussian Fit
Peak Name	ERK-PLCg
Apply To	All
ERK2 (MW kDa)	42
ERK2 Range (%)	10
ERK2 Control	no
ERK1 (MW kDa)	44
ERK1 Range (%)	10
ERK1 Control	no

- **Lane views (for all cycles on Peggy Sue and Sally Sue):**



- **Sample plots for each capillary of each cycle:**



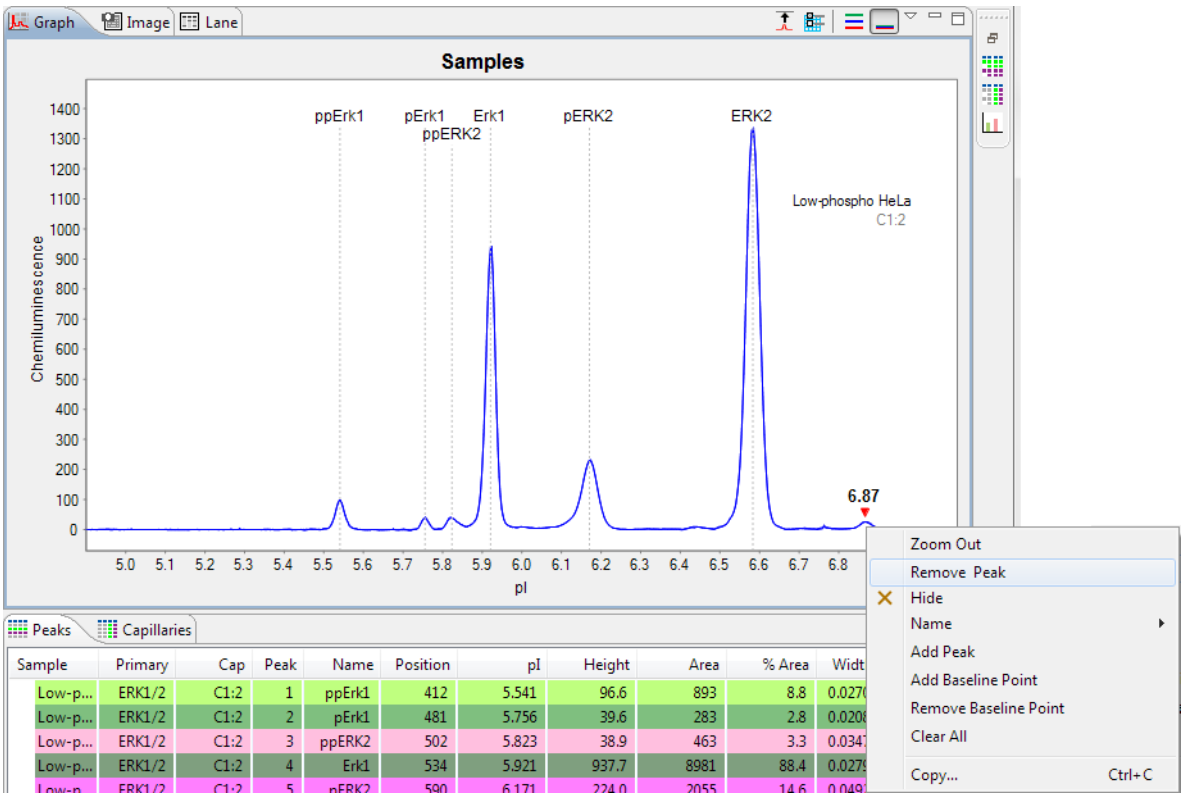
You can also include the fluorescent standards graphs for each capillary, registration graphs (Peggy Sue and Sally Sue only) and the standard peaks position and registration peak offsets in pixels in the report.

Changing Sample Protein Identification

Compass for Simple Western allows you to customize what sample proteins are reported in the results tables by making manual adjustments in the electropherogram or peaks table.

Adding or Removing Sample Data

1. Click **Show Samples** in the View bar.
2. Click **Single View** in the View bar.
3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.
    - **To remove a peak from the data -** Right click the peak in the electropherogram or peaks table and select **Remove peak**. Compass for Simple Western will no longer identify it as a sample peak in the electropherogram and the peak data will be removed in the results tables.



- **To add an unidentified peak to the data -** Right click the peak in the electropherogram or peaks table and select **Add Peak**. Compass for Simple Western will calculate and display the results for the peak in the results tables and identify the peak in the electropherogram.

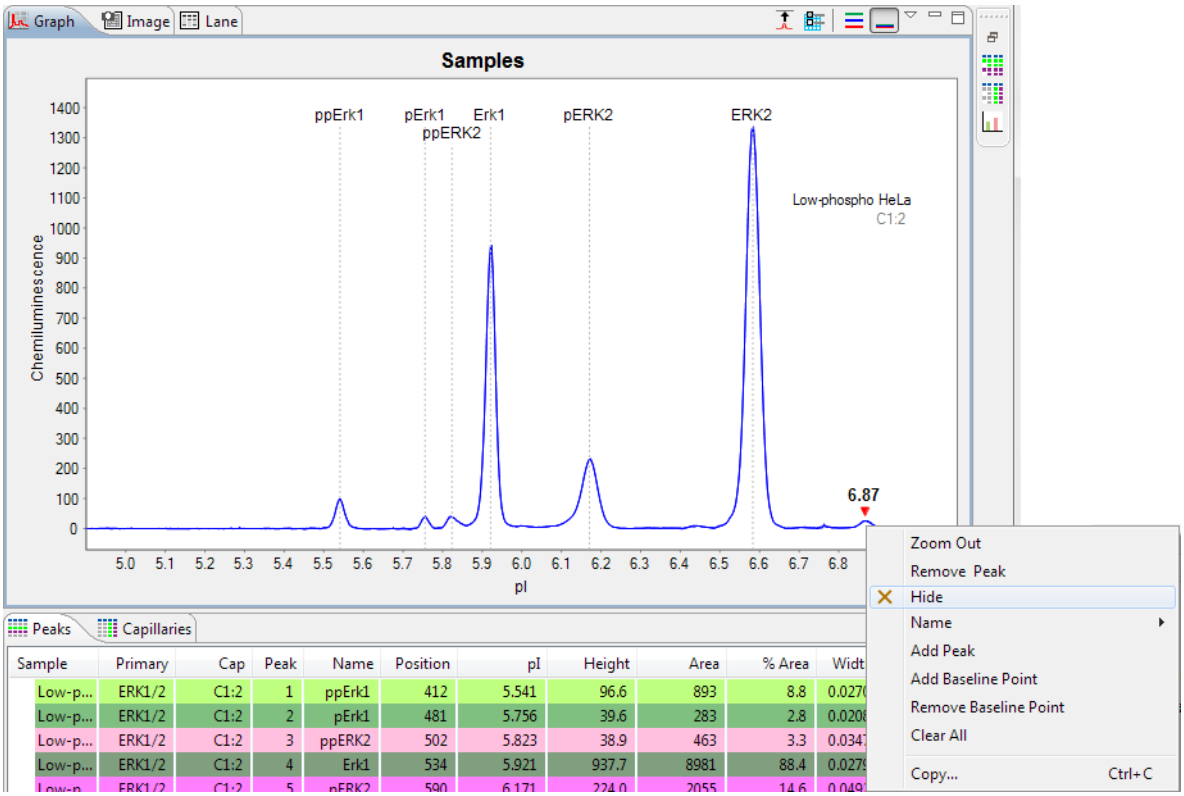
NOTES:
To remove sample peak assignments that were made manually and go back to the original view of the data, right click in the electropherogram and click ***Clear All****.*

Virtual blot data in the lane pane will also update to reflect changes made in the graph pane.

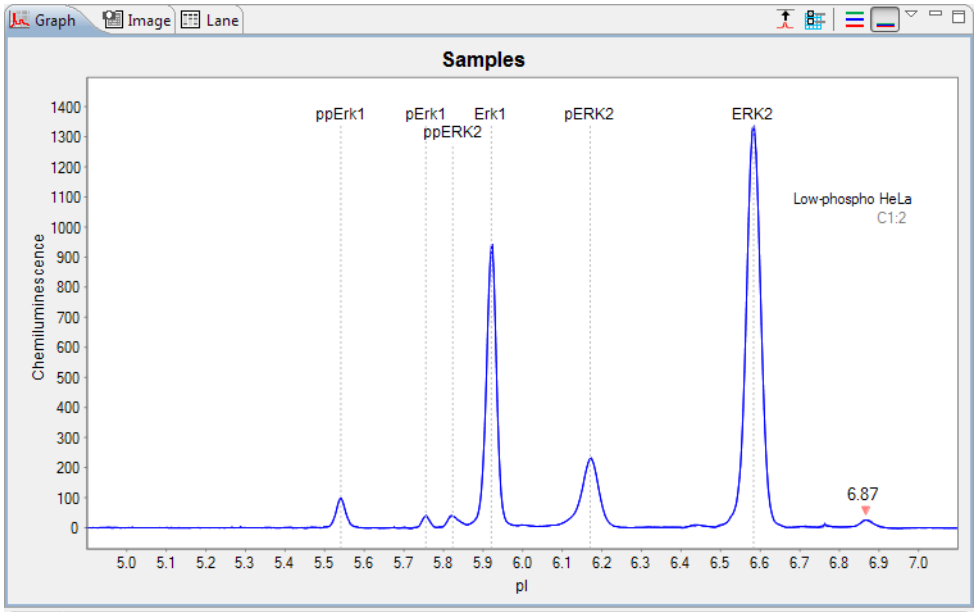
Hiding Sample Data

You can hide the results for a sample protein in the results tables without completely removing it from the reported results. To to this:

1. Click **Show Samples** in the View bar.
2. Click **Single View** in the View bar.
3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.
4. Right click the peak in the electropherogram or peaks table and select **Hide peak**. Compass for Simple Western will hide the peak data in the results tables.


Graph
Image
Lane
Samples
ppErk1
pErk1
ppERK2
Erk1
pERK2
ERK2
Low-phospho HeLa
C1:2
Chemiluminescence
6.87
pI
Zoom Out
Remove Peak
Hide
Name
Add Peak
Add Baseline Point
Remove Baseline Point
Clear All
Copy... Ctrl+C
Peaks
Capillaries
Sample Primary Cap Peak Name Position pI Height Area % Area Widt
Low-p... ERK1/2 C1:2 1 ppErk1 412 5.541 96.6 893 8.8
Low-p... ERK1/2 C1:2 2 pErk1 481 5.756 39.6 283 2.8
Low-p... ERK1/2 C1:2 3 ppERK2 502 5.823 38.9 463 3.3
Low-p... ERK1/2 C1:2 4 Erk1 534 5.921 937.7 8981 88.4

5. To view hidden peak data, click **View** in the main menu and click **Show Hidden**. Hidden peak data will display in the results table and be marked with an **X**.



Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
Low-p...	ERK1/2	C1:2	1	ppErk1	412	5.541	96.6	893	8.8	0.0270	231.2
Low-p...	ERK1/2	C1:2	2	pErk1	481	5.756	39.6	283	2.8	0.0208	103.8
Low-p...	ERK1/2	C1:2	3	ppERK2	502	5.823	38.9	463	3.3	0.0347	79.1
Low-p...	ERK1/2	C1:2	4	Erk1	534	5.921	937.7	8981	88.4	0.0279	2218.7
Low-p...	ERK1/2	C1:2	5	pERK2	590	6.171	224.0	2055	14.6	0.0492	542.4
Low-p...	ERK1/2	C1:2	6	ERK2	667	6.583	1345.5	11523	82.1	0.0398	3382.2
✕ Low-p...	ERK1/2	C1:2	7		724	6.869	25.6	212		0.0385	63.5

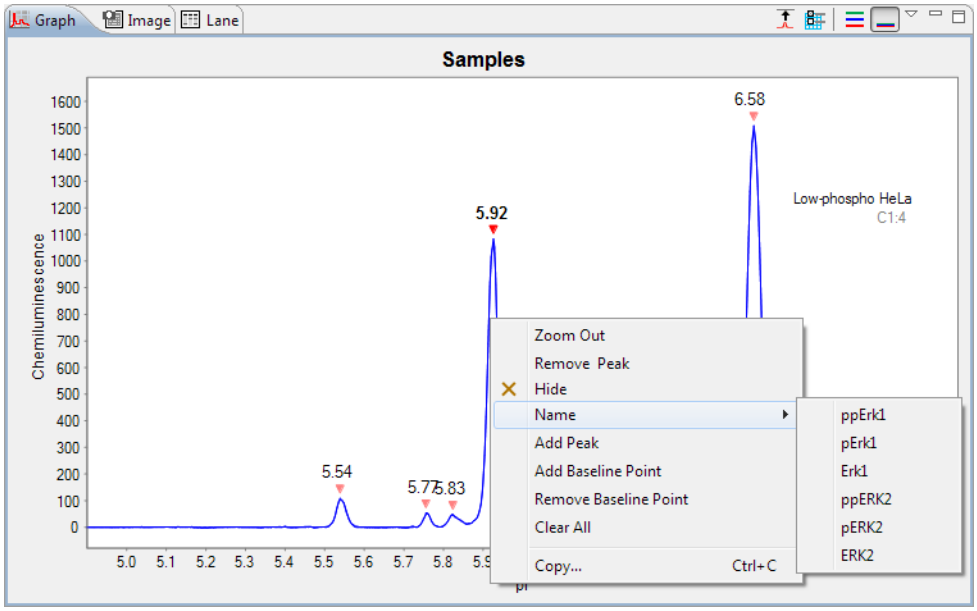
6. To unhide a peak, right click on the peak in the electropherogram or peaks table and select **Unhide Peak**.

Changing Peak Names for Sample Data

If Compass for Simple Western did not automatically name a sample protein associated with a specific primary antibody, this can be adjusted manually. To do this:

1. Click **Show Samples** in the View bar.
2. Click **Single View** in the View bar.
3. Click on the row in the experiment pane that contains the sample you wish to correct, then click the **Graph** tab.

4. Right click the peak in the electropherogram or peaks table and click **Name**, then click a name in the list. Compass for Simple Western will change the peak name in the electropherogram and results tables, and adjust peak names for other sample proteins accordingly.



NOTES:

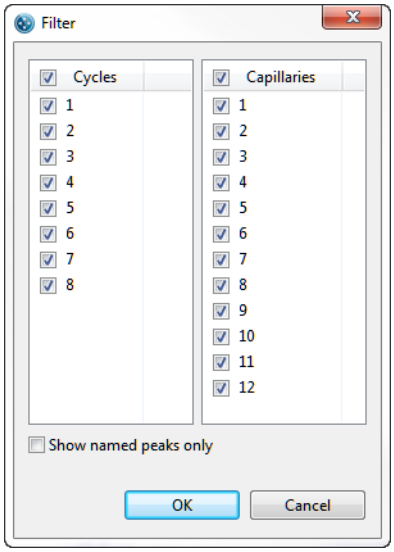
For details on how to specify peak name settings, see "Peak Names Settings" on page 441.

Virtual blot data in the lane pane will also update to reflect changes made in the graph pane.

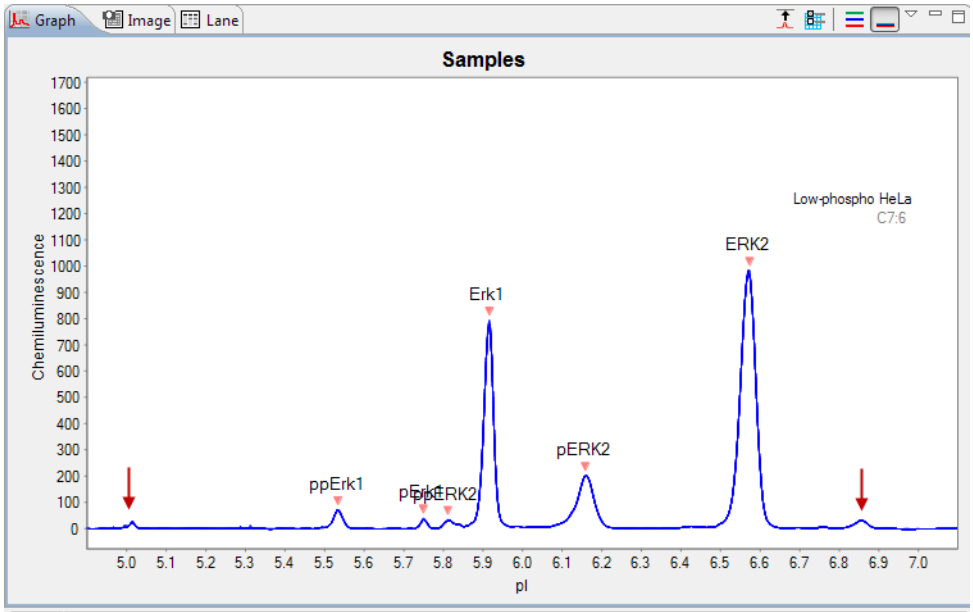
Displaying Sample Data for Named Peaks Only

You can adjust the sample data to display results only for user-specified named peaks. To do this:

1. Click **Show Samples** in the View bar.
2. Click **View** in the main menu and click **Filter**.
3. Check the **Show Named Peaks only** box and click **OK**.



Compass for Simple Western will hide peak data in the electropherogram and results tables for all unnamed peaks, and instead only display data for named peaks in the electropherogram and results tables.



Peaks | Capillaries

Sample	Primary	Cap	Peak	Name	Position	pI	Height	Area	% Area	Width	S/N
Low-p...	ERK1/2	C7:6	2	ppErk1	352	5.534	70.3	628	7.7	0.0259	159.9
Low-p...	ERK1/2	C7:6	3	pErk1	422	5.750	38.3	230	2.8	0.0174	89.8
Low-p...	ERK1/2	C7:6	4	ppERK2	442	5.815	30.0	375	3.3	0.0361	59.6
Low-p...	ERK1/2	C7:6	5	Erk1	476	5.914	788.7	7286	89.5	0.0268	1764.7
Low-p...	ERK1/2	C7:6	6	pERK2	531	6.159	194.8	1811	15.8	0.0498	436.5
Low-p...	ERK1/2	C7:6	7	ERK2	608	6.570	984.9	9257	80.9	0.0430	2195.6

Changing the Virtual Blot View

Options in the lane pane let you change the contrast or invert the virtual blot, remove baseline noise, change lane labels or overlay standards data on sample lanes.

The lane pane toolbar has the following options:


Contrast Adjustment


Invert

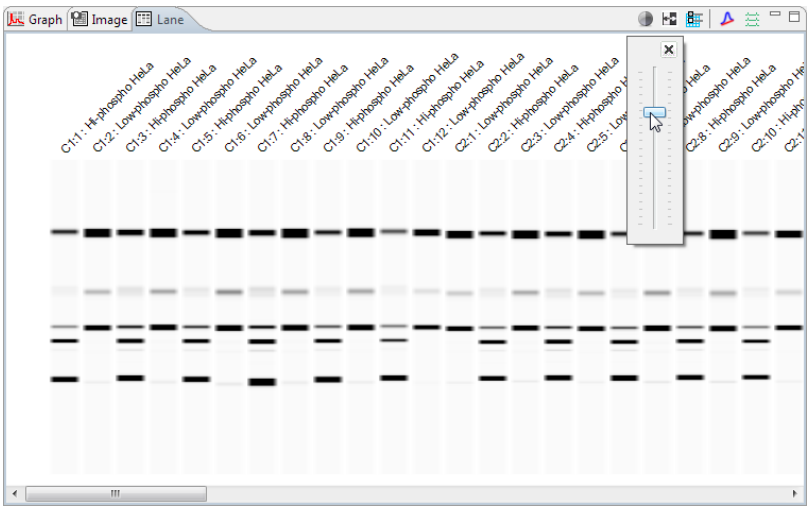

Lane Options


Remove Baseline


Overlay Standards Data

Adjusting the Contrast

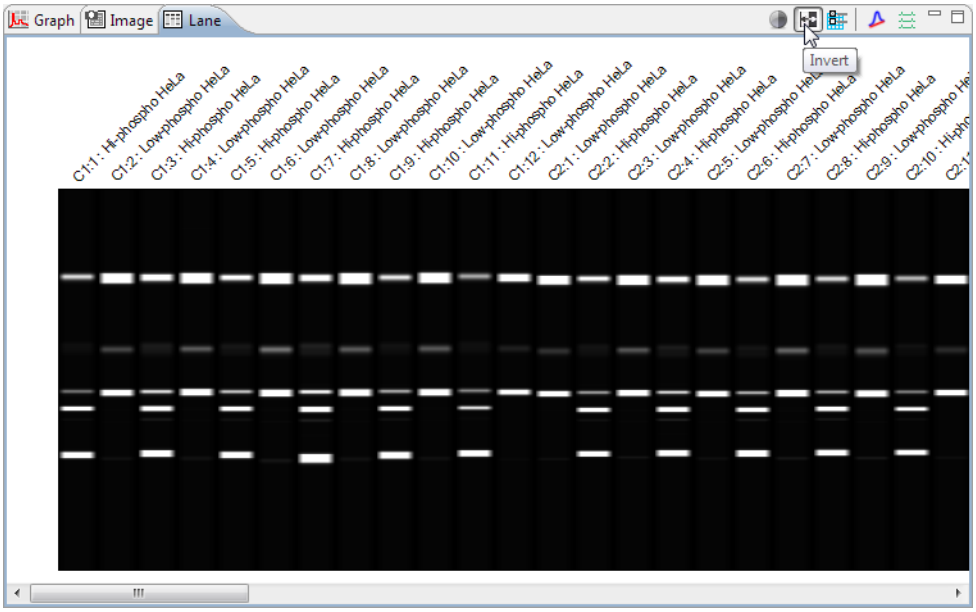
1. Click the **Contrast Adjustment** button. The contrast tool will display:



2. Click the bar and drag it up or down to adjust the contrast.
3. When finished, click **X** to close the tool.

Inverting the Virtual Blot

1. Click the **Invert** button. The virtual blot image will invert:

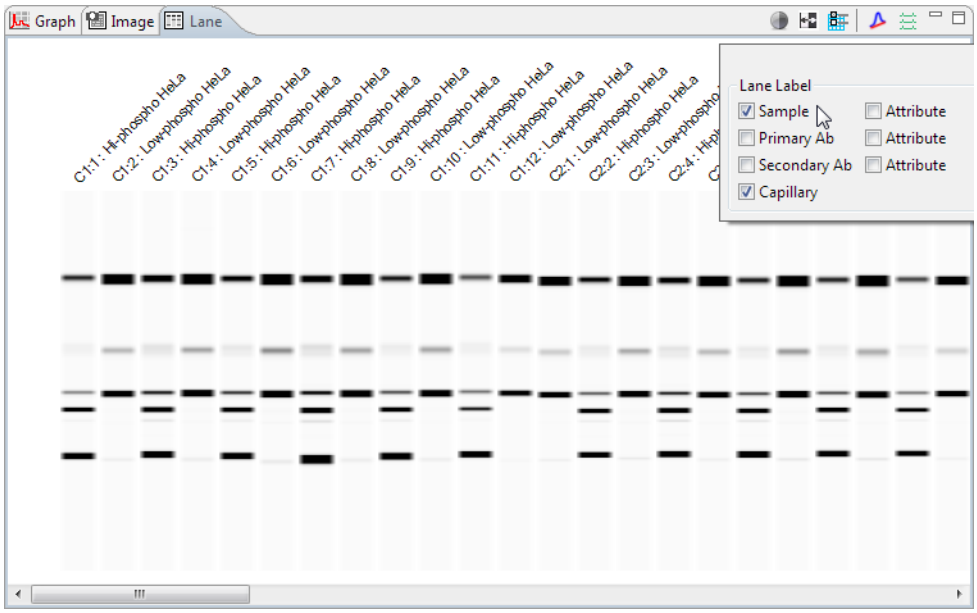


2. Click the **Invert** button again to return to the default view.

Selecting Lane Labels

The labels shown above the lanes in the virtual blot can be customized. To do this:

1. Click the **Lane Options** button. The label box will display:



2. Check one or multiple label boxes, and uncheck those you don't want to display. To remove labels completely, uncheck all boxes. The following label options are available:

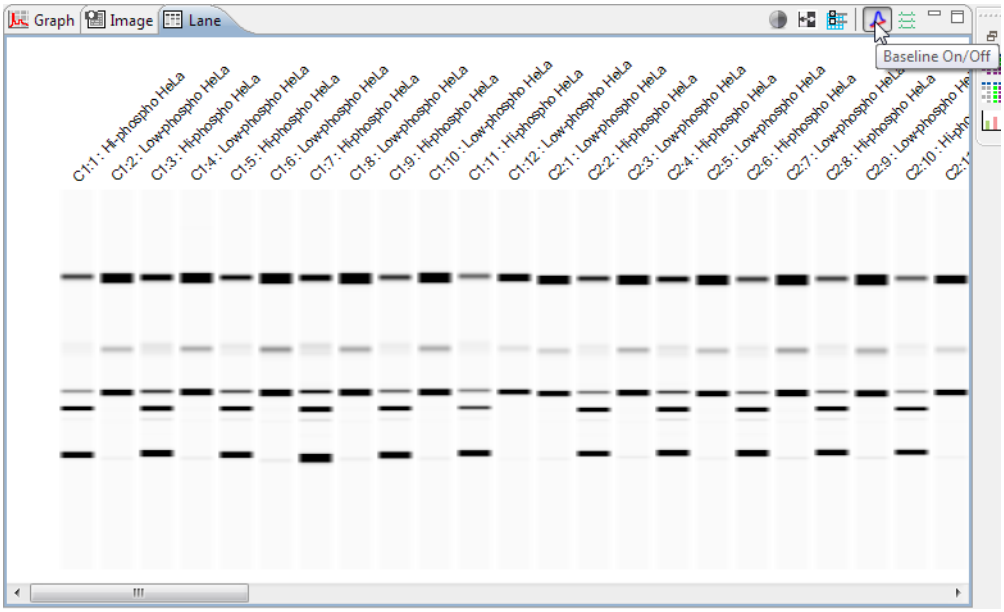
   - **Sample** - Sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
   - **Primary** - Primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
   - **Secondary Ab** - Secondary antibody name. If a secondary antibody name was entered in the assay template (Assay screen), those names will display here. Otherwise, Secondary (default name) will display.
   - **Capillary** - Cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3

NOTE: Peggy Sue runs up to eight cycles (12 capillaries at a time), so the cycle number displayed will always be C1-C8 depending on the number of cycles programmed.

   - **Attributes** - Attribute text. If attribute information was entered for any of the rows or wells in the assay template (Assay screen), up to four can be selected and displayed as lane labels.

Viewing the Uncorrected Sample Baseline

1. Click the **Remove Baseline** button (active for sample data only). This will remove the automatic baseline correction.

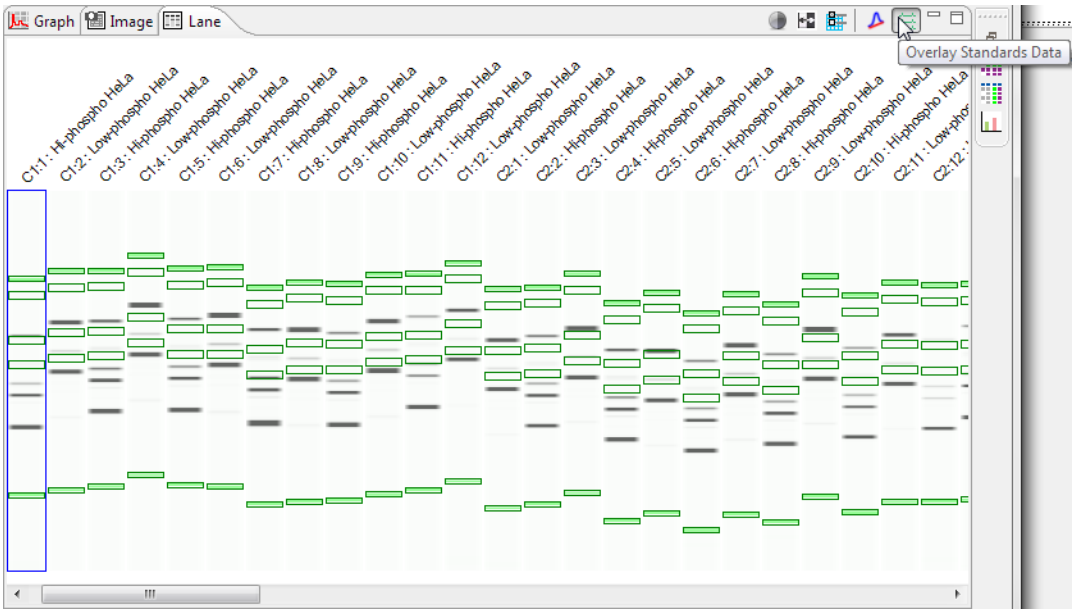


2. Click the **Remove Baseline** button again to return to the default, baseline corrected view.

Overlaying Standards Data on Sample Lanes

Data for the standards can be overlaid on the sample data in the virtual blot so you can view the raw, unaligned (uncorrected) lane data. To do this:

1. Click the **Overlay Standards Data** button (active for sample data only). An overlay of the raw sample and standards data will display:

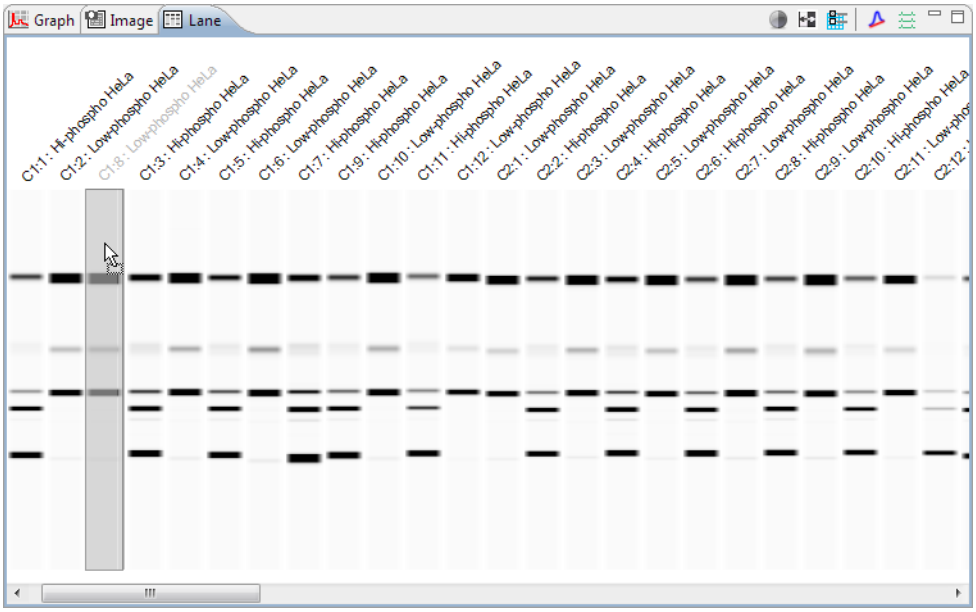


2. Click the **Overlay Standards Data** button again to return to the default, auto-aligned view.

Moving Lanes in the Virtual Blot View

Lanes in the virtual blot view can be moved to make it easier to compare specific lanes of data. To do this:

1. Click a lane in the virtual blot. Hold the mouse button down and drag the lane to a new position.



2. Release the mouse button. The lane will now be repositioned in the virtual blot view.

Changing the Electropherogram View

Options in the graph pane let you zoom and scale electropherograms, overlay or stack plots and change the peak and plot information displayed.

The graph pane toolbar has the following options:


Auto Scale

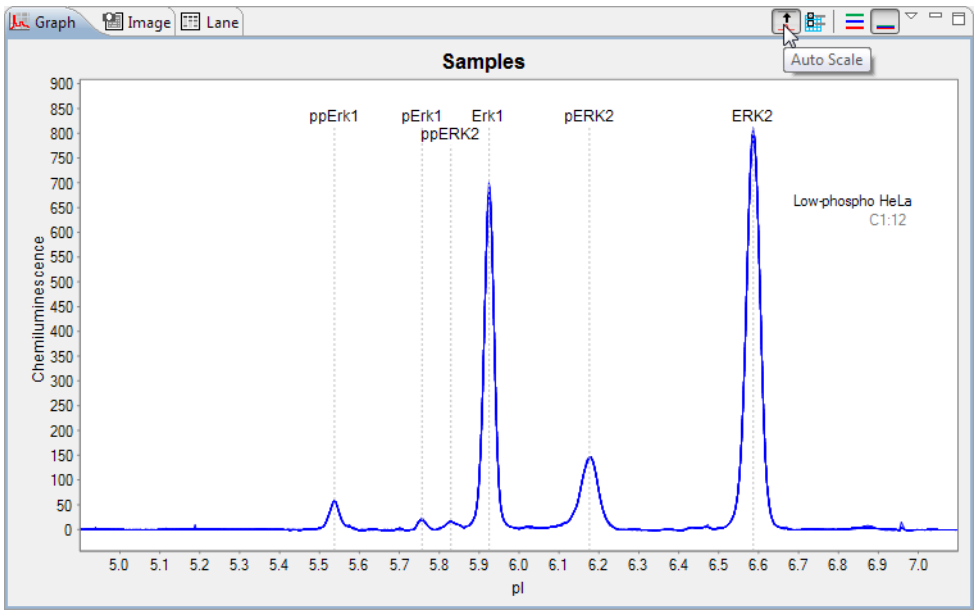

Graph Options


Stack the Plots

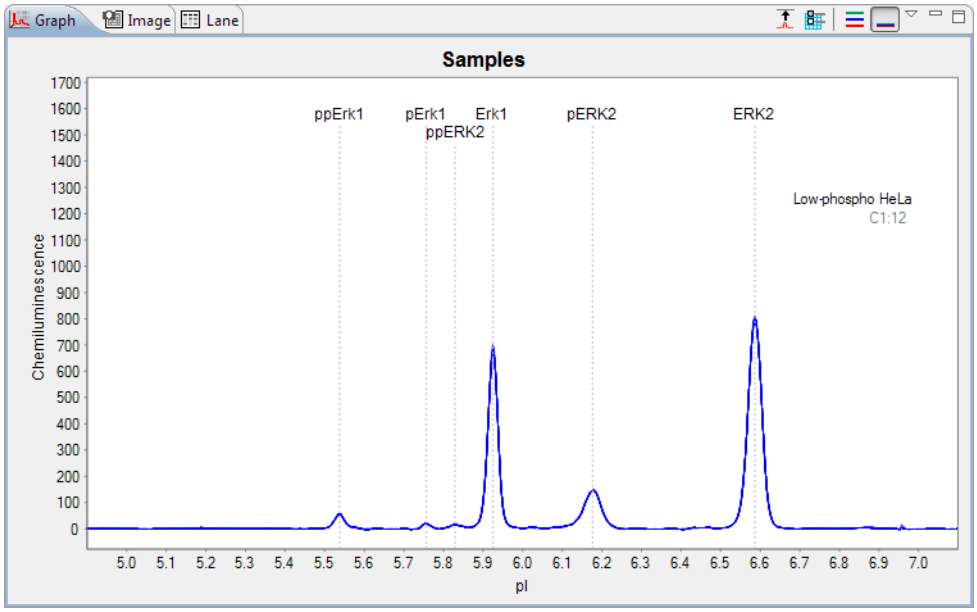

Overlay the Plots

Autoscaling the Electropherogram

Click the **Autoscale** button to scale the y-axis to the largest peak in the electropherogram.



Click the **Autoscale** button again to return to default scaling.

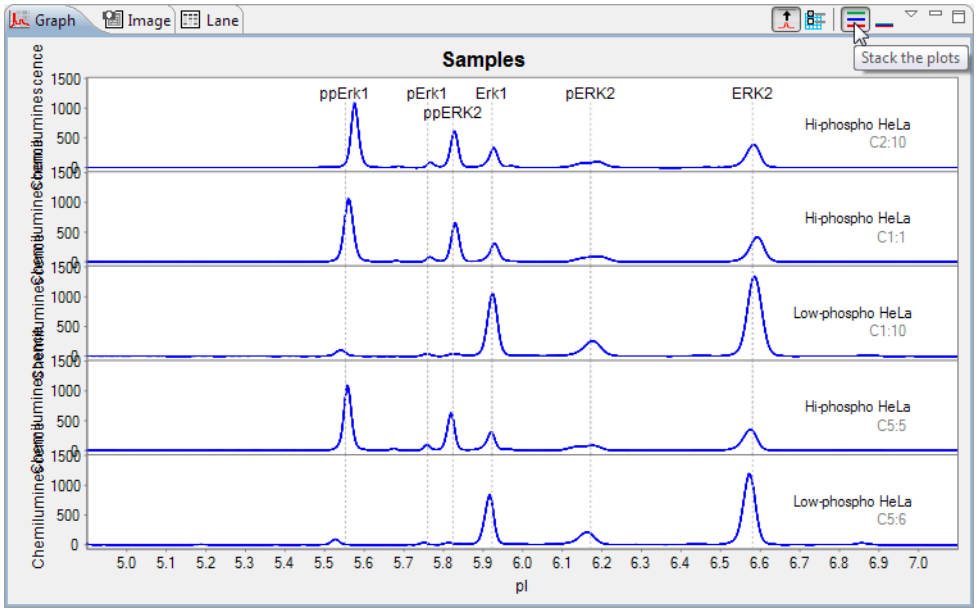


Stacking Multiple Electropherograms

Electropherogram data for multiple capillaries can be stacked vertically in the graph pane for comparison. To do this:

1. Click **Single View**.
2. Select multiple rows in the experiment pane.

3. Click the **Stack the Plots** button. The individual electropherograms for each row selected will stack in the graph pane.

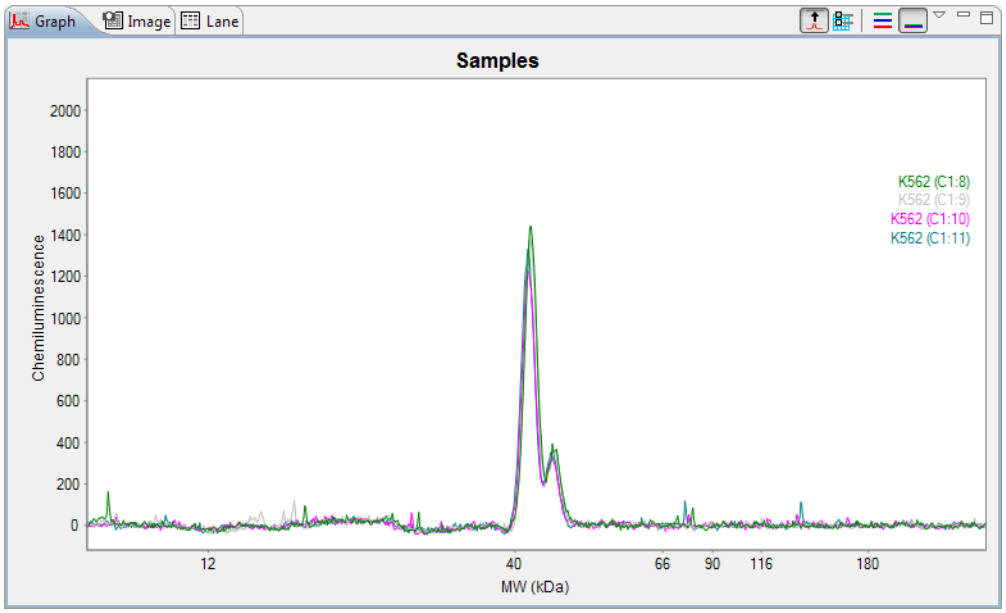


You can customize the colors used for the stacked plot display. For more information see "Selecting Custom Plot Colors for Graph Overlay" on page 466.

Overlaying Multiple Electropherograms

The electropherogram data for multiple capillaries can also be overlaid on top of each other for comparison in the graph pane. To do this:

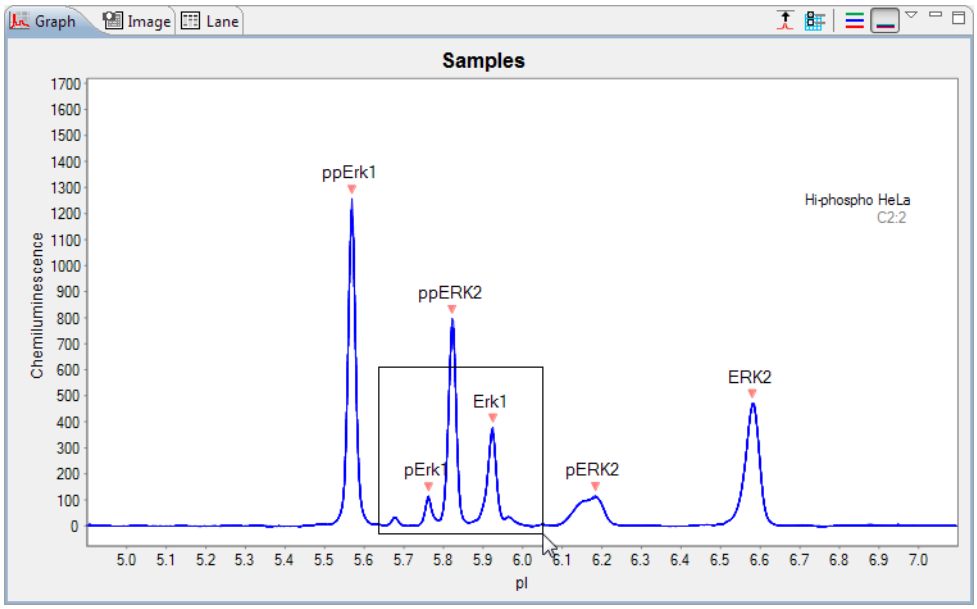
1. Click **Single View**.
2. Select multiple rows in the experiment pane.
3. Click the **Overlay the Plots** button. The individual electropherograms for each row selected will overlay in the graph pane.



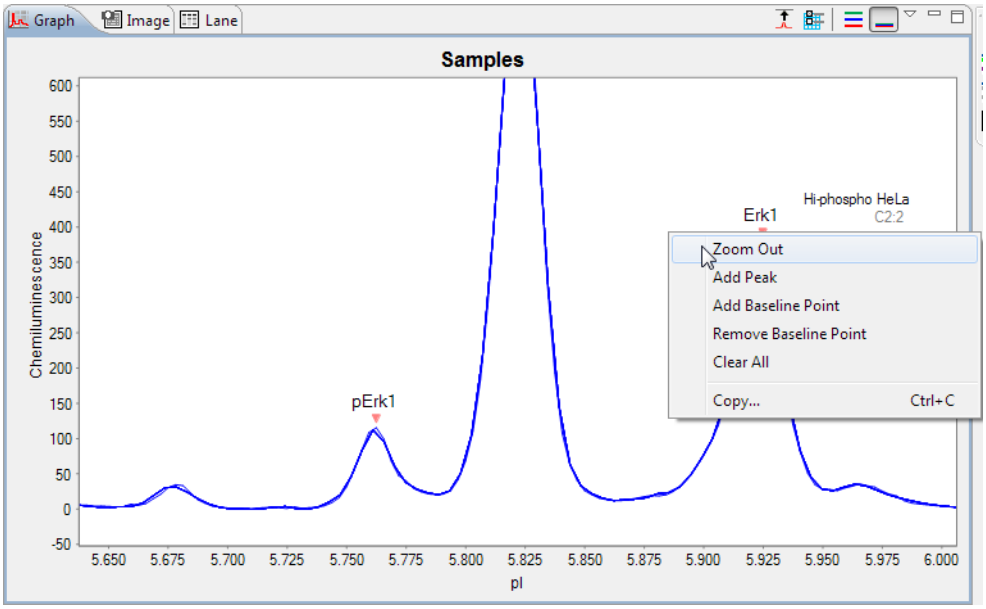
You can customize the colors used for the overlay plot display. For more information see "Selecting Custom Plot Colors for Graph Overlay" on page 466.

Zooming

To zoom in on a specific area of the electropherogram, hold the mouse button down and draw a box around the area with the mouse:

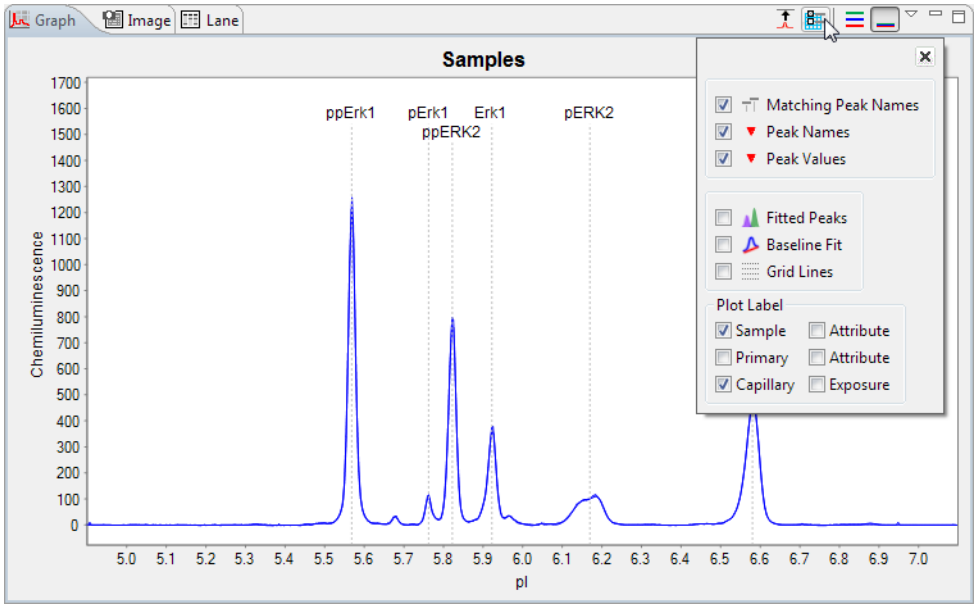


To return to default scaling, right click in the electropherogram and click **Zoom Out**.



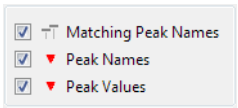
Customizing the Data Display

You can customize electropherogram peak labels, plot labels and display options. To do this, select the **Graph Options** button.

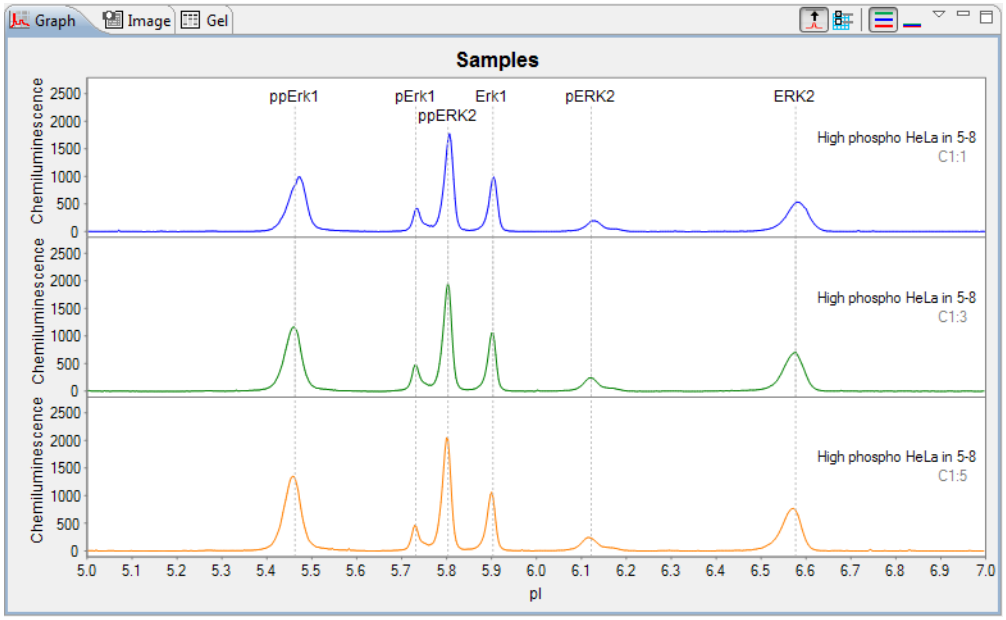


Peak Labels

You can customize the labels used to identify peaks in the electropherogram with these options:

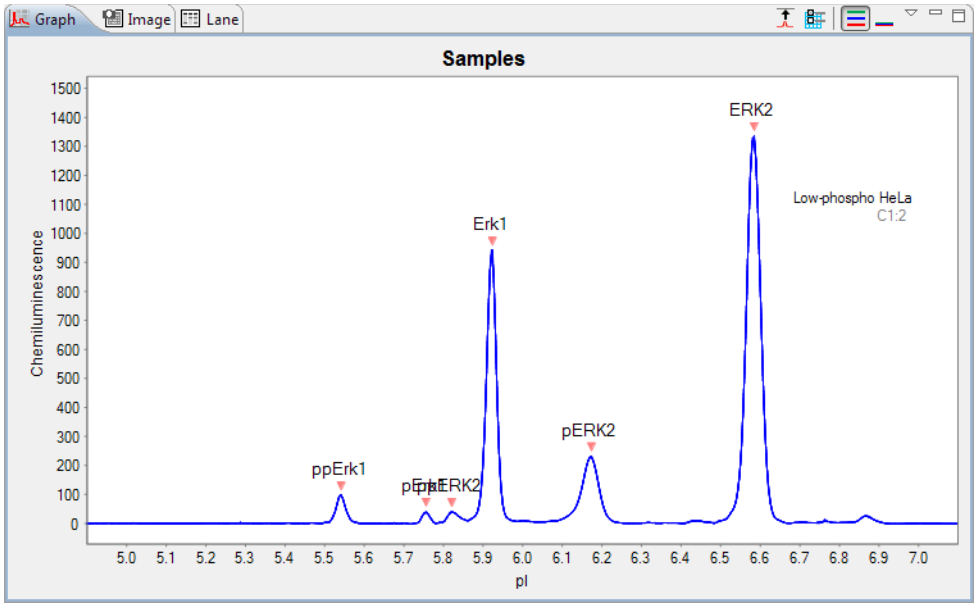


Matching Peak Names - Checking this box will draw vertical lines through each named peak. Using this option with Stack the Plots or Overlay the Plots features is useful for visual comparison of named peaks across multiple capillaries.



- **Peak Names** - Checking this box will display peak name labels on all named peaks in the electropherogram.

NOTE: If more than one peak label option is selected, peak name labels will always be used for named peaks.

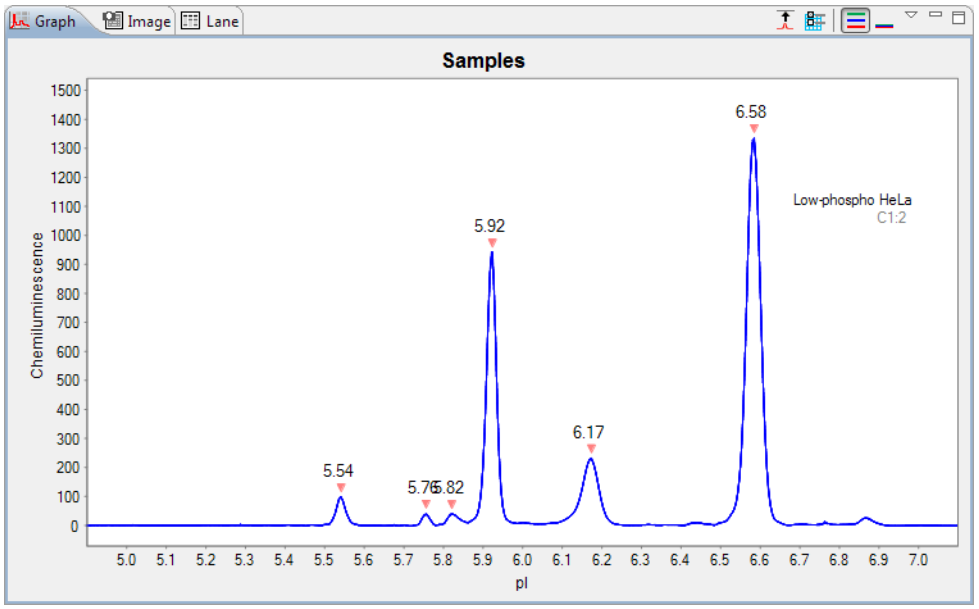


- **Peak Values** - Checking this box will display the pI labels on all peaks in the electropherogram.

NOTES:
When viewing standards and registration data, this option is called Peak Positions. Labels displayed are peak positions rather than pI.

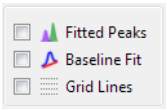
If more than one peak label option is selected, peak name labels will always be used for named peaks.

The reported pI for immunodetected sample proteins in Compass for Simple Western may vary slightly from predicted pIs based on sample, buffer and assay conditions.



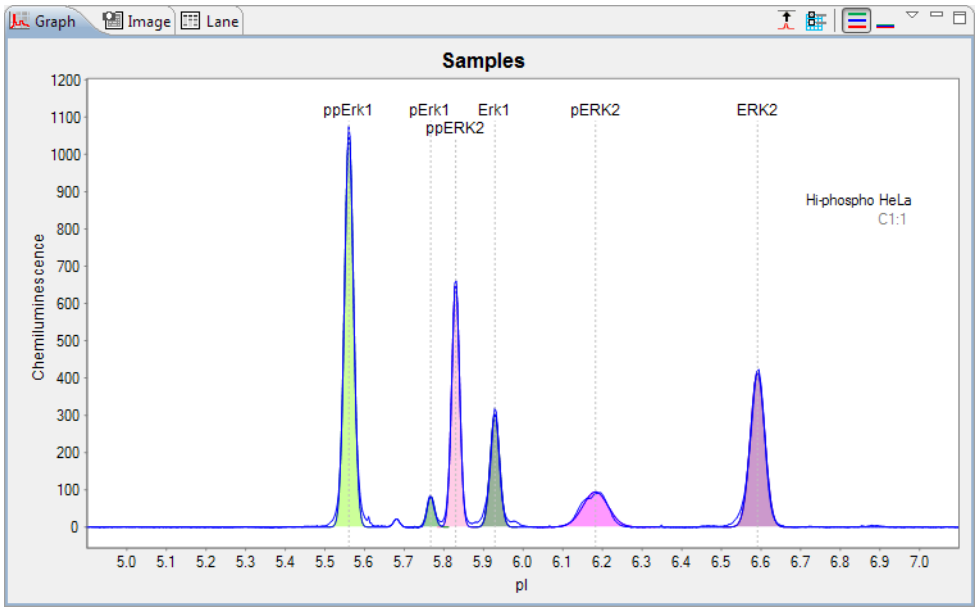
Baseline and Grid Options

You can view the calculated baseline fit, peak integration and show grid lines with these options.



- **Fitted peaks** - Checking this box will display how the peaks were fit by the software.

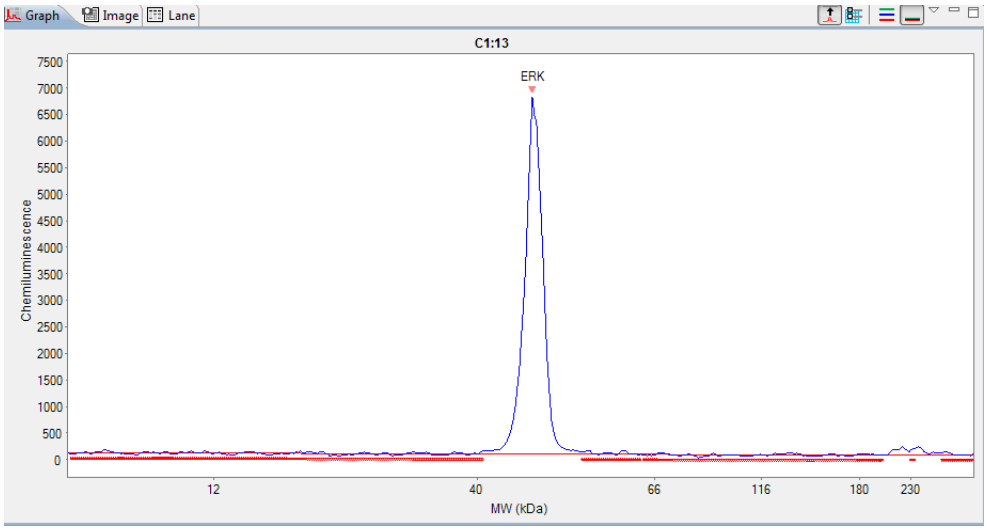
NOTE: This option is only available for sample data.



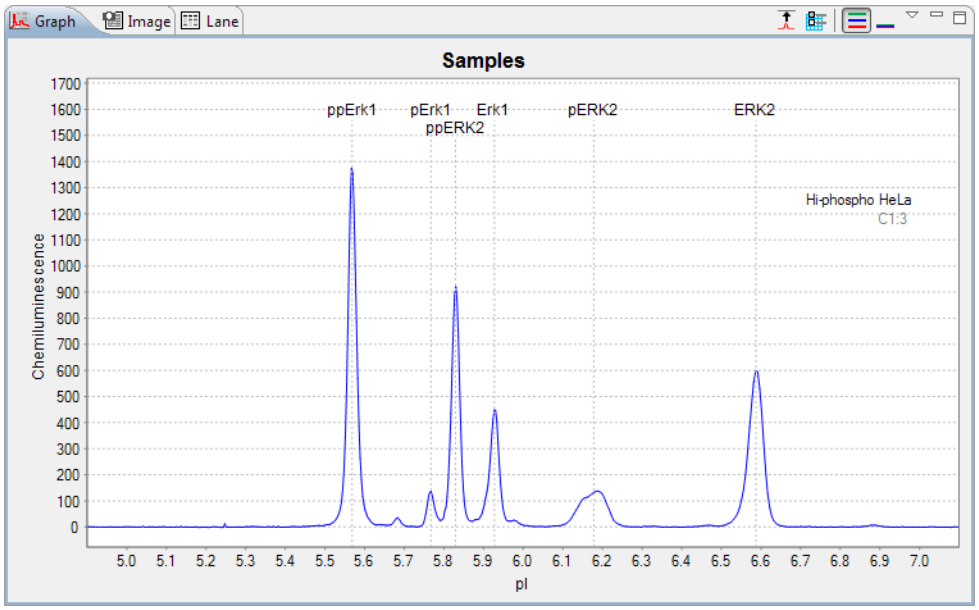
- **Baseline Fit** - Checking this box will display the calculated baseline for the peaks. Baseline points will also display for regions of the electropherogram considered to be at baseline.

NOTE: Compass for Simple Western v3.0 improves how the raw baseline is calculated. With this version of the software you may notice that the raw baseline for the shortest exposures is shifted (typically lower than previously observed when looking at data obtained with a prior software version). Peak areas for all exposures will stay the same between versions and won't be affected by the shift of the raw baseline. But, area to baseline ratio will change from what was previously calculated with earlier versions of the software.

NOTE: This option is only available for sample data.



- **Grid Lines** - Checking this box will display grid lines in the graph area.



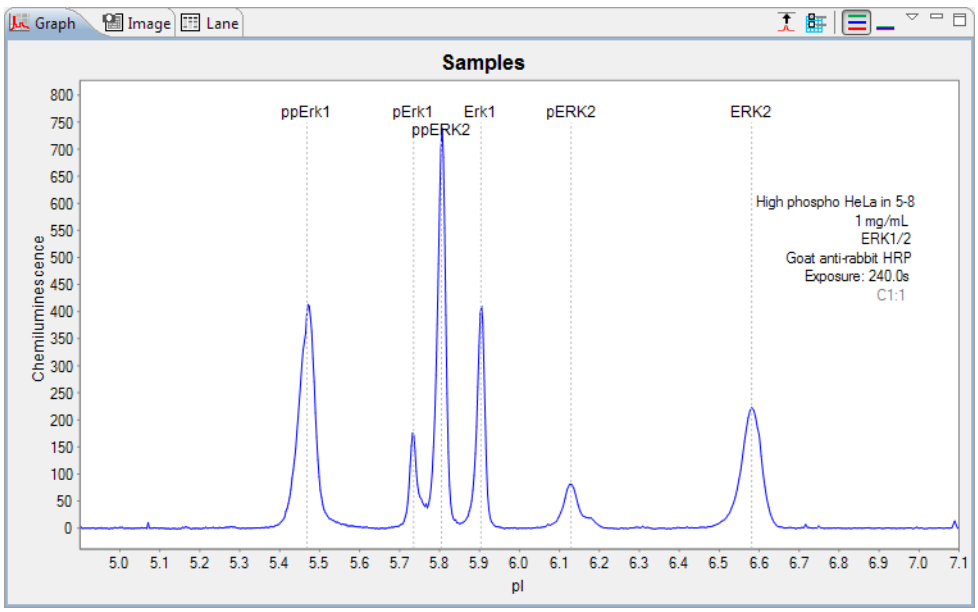
Plot Labels

You can customize the plot labels displayed on the electropherogram with these options.

Plot labels are shown on the right side of the graph pane.

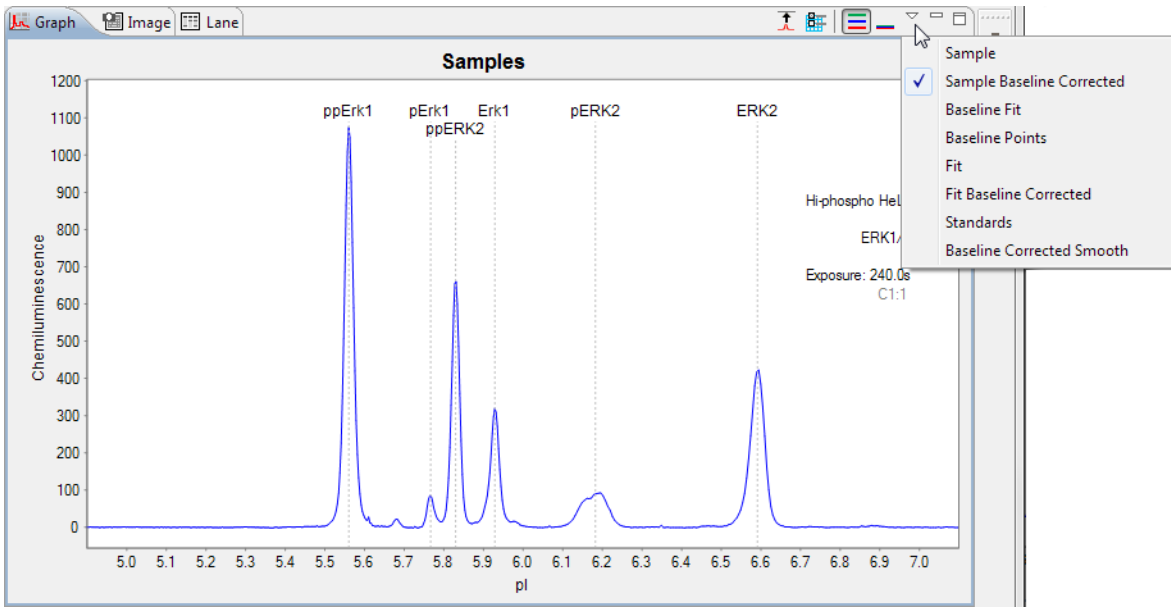
- **Sample** - Checking this box will display the sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Checking this box will display the primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Capillary** - Checking this box will display the cycle and capillary number. For example, C1:3 indicates cycle 1, capillary 3.
- **Attributes** - Checking this box will display attribute text. If attribute information was entered for samples or primary antibodies in the assay template (Assay screen), they can be selected as plot labels.
- **Exposure** - Checking this box will display the exposure time(s) used for the data.

The following example shows an electropherogram with all plot labels selected:



Selecting Data Viewing Options

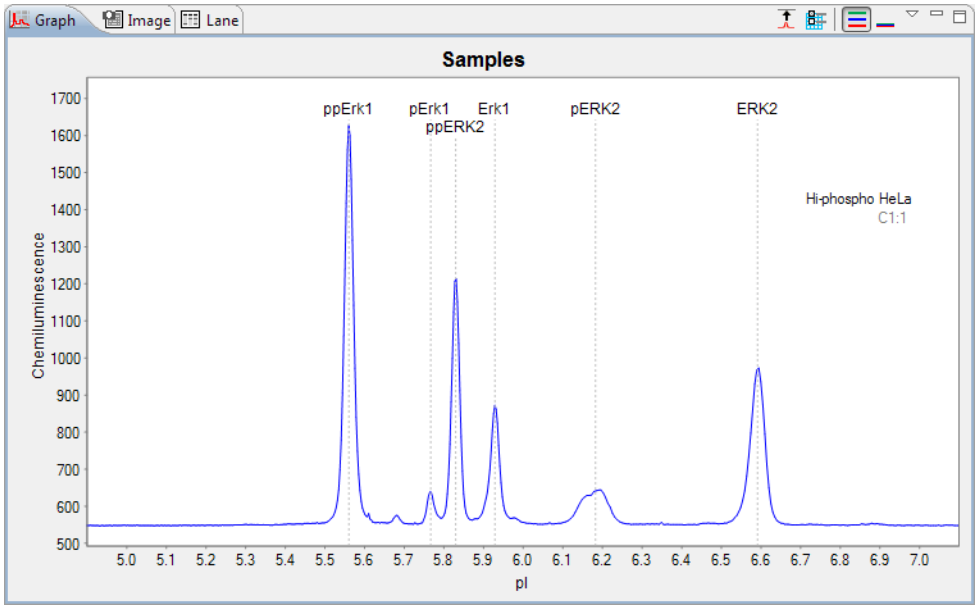
The graph view menu provides multiple options for changing what type of electropherogram data is displayed. To access the view menu, click the down arrow next to the graph pane toolbar:



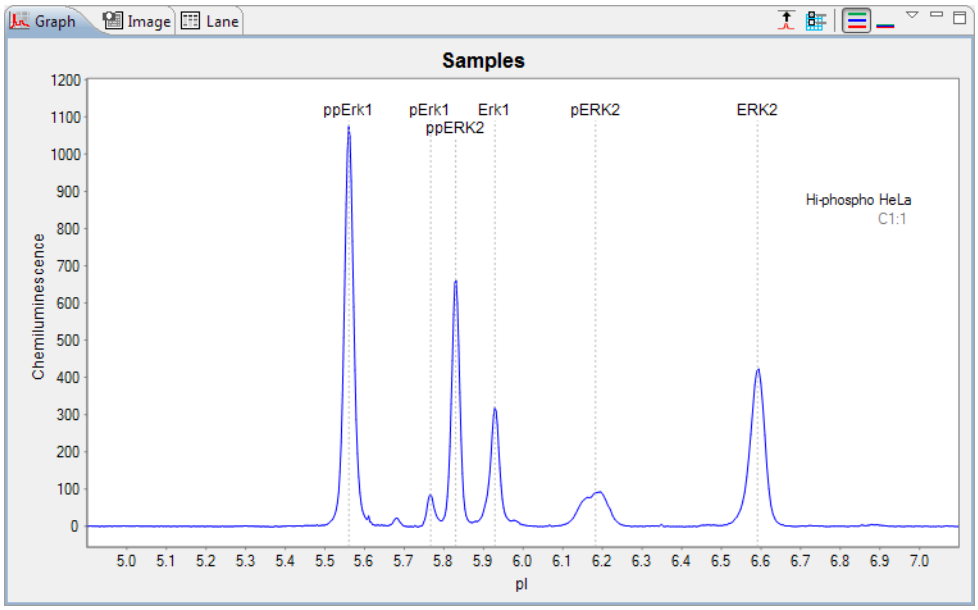
A check mark next to the menu option indicates it is currently selected. Multiple options can be selected at a time.

NOTE: Unless otherwise noted, graph view menu options are available for sample data only.

- **Sample** - Clicking this option will display raw, uncorrected sample data.

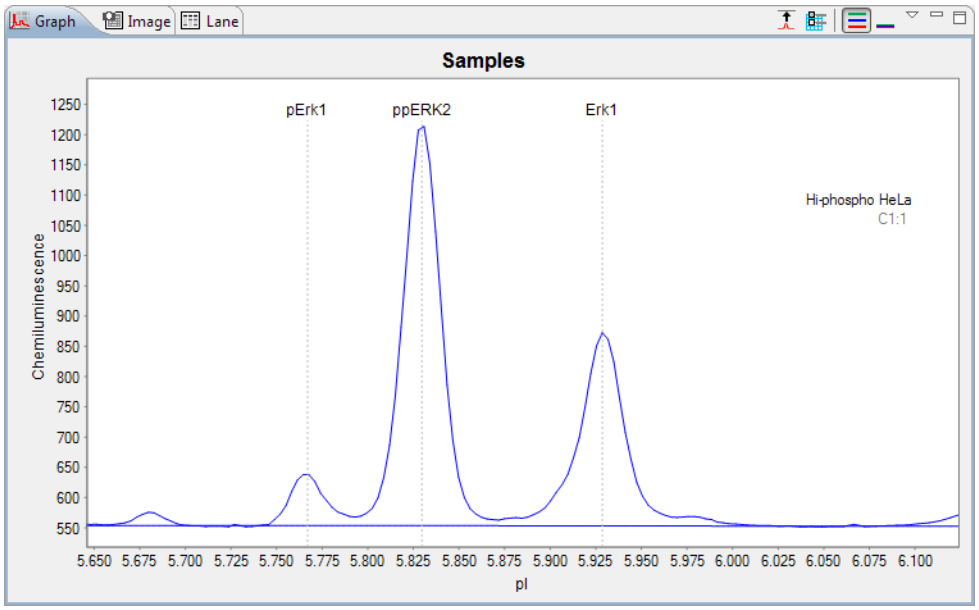


- **Sample Baseline Corrected** - Clicking this option will display sample data with the baseline subtracted (zeroed). This is the default view.



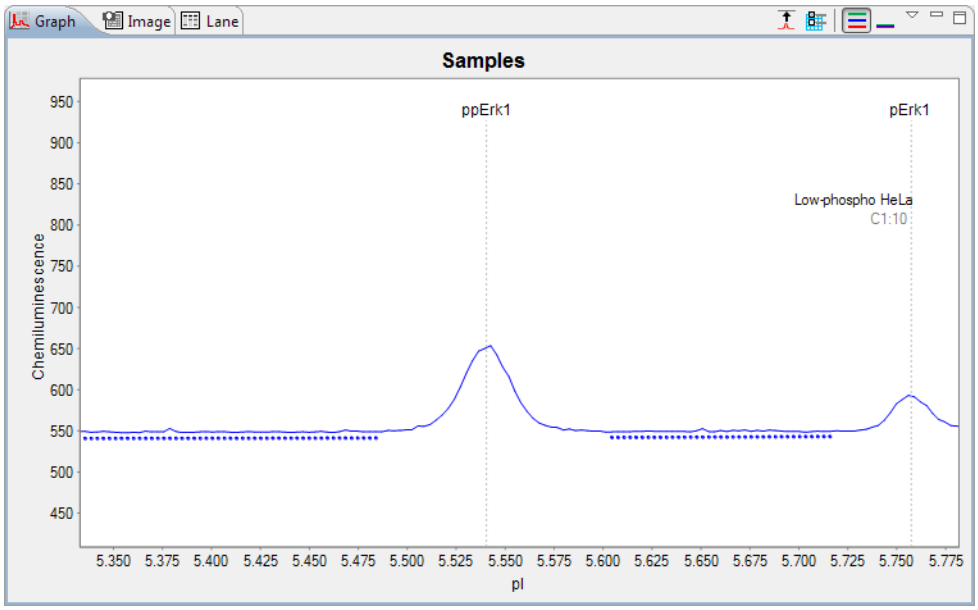
- **Baseline Fit** - Clicking this option will display the calculated baseline for the raw sample data. In the example that follows, both Baseline fit and Sample are selected.

NOTE: This option is selected automatically when Baseline Fit is selected in graph options.

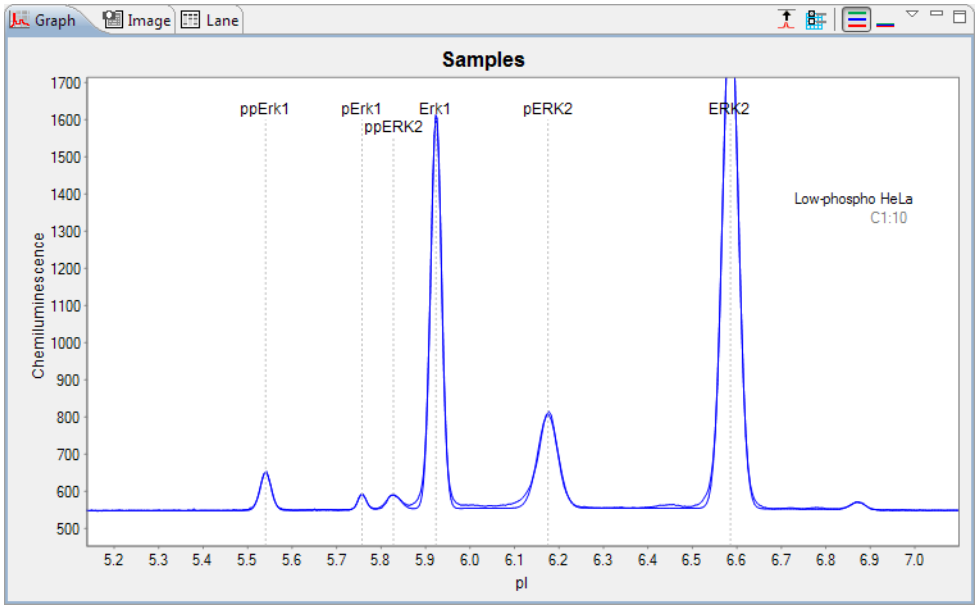


- **Baseline Points** - Clicking this option will display regions of the electropherogram considered to be at baseline. In the example that follows, both Baseline Points and Sample are selected.

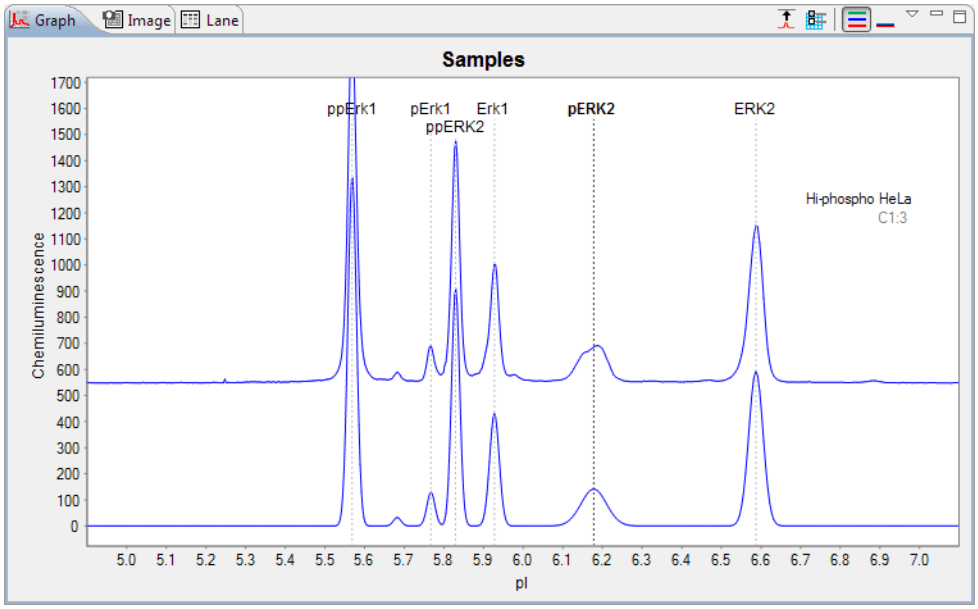
NOTE: This option is selected automatically when Baseline Fit is selected in graph options.



- **Fit** - Clicking this option will display the bounding envelope of the fitted peaks as calculated by the software for the raw sample data. In the example that follows, both Fit and Sample are selected.

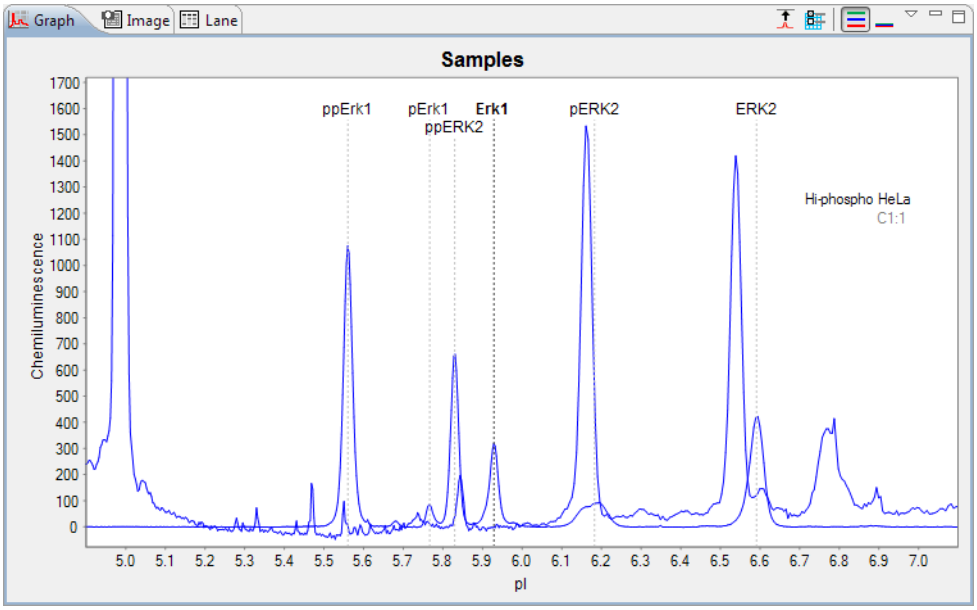


- **Fit Baseline Corrected** - Clicking this option will display the fitted peaks as calculated by the software for the sample baseline corrected data. In the example that follows, both Fit Baseline Corrected and Sample are selected, the fit plot is on the bottom.



NOTE: When viewing standards or registration data, this option is called Standards Fluorescence or Registration Fluorescence, respectively.

- **Standards** - Checking this box aligns the pI of the raw standards data to the sample data and overlays both electropherograms in the graph pane.

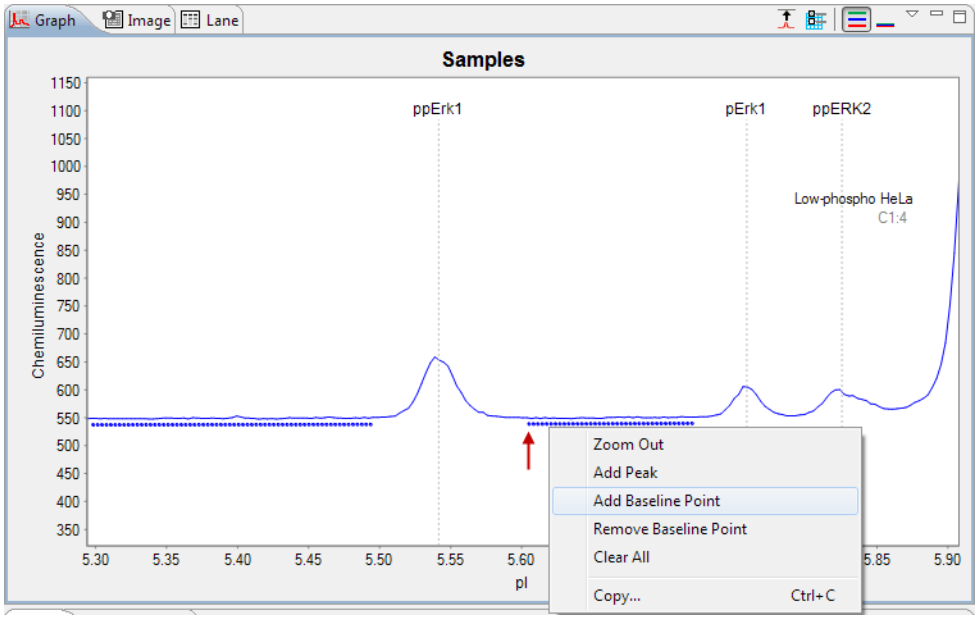


Adding and Removing Baseline Points

Points in the baseline can be added or removed as needed. To do this:

1. Click the **Graph Options** button in the graph pane toolbar and check **Baseline Fit**. This will display baseline points for the raw sample data.
2. Use the mouse to draw a box around the area you want to correct. This will zoom in on the area.

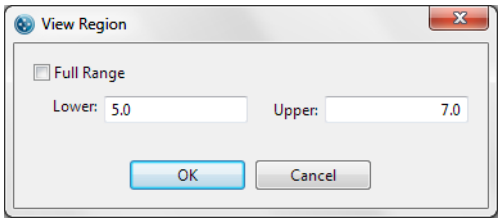
3. Right click a baseline point and click **Add Baseline Point** or **Remove Baseline Point**.



*NOTE: To clear the manual addition or removal of baseline points and go back to the original view of the data, right click in the electropherogram and click **Clear All**.*

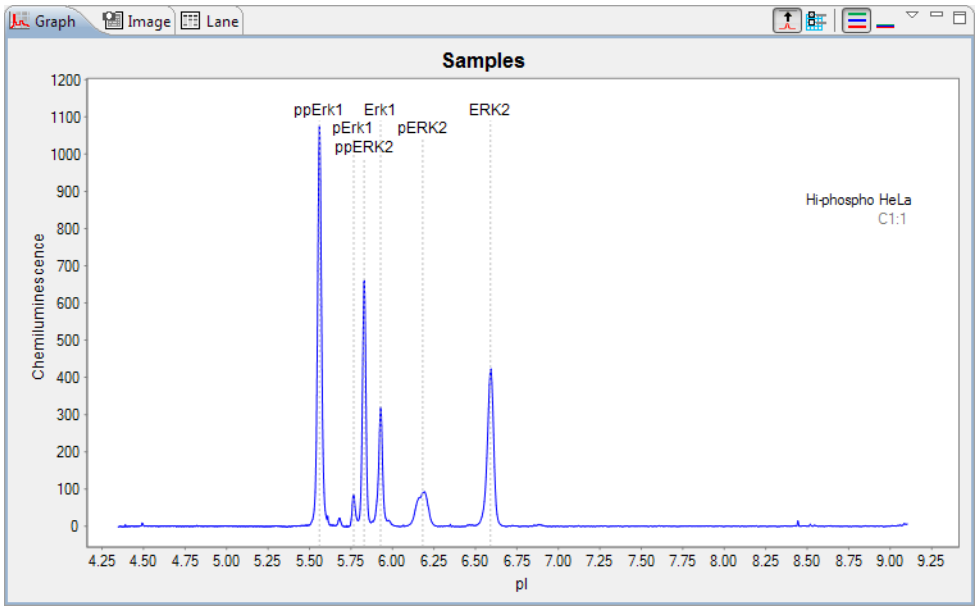
Selecting the X-Axis pI Range

The pI range used for the x-axis can be changed. To do this, Select **View** in the main menu and click **View Region**. The following pop-up window will display:



- **To change the x-axis pI range displayed for the run data** - Enter new values in the Lower or Upper range in pI and click **OK**. Electropherogram and virtual blot data will update to display only the data in the entered range.

- **To see the full x-axis pI range included in the run data** - Check **Full Range**. Electropherogram and virtual blot data will update to display the full range of available data.



NOTE: You can change the default x-axis range that Compass for Simple Western uses. For more information, see "Peak Fit Analysis Settings" on page 434.

Closing Run Files

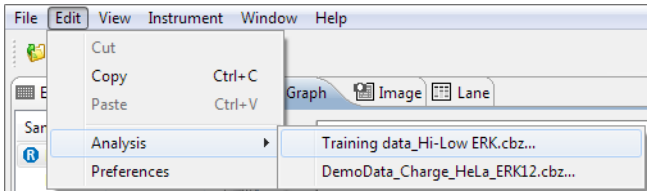
If more than one run file is open, you can close just one file or all the open files at the same time.

- **To close one of multiple open run files** - In the experiment pane, click on one of the sample rows in the file. Next click **File** from the main menu and click **Close**.
- **To close all open run files** - Select **File** from the main menu and click **Close All**.

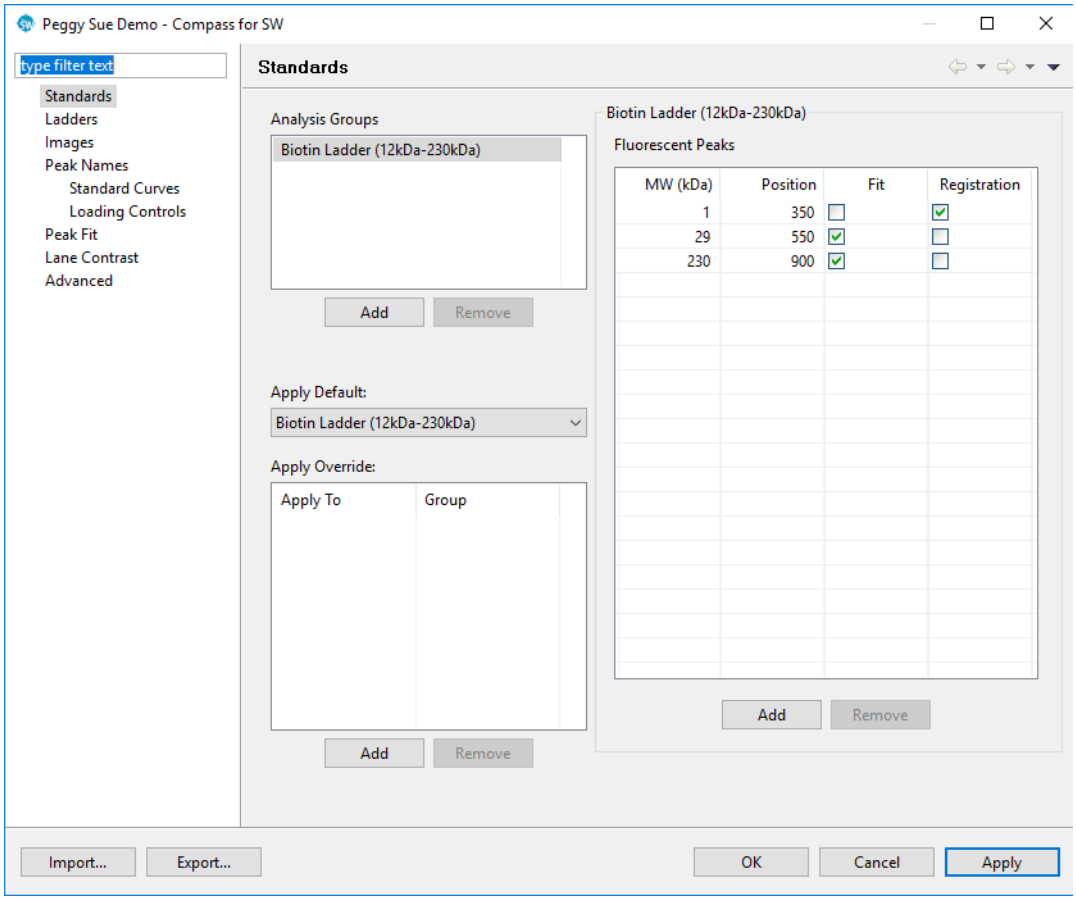
Analysis Settings Overview

Compass for Simple Western has a variety of analysis features and settings that you can modify as needed to enhance run data.

To access these settings, select **Edit** in the main menu and click **Analysis**. If more than one run file is open, select the run file you want to view analysis settings for from the list:



The following screen will display:



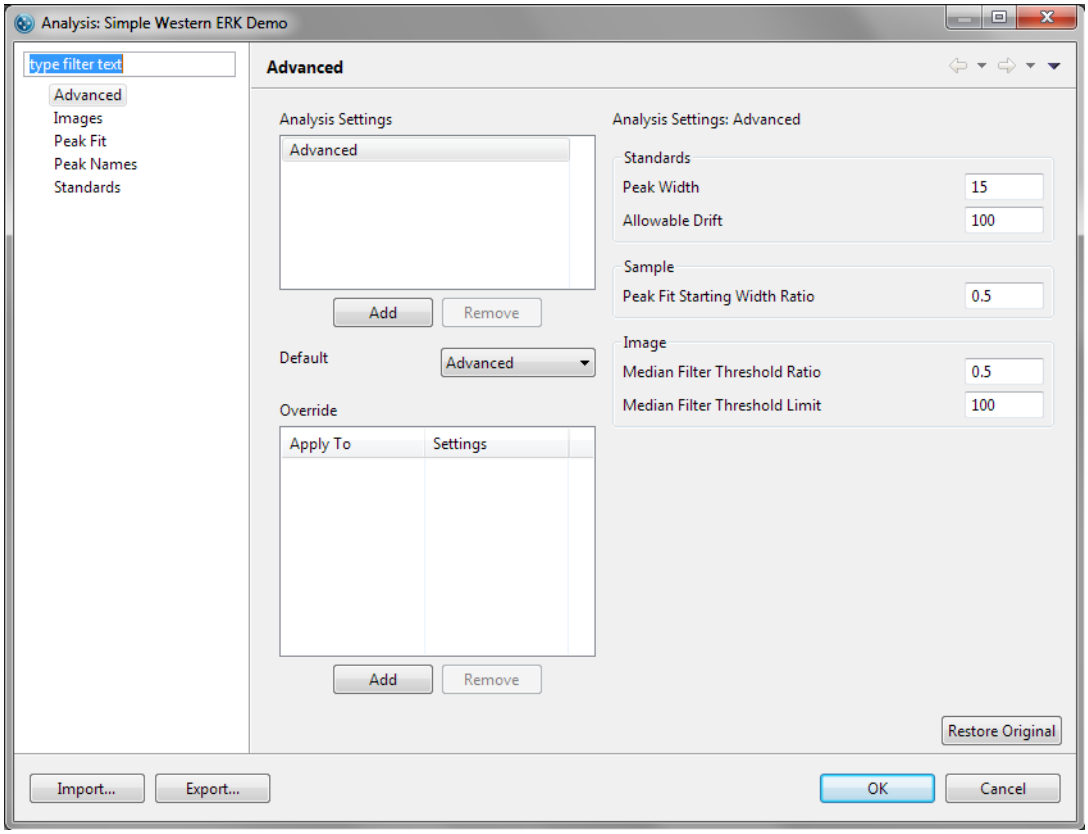
To move between analysis pages in this window, click on an option in the list on the left. The following analysis settings can be user-customized in Compass for Simple Western:

- **Advanced** - Lets you customize analysis settings for samples, standards and image data.
- **Images** - Lets you view the chemiluminescent exposures taken during the run and view data for different exposures in the Analysis screen.
- **Peak Fit** - Lets you customize peak fit settings for sample data.
- **Peak Names** - Lets you enter custom naming settings for sample proteins associated with specific primary antibodies or attributes and have Compass for Simple Western automatically label the peaks in the run data.
- **Standards** - Lets you customize the pI and positions Compass for Simple Western uses to identify fluorescent standards and registration peaks.

Advanced Analysis Settings

The advanced analysis settings page lets you view and change analysis settings for samples, standards and image data. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 460.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 460.
- Click **Restore Original** to restore Compass for Simple Western default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Standards Settings

- **Peak Width** - The approximate width (at full width half max) used to filter out fluorescence artifacts which improves recognition of standards. The default value is 15.
- **Allowable Drift** - The distance in pixels that standards are expected to move compared to their entered positions in the standards analysis page. This setting assists in recognition of standards. The default value is 100.

Sample Settings

- **Peak Fit Starting Width Ratio -** Focuses the peak fit towards the peak center and aids the overall peak fitting. The default value is 0.5.

Image Settings

- **Median Filter Threshold Ratio** - Pixel ratio used to filter out camera artifacts. The default value is 0.5.
- **Median Filter Threshold Limit** - Pixel threshold value used to filter out camera artifacts. The default value is 100.

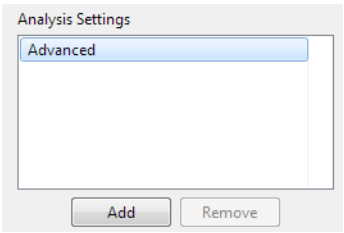
Advanced Analysis Settings Groups

Advanced analysis settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:
We recommend using the Compass for Simple Western default values for advanced analysis settings. These settings are included in the default Advanced group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 460.

Analysis groups are displayed in the analysis settings box:

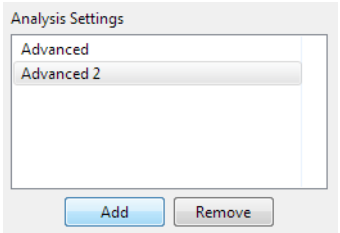


The Advanced group shown contains the Compass for Simple Western default analysis settings. You can make changes to this group and create new groups.

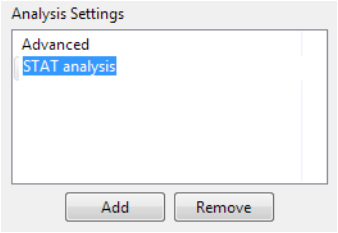
To view settings for a group, click on the group name in the analysis settings box.

Creating a New Analysis Group

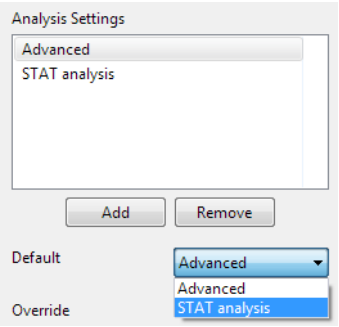
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



3. Click on the new group and enter a new name.



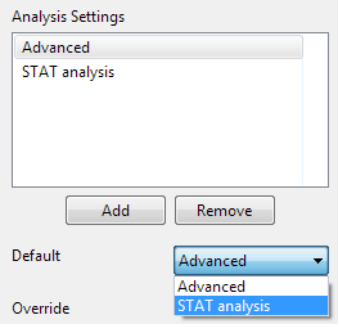
4. Modify standards, sample or image parameters as needed.
5. To use the new group as the default analysis settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Analysis settings in the new group will then be applied to the run data.



6. Click **OK** to save changes.

Changing the Default Analysis Group

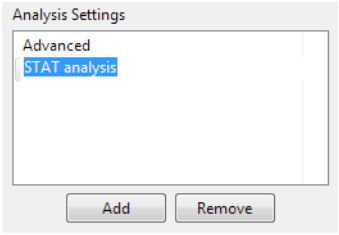
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click the arrow in the drop down list next to Default, then click a new default group from the list.



3. Click **OK** to save changes. Analysis settings in the group selected will be applied to the run data.

Modifying an Analysis Group

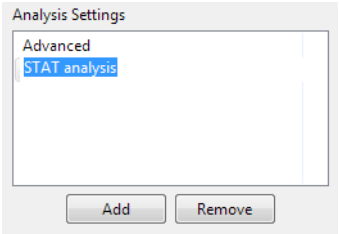
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Modify standards, sample or image parameters as needed.
4. Click **OK** to save changes. The new analysis settings will be applied to the run data.

Deleting an Analysis Group

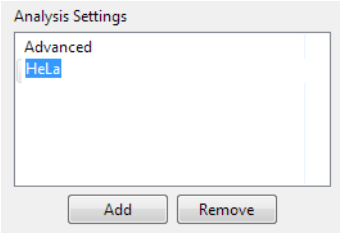
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



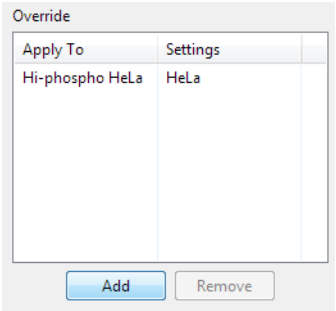
3. Click **OK** to save changes.

Applying Analysis Groups to Specific Run Data

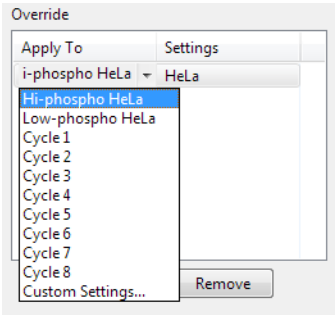
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of analysis groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

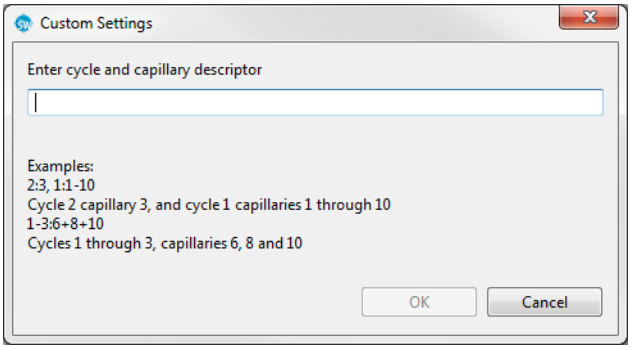


4. Click the cell in the **Apply To** column, then click the down arrow.

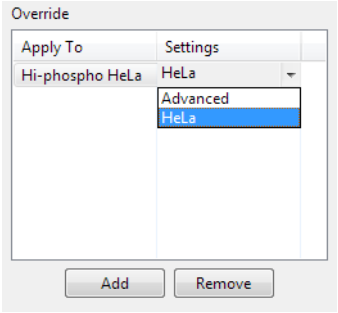


5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:
   - **Sample names** - All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a sample name to apply group settings to all capillaries that use this sample name in the run file.
   - **Attributes** - All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.

- **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
- **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:



6. If you need to change the analysis group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.



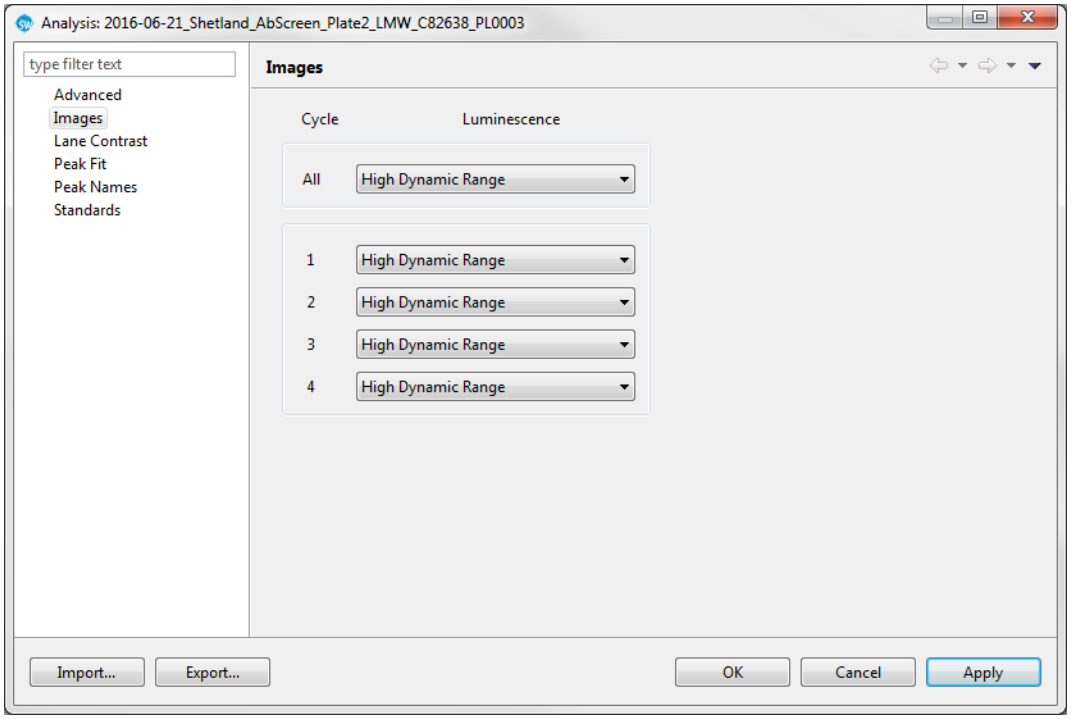
7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **OK** to save changes.

Images Analysis Settings

The Images settings in the Analysis window lets you see what Detection Profile was selected, what exposures were taken during the run, and view data for different exposures in the Analysis screen. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Images** in the options list.

NOTES:
If you're using Compass for Simple Western v3.1 or higher, High Dynamic Range is the default setting.

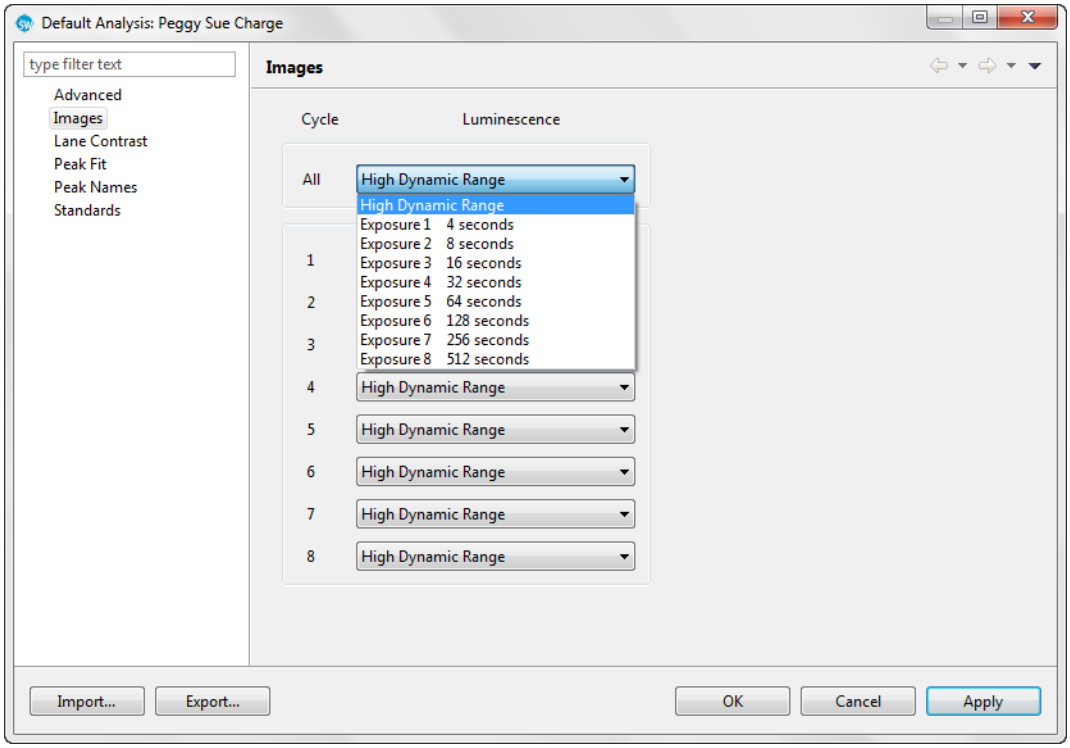
If the run was performed with HDR unchecked, the data can still be analyzed with the improved HDR algorithm by selecting High Dynamic range from the drop down menu. For Wes runs, the increase in dynamic range will not be as noticeable since the substrate refresh was not performed.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 460.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 460.
- Click **Apply** to see effects of changes before saving.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Exposure Settings

The exposure used for the sample data displayed in the Analysis screen is shown in the All box shown below:

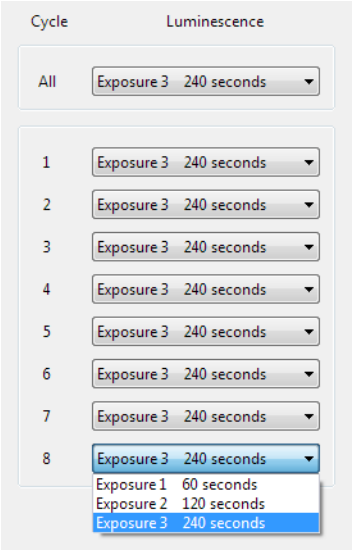


Even when the exposures for HDR are set and can't be edited from the assay protocol, here you can choose to view each one separately including High Dynamic Range (HDR multi-image).

NOTE: Peggy Sue runs up to eight cycles (12 capillaries per cycle). The individual exposure setting selected for a specific cycle is applied for all 12 capillaries in that cycle unless selected for All cycles.

- **High Dynamic Range** - The HDR method uses information from multiple exposures to achieve good signal-to-noise for low protein concentration while simultaneously maintaining signal monotonicity at high protein concentration (*i.e.* avoiding "burnout").
- **Exposure #** - Sample data displayed in the Analysis screen is for this specific exposure only.

  To see the number of exposures and exposure times used for the run data, click the arrow in the drop down list next to **All**.

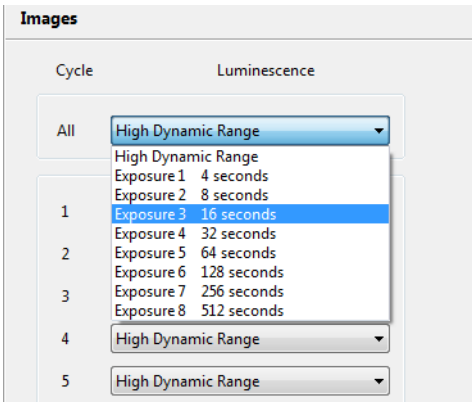


NOTE: The number of exposures taken and exposure times shown were specified in the assay protocol (Assay screen) prior to executing the run and cannot be modified.

Changing the Sample Data Exposure Displayed

The exposure used for the sample data displayed in the Analysis screen can be changed. To do this:

1. Select **Edit** in the main menu and click **Analysis**, then click **Images** in the options list.
2. Click the arrow in the drop down list next to **All** and select an exposure setting:

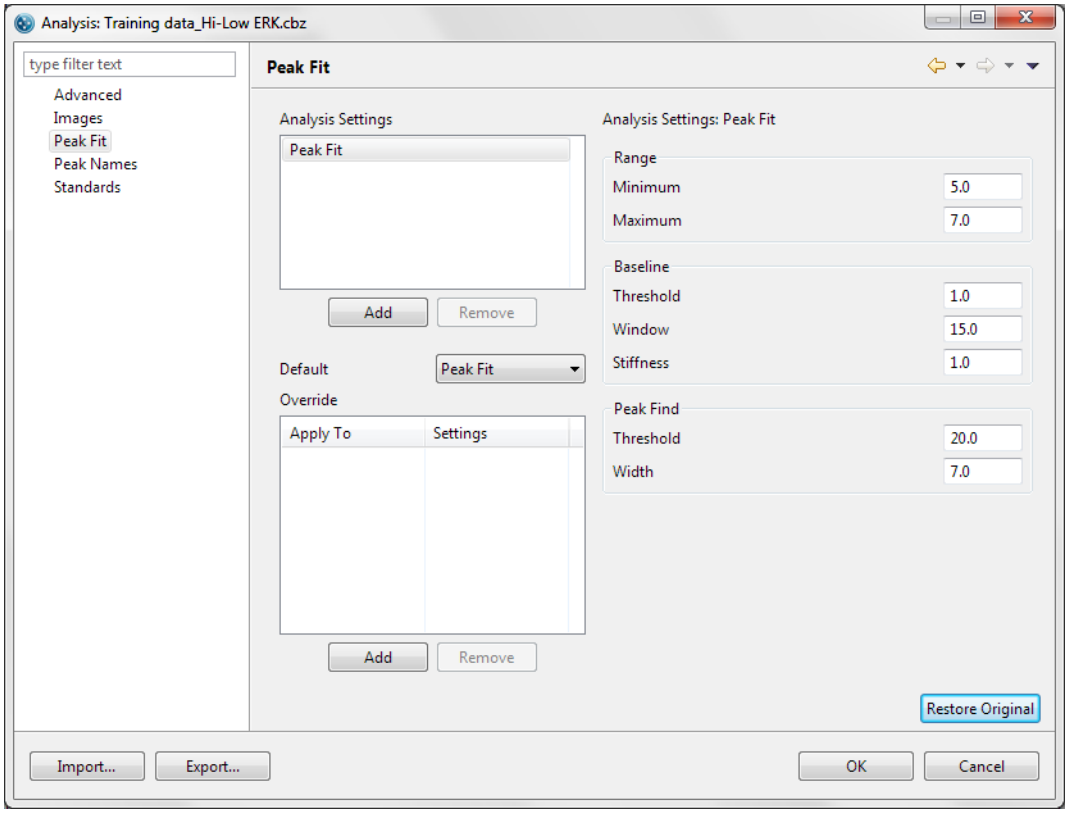


3. Click **OK** to save changes and exit. Sample data for the exposure selected will display in the Analysis screen.

Peak Fit Analysis Settings

The peak fit analysis settings page lets you view and change peak fit settings for sample data. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 460.

- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 460.
- Click **Restore Original** to restore Compass for Simple Western default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Range Settings

- **Minimum** - The pI value below which peaks will not be identified. This value will also be used as the default lower pI range for the data displayed in the electropherogram and virtual blot. The default value is 5.
- **Maximum**: The pI value above which peaks will not be identified. This value will also be used as the default upper pI range for the data displayed in the electropherogram and virtual blot. The default value is 7.

Baseline Settings

- **Threshold** - The variance, or roughness, in a baseline data segment below which a point will be called part of the baseline. The default value is 1.0.
- **Window** - How long baseline data segments are expected to be in pixels. Shorter segments will allow the baseline to follow plateau sections of the signal. The default value is 15.
- **Stiffness** - The amount the baseline is allowed to vary from a straight line. Settings between 0.1 and 1.0 make the baseline fit closer to a straight line. Settings from 1.0 to 10.0 will make the baseline fit follow the data more closely. The default value is 1.0.

Peak Find Settings

- **Threshold** - The minimum signal to noise ratio required for a peak to be identified. A setting of 1.0 will detect many peaks, a setting of 30.0 will detect fewer peaks. The default value is 20.0.
- **Width** - The approximate peak width (at full width half max) in pixels used to detect peaks. The minimum value for this setting is 3.0. Larger widths help to eliminate the detection of shoulder peaks and noise peaks. The default value is 7.0.

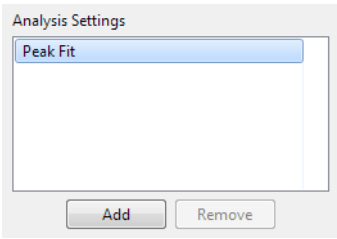
Peak Fit Analysis Settings Groups

Peak fit settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:
We recommend using the Compass for Simple Western default values for peak fit analysis settings. These settings are included in the default Peak Fit group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 460.

Peak fit groups are displayed in the analysis settings box:

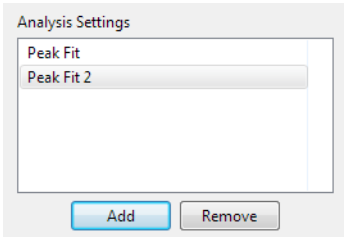


The Peak Fit group shown contains the Compass for Simple Western default analysis settings. You can make changes to this group and create new groups.

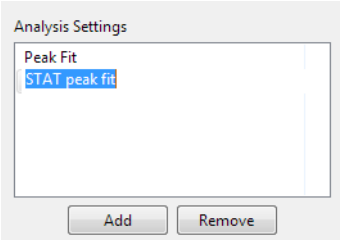
To view settings for a group, click on the group name in the analysis settings box.

Creating a New Peak Fit Group

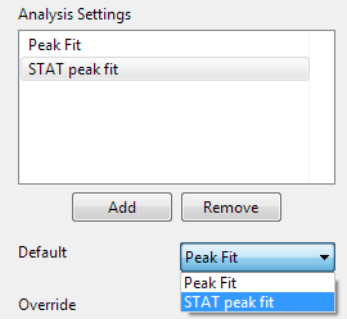
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



3. Click on the new group and enter a new name.



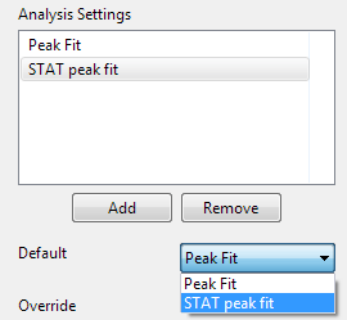
4. Modify range, baseline or peak find parameters as needed.
5. To use the new group as the default peak fit settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Peak fit settings in the new group will then be applied to the run data.



6. Click **OK** to save changes.

Changing the Default Peak Fit Group

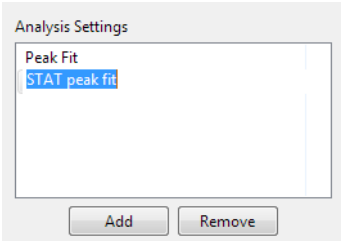
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click the arrow in the drop down list next to Default, then click a new default group from the list.



3. Click **OK** to save changes. Peak fit settings in the group selected will be applied to the run data.

Modifying a Peak Fit Group

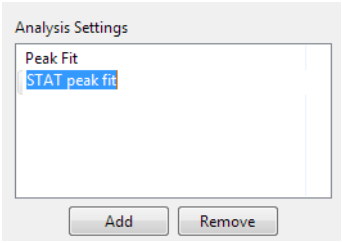
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Modify range, baseline or peak find parameters as needed.
4. Click **OK** to save changes. The new peak fit settings will be applied to the run data.

Deleting a Peak Fit Group

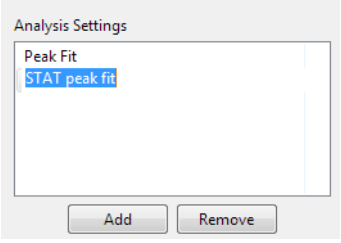
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



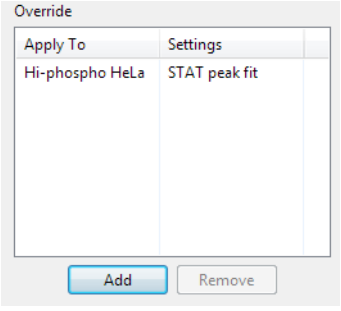
3. Click **OK** to save changes.

Applying Peak Fit Groups to Specific Run Data

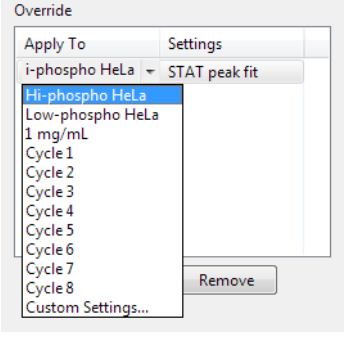
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of peak fit groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.



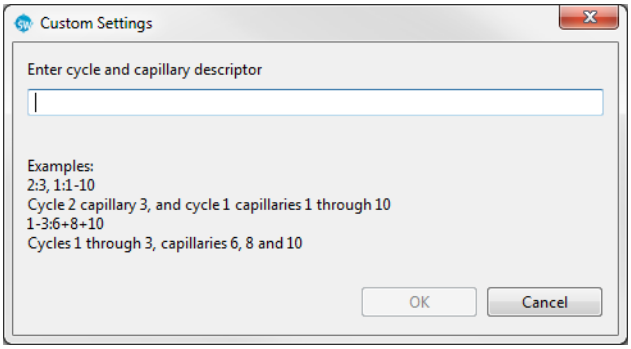
4. Click the cell in the **Apply To** column, then click the down arrow.



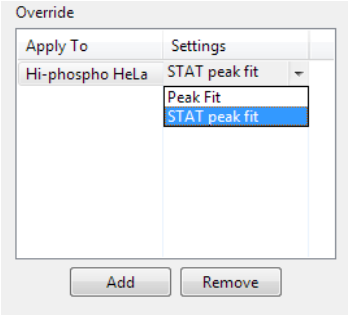
5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

   - **Sample names** - All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a sample name to apply group settings to all capillaries that use this sample name in the run file.
   - **Attributes** - All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.

- **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
- **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:



6. If you need to change the peak fit group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.

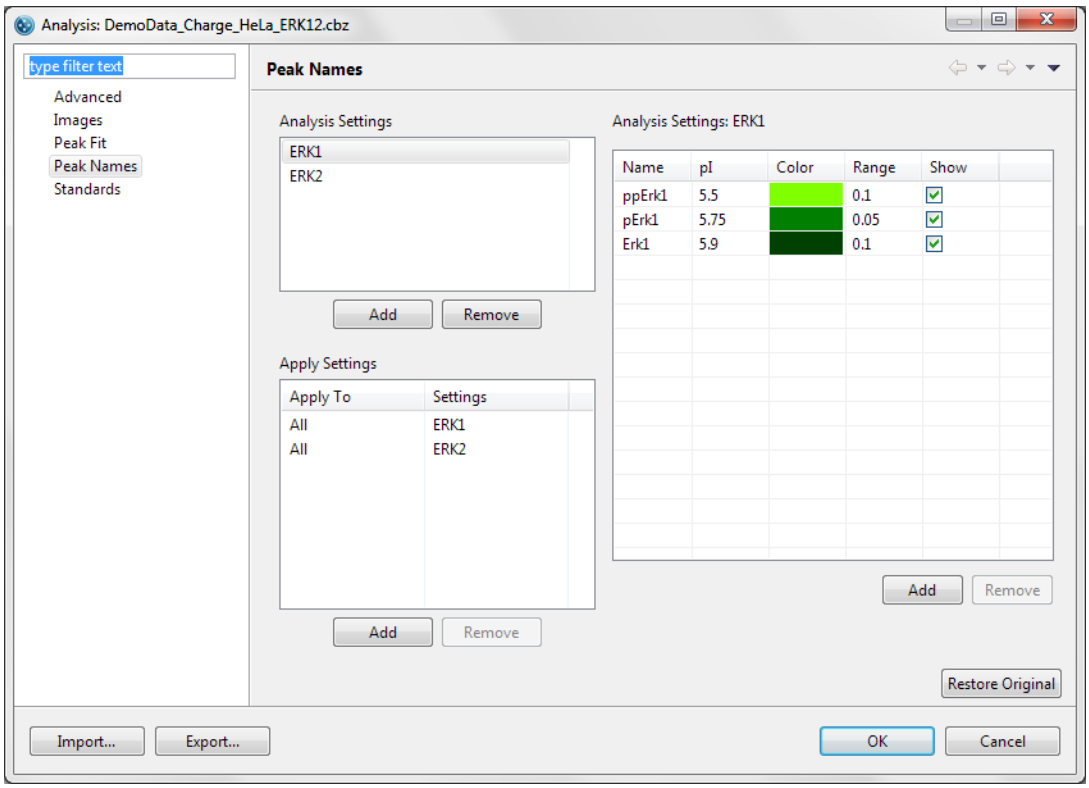


7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **OK** to save changes.

Peak Names Settings

The peak names analysis settings page lets you enter custom naming settings for sample proteins. Compass for Simple Western can automatically label sample peaks in the run data associated with specific blocking reagent names, primary antibody names or attributes. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 460.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 460.
- Click **Restore Original** to restore Compass for Simple Western default settings.
- Click **OK** to save changes and exit.

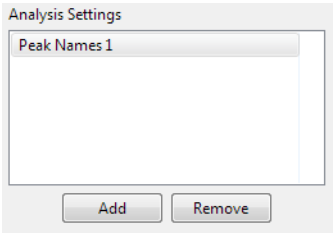
- Click **Cancel** to exit without saving changes.

Peak Names Analysis Settings Groups

Peak name settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, blocking reagent names or primary antibody names and attributes in the run data.

NOTE: Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 460.

Peak name groups are displayed in the analysis settings box:

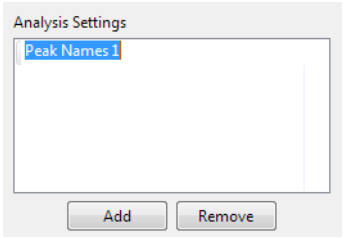


The Peak Names group shown is a Compass for Simple Western template group. You can make changes to this group and create new groups.

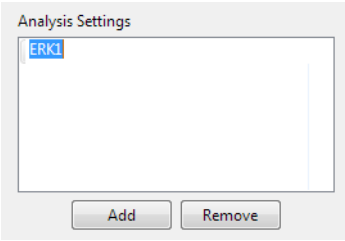
To view settings for a group, click on the group name in the analysis settings box.

Creating a Peak Names Group

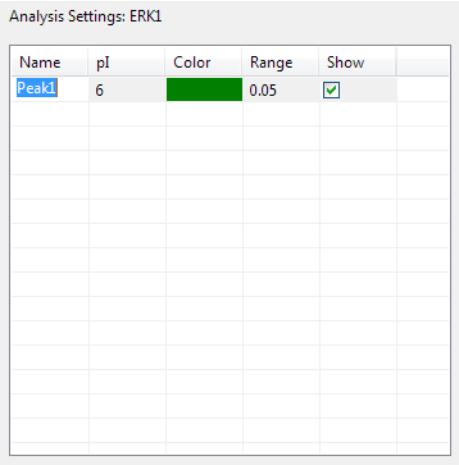
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the **Peak Names 1** template group in the analysis settings box.



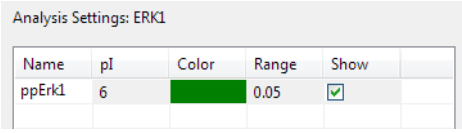
3. Enter a new name for the group.



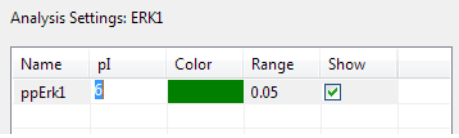
4. Click in the first cell in the **Name** column in the analysis settings peak table.



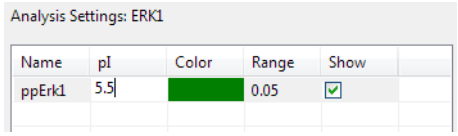
5. Enter a sample protein name associated with the primary antibody used in the run.



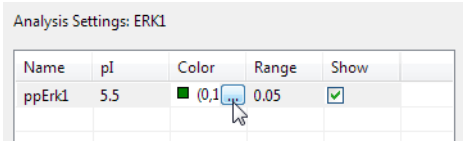
6. Click in the first cell in the **pI** column.



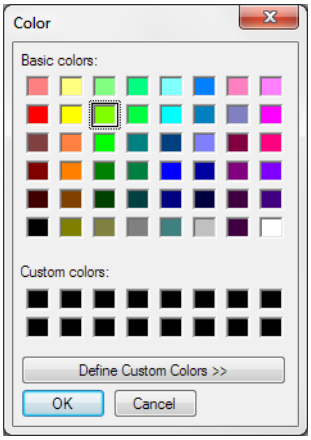
7. Enter the pI for the sample protein.



8. Click in the first cell in the **Color** column, then click the button.

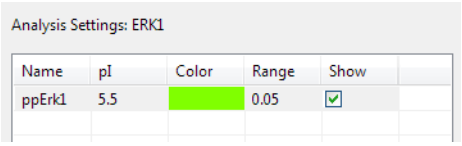


The color selection box will display:

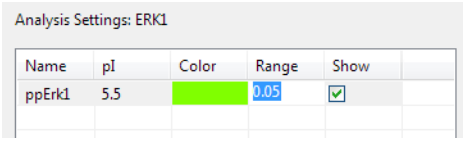


The color selected will be used to identify the sample protein peak in the peaks and capillaries table in the Analysis screen.

9. Click a color or define a custom color and click **OK**. The color selection will update in the table:



10. Click in the first cell in the **Range (%)** column.



11. Enter a range window for the pI entered. Compass for Simple Western will automatically name peaks found within this percent of the pI. For example, if the pI entered is 5.5 and a 0.1 pI range is used, all peaks between pI 5.4 and 5.6 will be identified with this peak name.

NOTE: The reported pI for immunodetected sample proteins in Compass for Simple Western may vary slightly from predicted pIs based on sample, buffer and assay conditions.

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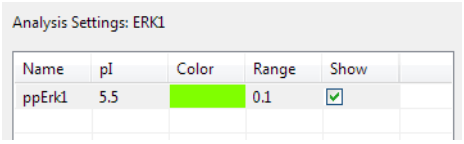
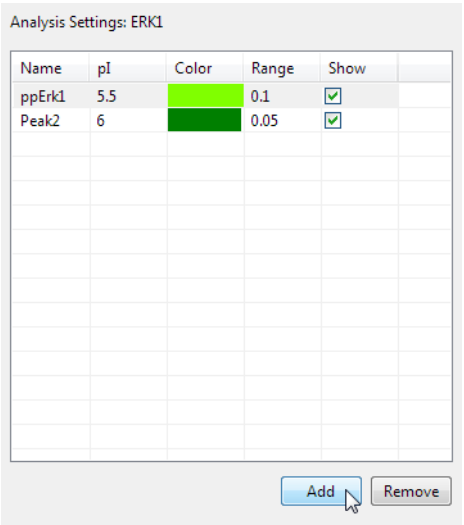
12. Select the checkbox in the first cell of the **Show** column. This will turn peak naming on for the sample protein.

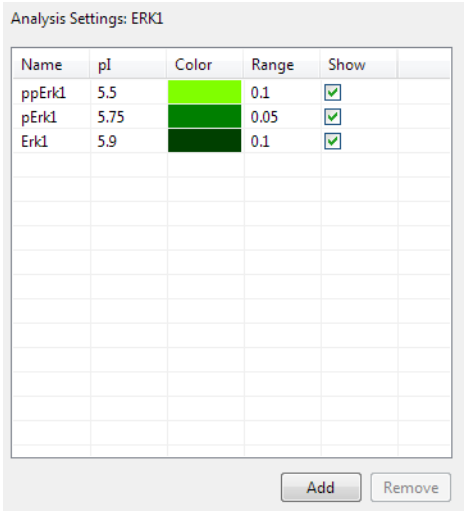


To turn peak naming off for a particular sample protein, deselect the checkbox in the Show column.

13. To add another sample protein, click **Add** under the analysis settings peak table:



14. Repeat the previous steps to enter information for other sample proteins. In the following example, three sample proteins were entered:

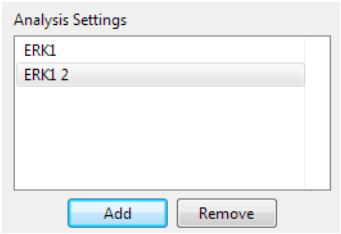


To remove a sample protein, select its row and click **Remove**.

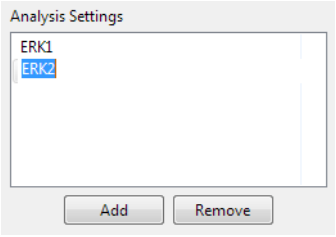
15. Click **OK** to save changes.

Adding Peak Names Groups

1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



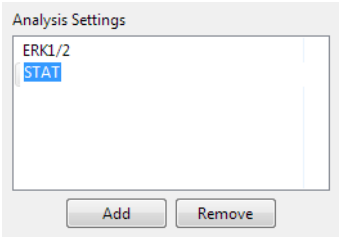
3. Click on the new group and enter a new name.



4. Enter information in the analysis settings peak table as described in "Creating a Peak Names Group" on page 442.
5. Click **OK** to save changes.

Modifying a Peak Names Group

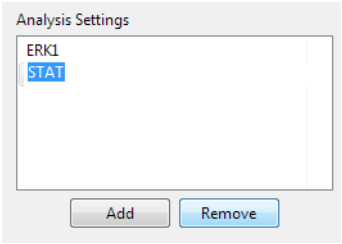
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Change the information in the analysis settings peak table as described in "Creating a Peak Names Group" on page 442.
4. Click **OK** to save changes.

Deleting a Peak Names Group

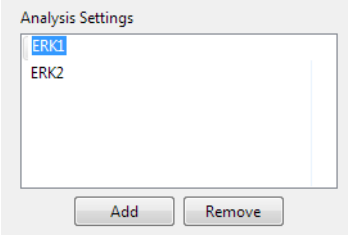
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



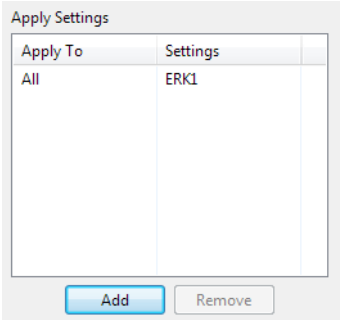
3. Click **OK** to save changes.

Applying Peak Names Groups to Run Data

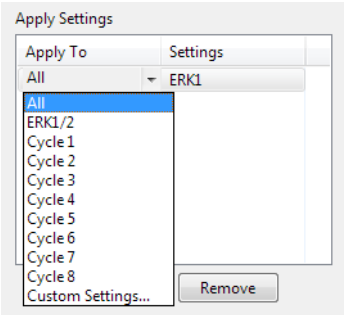
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of peak names groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created.

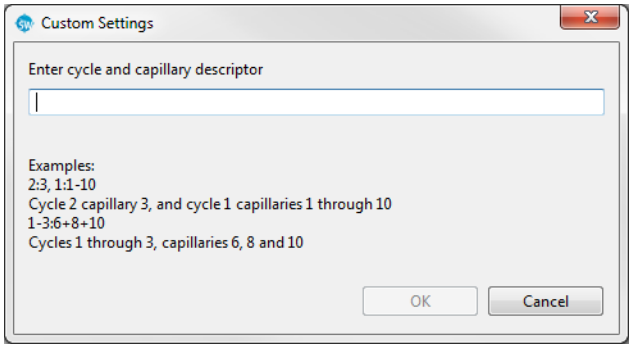


4. Click the cell in the **Apply To** column, then click the down arrow.

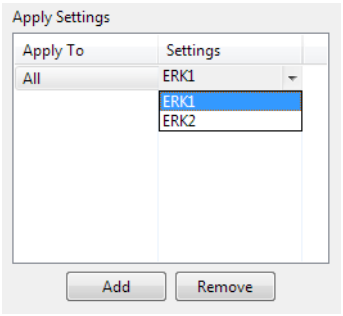


5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

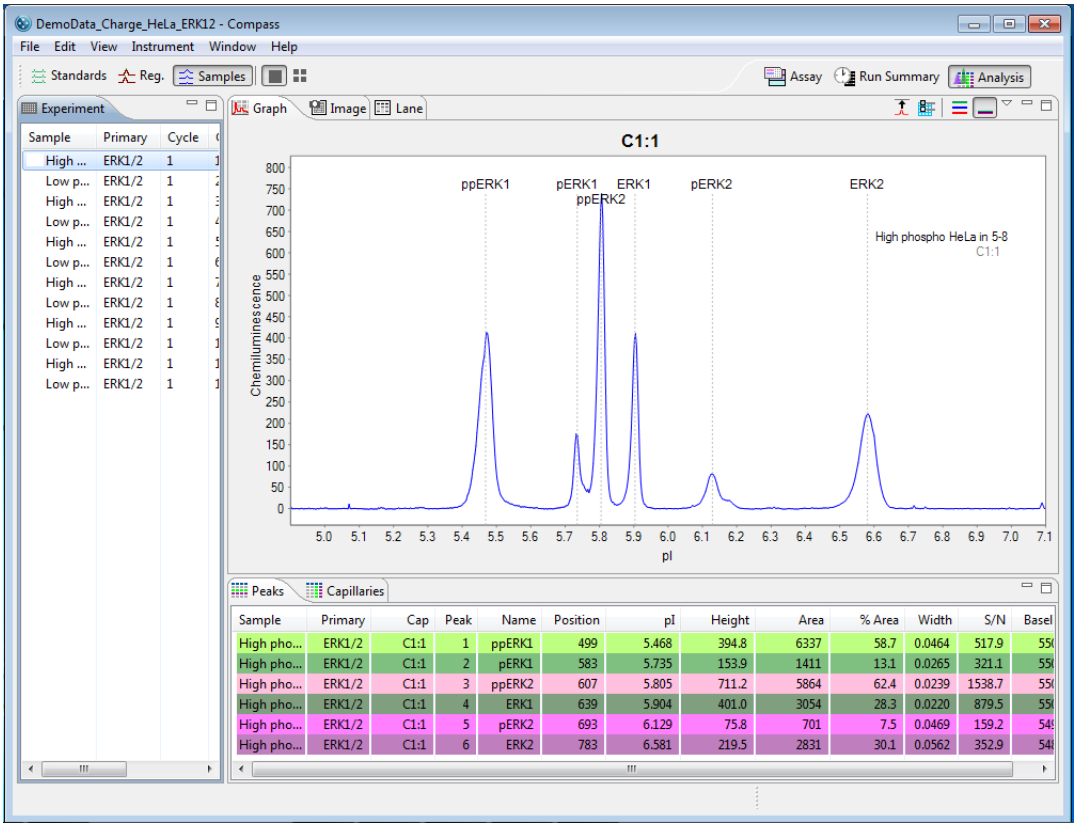
   - **All** - When this option is selected, peak names group settings will be applied to all capillaries in the run file.
   - **Primary antibody names** - All primary antibody names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Primary will be shown. Select a name to apply group settings to all capillaries that use the primary antibody name in the run file.
   - **Attributes** - All primary antibody attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
   - **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
   - **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:



6. If you need to change the peak names group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.



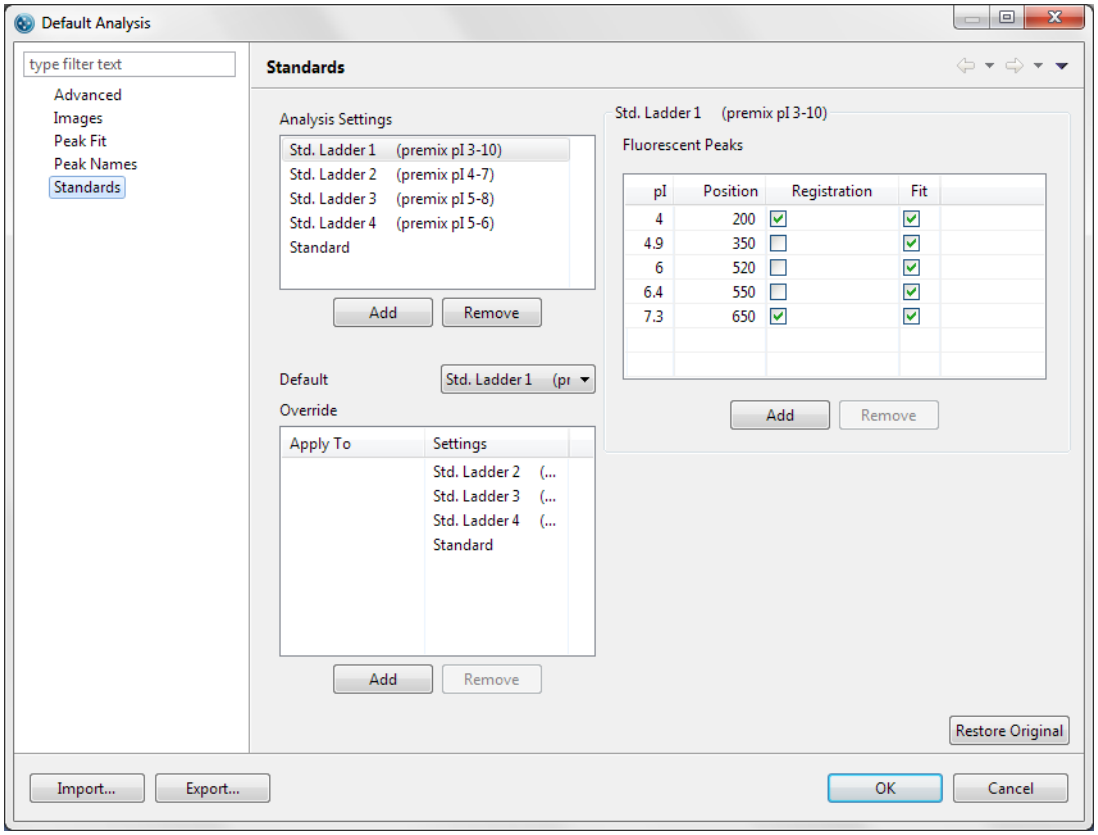
7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **OK** to save changes. Named peaks will be identified with a peak name label in the electropherogram and virtual blot, and will be color coded in the peaks and capillaries tables:



Standards Settings

The standards analysis settings page lets you view and change the pI and position for fluorescent standards and set the registration peaks. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.

NOTE: Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.



- Click **Import** to import an analysis settings file. This will be explained in more detail in "Importing Analysis Settings" on page 460.
- Click **Export** to export the current analysis settings file. This will be explained in more detail in "Exporting Analysis Settings" on page 460.
- Click **Restore Original** to restore Compass for Simple Western default settings.
- Click **OK** to save changes and exit.

- Click **Cancel** to exit without saving changes.

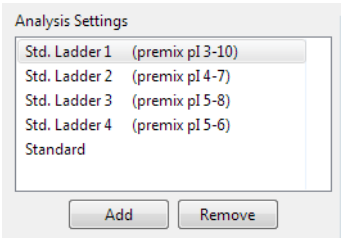
Standards Analysis Settings Groups

Standards settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual capillaries, sample names or attributes in the run data.

NOTES:
We recommend using the Compass for Simple Western default values for standards analysis settings. These settings are included in the default Standards group.

Analysis settings are run-file specific. However, settings can be imported or exported for use with other run files. For more information see "Importing and Exporting Analysis Settings" on page 460.

Standards groups are displayed in the analysis settings box:

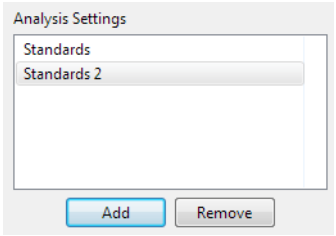


The Std. Ladder groups shown contains the Compass for Simple Western default analysis settings for pI Standard Ladders used with each of the premixes (separation gradients) for charge assays on Peggy Sue. You can select and use one of these default groups, make changes to groups or create new groups.

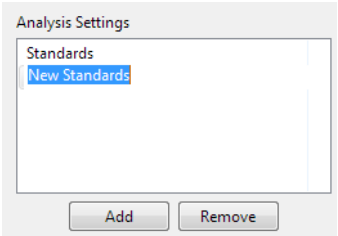
To view settings for a group, click on the group name in the analysis settings box.

Creating a New Standards Group

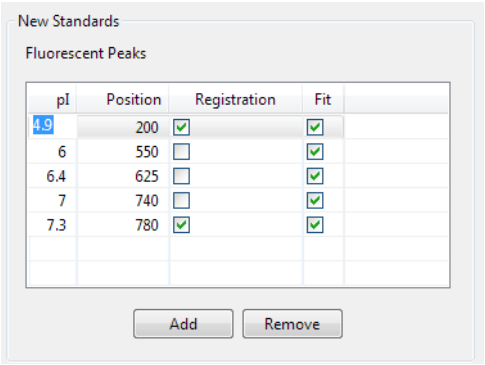
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click **Add** under the analysis settings box. A new group will be created:



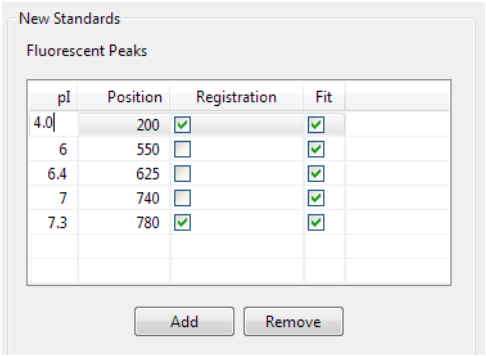
3. Click on the new group and enter a new name.



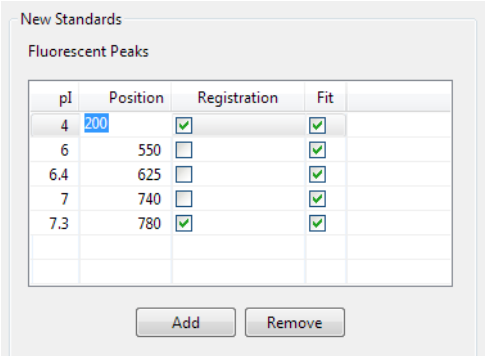
4. Click in the first cell in the **pI** column in the Fluorescent Peaks table.



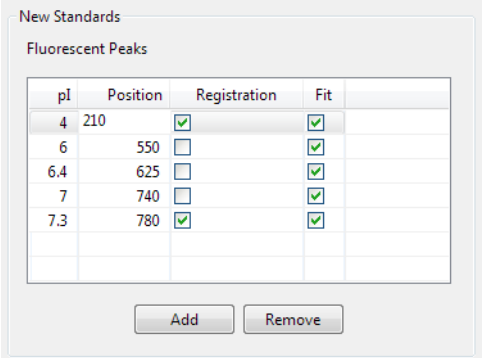
5. Enter the pI for the fluorescent standard.



6. Click in the first cell in the **Position** column.

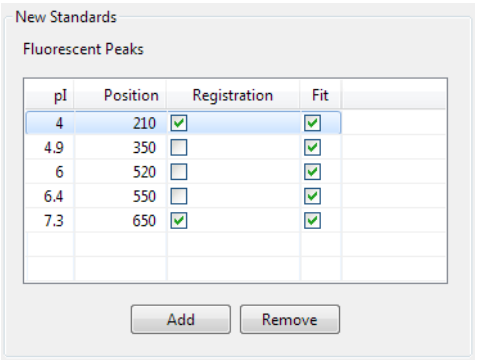


7. Enter the position of the fluorescent standard peak.



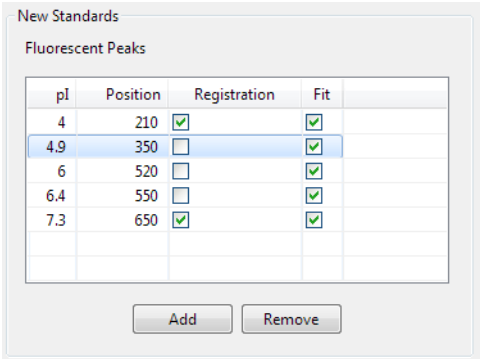
NOTE: Standards peak positions are relative to each other. Only the difference in their position is used to help identify the standard peaks. When entering standard peak information for the first time, review the standards data in the Analysis screen to find the correct peak position.

8. Repeat the steps above for the remaining standards in the table.
   - **To add another standard** - Click **Add** under the peak table, then modify the information in the new row.
   - **To remove a standard** - Select its row and click **Remove**.
9. Select which standard should be used for capillary registration by clicking the checkbox in the **Registration** column. The first and last standards are typically used for the registration.

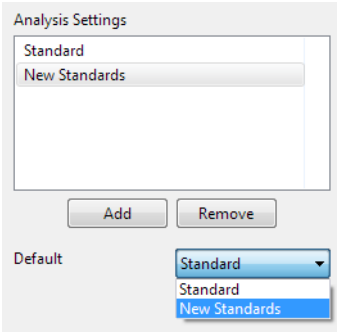


NOTE: In order for Compass for Simple Western to perform data analysis, at least one peak must be selected for registration.

10. Select which standards should be used for pI determination of sample proteins by clicking the checkbox in the **Fit** column. The standards not used for registration are typically also used for fit.



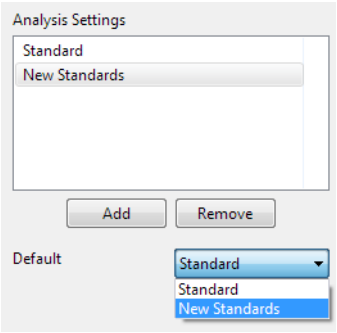
11. To use the new group as the default analysis settings for the run file data, click the arrow in the drop down list next to Default, then click the new group from the list. Analysis settings in the new group will then be applied to the run data.



12. Click **OK** to save changes.

Changing the Default Standards Group

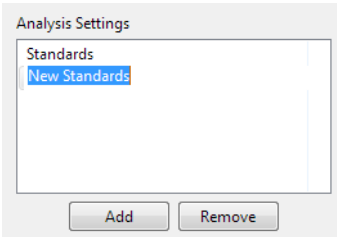
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click the arrow in the drop down list next to Default, then click a new default group from the list.



3. Click **OK** to save changes. Analysis settings in the group selected will be applied to the run data.

Modifying a Standards Group

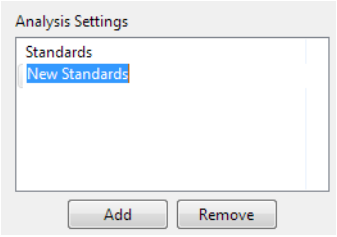
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click on the group in the analysis settings box you want to modify.



3. Modify fluorescent standards information as described in "Creating a New Standards Group" on page 453.
4. Click **OK** to save changes. The new analysis settings will be applied to the run data.

Deleting an Analysis Group

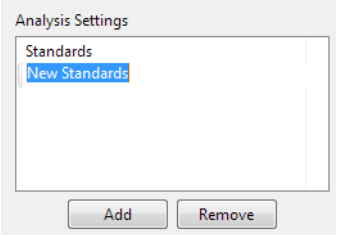
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click on the group in the analysis settings box you want to delete and click **Remove**.



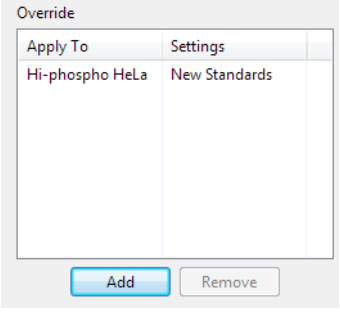
3. Click **OK** to save changes.

Applying Analysis Groups to Specific Run Data

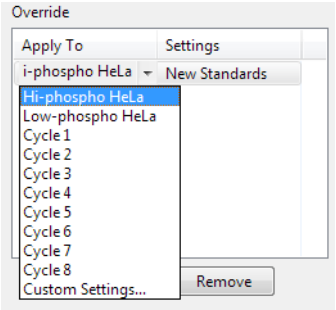
1. Select **Edit** in the main menu and click **Analysis**, then click **Standards** in the options list.
2. Click on the group in the analysis settings box you want to apply to specific run data.



3. Application of analysis groups to specific run data is done in the override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

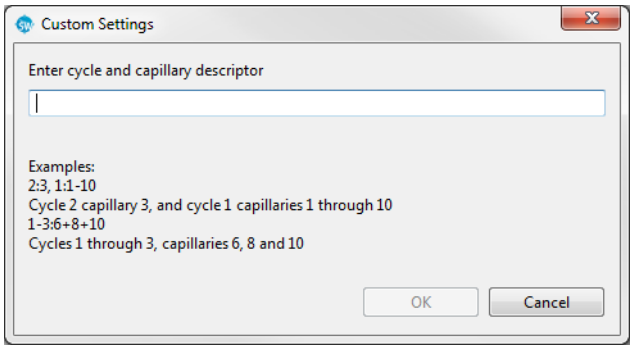


4. Click the cell in the **Apply To** column, then click the down arrow.

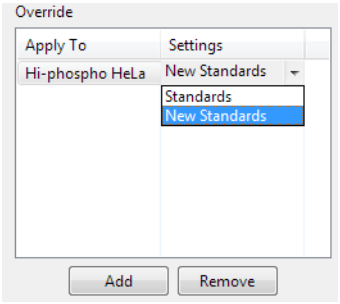


5. Select an option from the drop down list. This will apply the settings group selected to specific run data as follows:

   - **Sample names** - All sample names entered in the assay template (Assay screen) will display in the drop down list, otherwise the default name of Sample will be shown. Select a name to apply group settings to all capillaries that use this sample name in the run file.
   - **Attributes** - All sample attributes entered in the assay template (Assay screen) will display in the drop down list. Select an attribute to apply group settings to all capillaries that use this attribute in the run file.
   - **Cycle 1-8** - When this option is selected, group settings will be applied to all capillaries in the cycle.
   - **Custom settings** - Lets you choose specific capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries:



6. If you need to change the analysis group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop down list.



7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **OK** to save changes.

Importing and Exporting Analysis Settings

The analysis settings in a run file can be exported as a separate file. This allows the same analysis settings to be imported into other assays or run files at a later time, rather than having to re-enter settings manually.

Importing Analysis Settings

NOTE: Importing an analysis settings file populates the settings in all Analysis pages.

---

1. Open the run file or assay you want to import analysis settings to.
2. Select **Edit** in the main menu and click **Default Analysis** (Assay screen) or **Analysis** (Analysis screen).
3. Click **Import** on any page.
4. Select a settings file (*.settings) and click **OK**. The imported settings will display in all analysis pages.

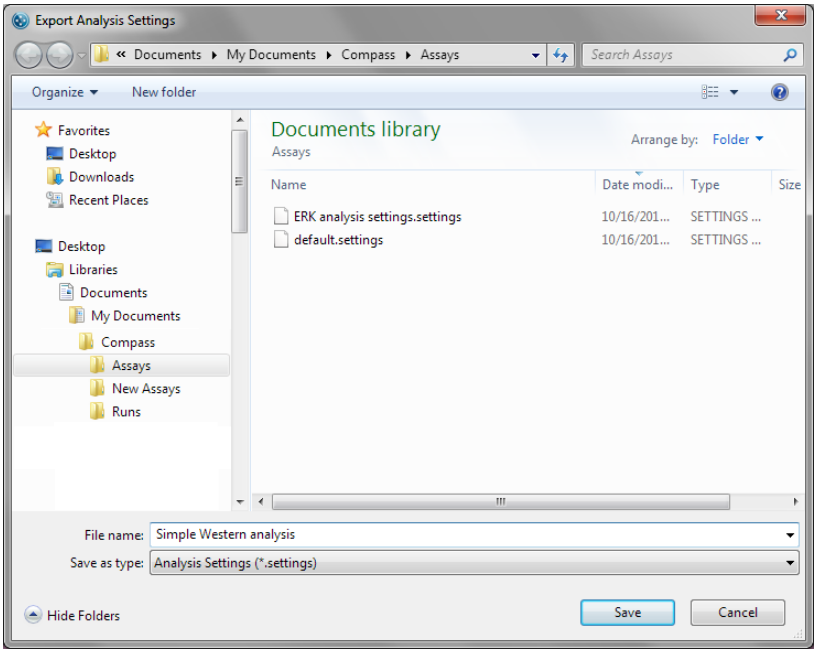
Exporting Analysis Settings

NOTE: Exporting an analysis settings file exports the settings in all Analysis pages.

---

1. Open the run file or assay you want to export analysis settings from.
2. Select **Edit** in the main menu and click **Default Analysis** (Assay screen) or **Analysis** (Analysis screen).

3. Click **Export** on any page. The following window displays:



4. The default directory is Compass/Assays. Change the directory if needed.
5. Enter a file name and click **Save**. The settings will be saved as a *.settings file.

Chapter 11:

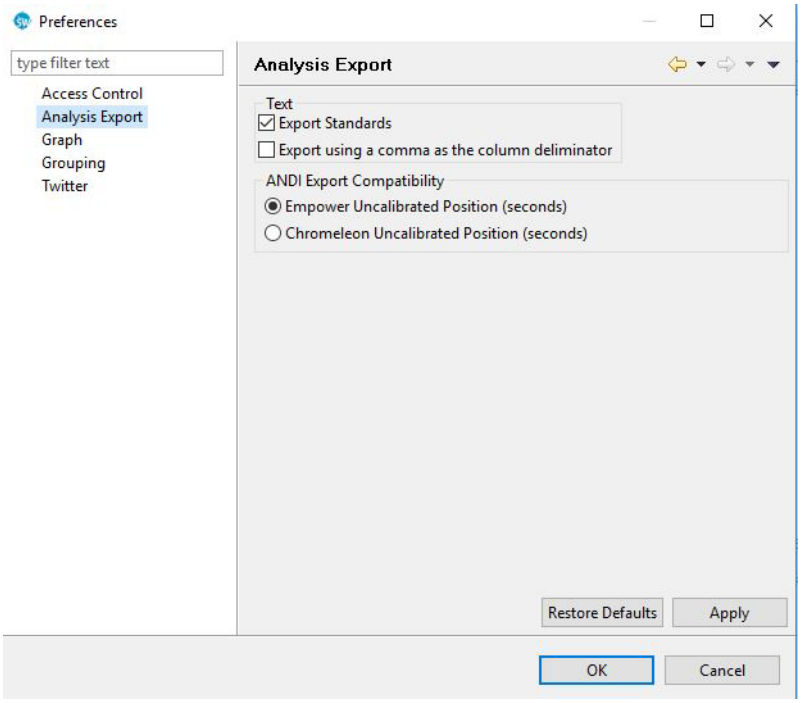
Setting Your Preferences

Chapter Overview

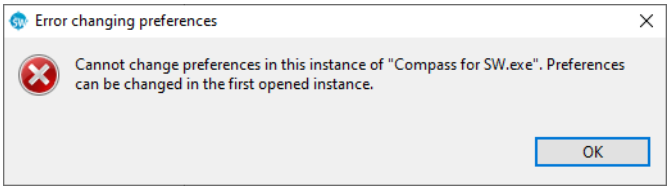
- Custom Preference Options
- Setting Data Export Options
- Selecting Custom Plot Colors for Graph Overlay
- Setting Up Jess, Wes, Sally Sue and Peggy Sue to Send Tweets

Custom Preference Options

You can set and save custom preferences for Access Control, data export, plot colors in the graph and Twitter communication. To access these settings, select **Edit** in the main menu and click **Preferences**.



NOTE: In Compass for Simple Western version 5.0 and higher, you can no longer change or save Custom Preferences outside of the first instance of the software that's been opened. When running multiple instances of the software on the same computer, the following message displays if you try to change Custom Preferences in any instance except the first instance opened.



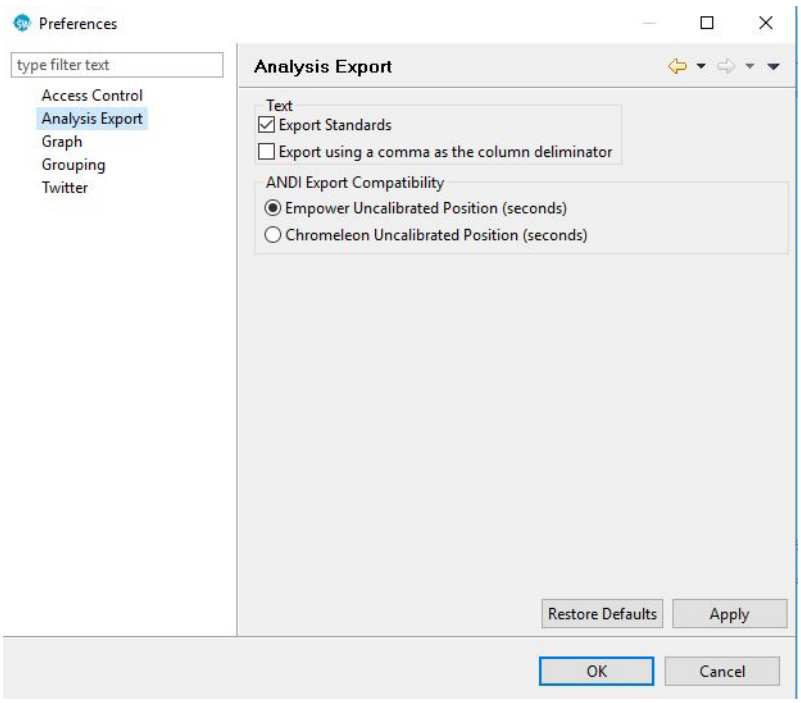
To move between preferences pages in this window, click on any option in the list on the left. The following items can be user-customized in Compass for Simple Western:

- **Analysis Export** - Lets you customize data export options.

- **Graph** - Lets you customize graph color displays.
- **Twitter** - Lets you configure Jess, Wes, Sally Sue or Peggy Sue to Tweet run status.

Setting Data Export Options

Select **Edit** in the main menu and click **Preferences**, then click **Analysis Export** in the options list.

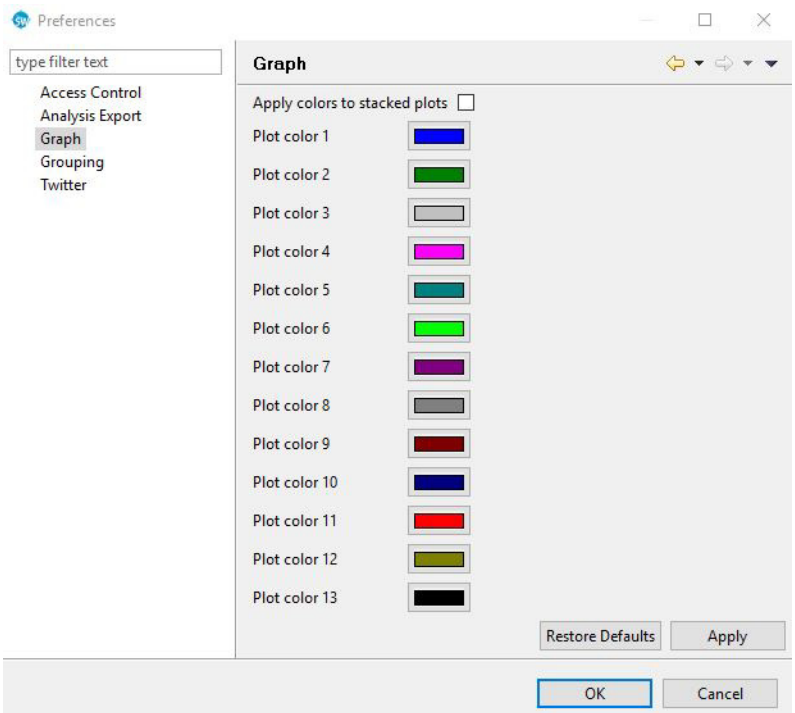


- **Export Standards** - Selecting this option includes data for the standards in each sample when run data is exported. When this option is deselected, only sample data will be exported. This option is selected by default.
- **Export using a comma as the column delimiter -** Selecting this option exports run data in .csv format. When this option is deselected, the data is exported in .txt format.
- **Empower** - This option exports uncalibrated run data in a format that is compatible for further analysis in Empower and other analysis programs that use standard formats.
- **Chromeleon** - This option exports uncalibrated run data in a format that is compatible for further analysis in Chromeleon and other analysis programs that use standard formats.
- Click **Apply** to apply changes to any open run files in Compass for Simple Western.
- Click **Restore Defaults** to restore Compass for Simple Western default settings.

- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Selecting Custom Plot Colors for Graph Overlay

Select **Edit** in the main menu and click **Preferences**, then click **Graph** in the options list.



- **Apply colors to stacked plots** - Selecting this option applies the color scheme to individual electropherograms when the Stack the Plots option is selected in the Analysis screen graph pane.

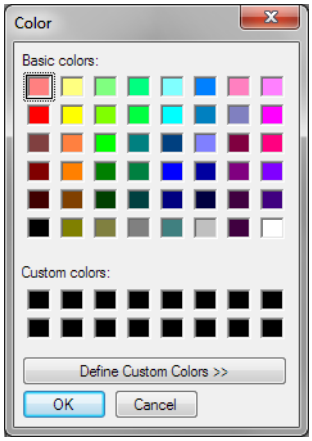
NOTE: If Apply colors to stack plots is not checked, the colors shown in the preferences screen will be applied only to overlaid electropherograms when the Overlay the Plot option is selected in the graph pane.

- When this option is deselected, plots will use Compass for Simple Western default colors.
- Click **Apply** to apply changes to any open run files in Compass for Simple Western.
- Click **Restore Defaults** to restore Compass for Simple Western default settings.

- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

Changing Plot Colors

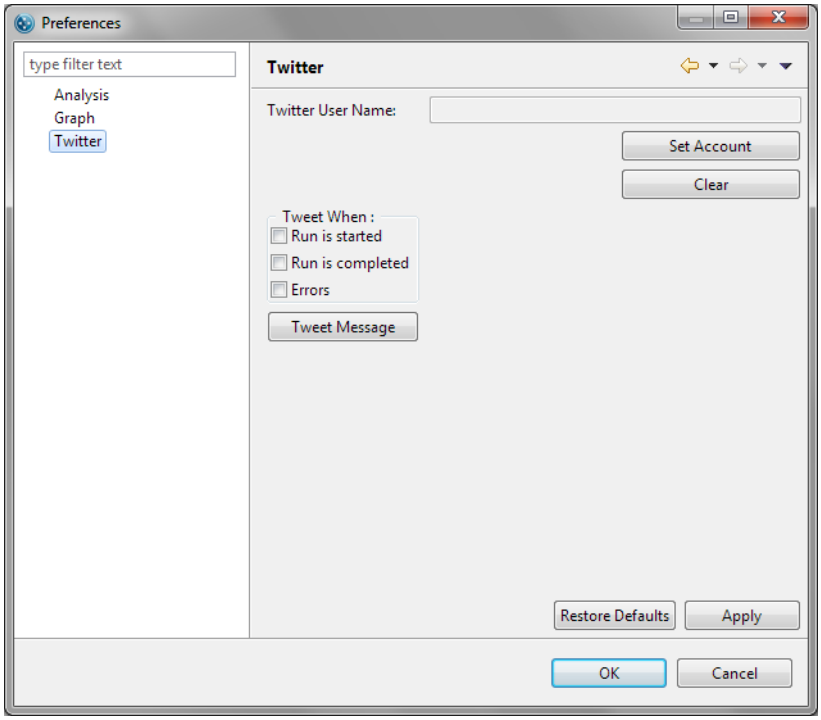
1. Select **Edit** in the main menu and click **Preferences**, then click **Graph** in the preferences list.
2. Click the color button next to a plot number. The color selection box displays:



3. Select a color or define a custom color and click **OK**. The color button will update to the new color selected.
4. Repeat the steps above for any other plot colors.
5. Check **Apply Colors to Stacked Plots** if you want the new color scheme to also be used for the Stack the Plots option in the graph pane.
6. Click **Apply** to apply changes to plots currently displayed in the graph pane.
7. Click **OK** to save changes and exit. When the Overlay the Plots option is selected in the graph pane, the new color scheme will be used.

Setting Up Jess, Wes, Sally Sue and Peggy Sue to Send Tweets

Select **Edit** in the main menu and click **Preferences**, then click **Twitter** in the options list.



- Click **Apply** to apply changes.
- Click **Restore Defaults** to restore Compass for Simple Western default settings.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

To have Jess, Wes, Sally Sue or Peggy Sue tweet a Twitter account:

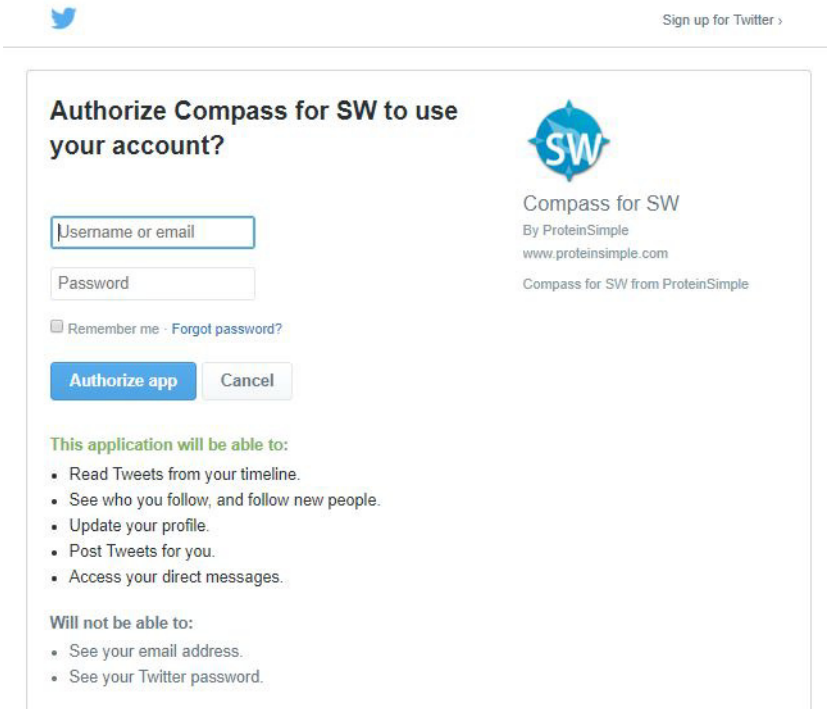
NOTES:
To set Jess, Wes, Sally Sue or Peggy Sue up to tweet, the computer you are using must have an internet connection.

To tweet, Jess, Wes, Sally Sue or Peggy Sue must be connected to the internet through a network connection or via the local lab computer.

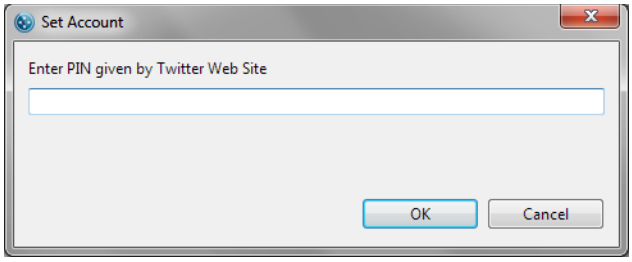
We recommend setting up a separate Twitter account for Jess, Wes, Sally Sue or Peggy Sue. This lets multiple people in the lab follow run progress. It also lets you send tweets directly from Jess, Wes, Sally Sue or Peggy Sue to all users, for example to notify others when the instrument is available or when an error has been reset, etc.

---

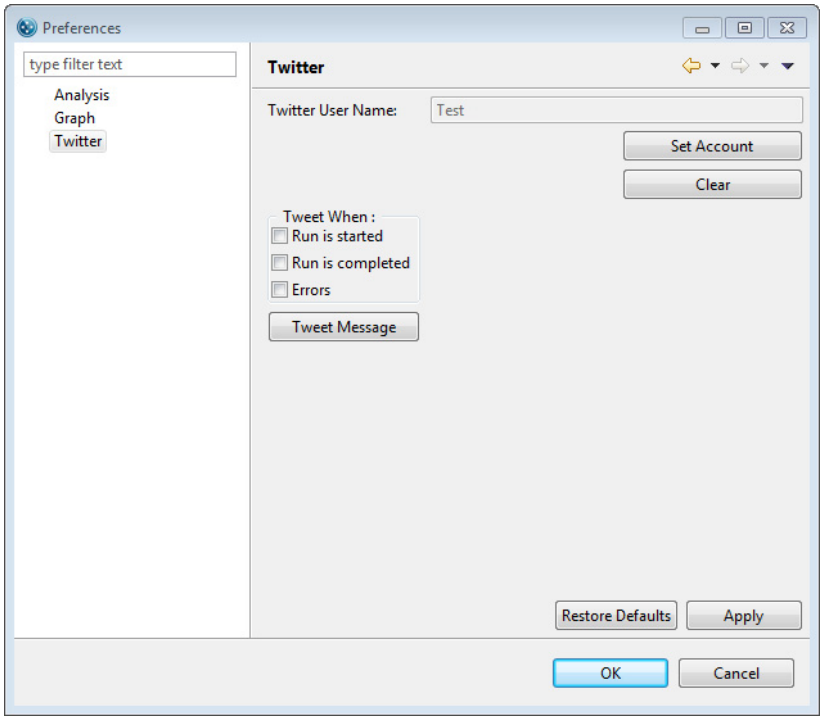
1. Click **Set Account**. A set account window will display in Compass for Simple Western and the following browser window will open:



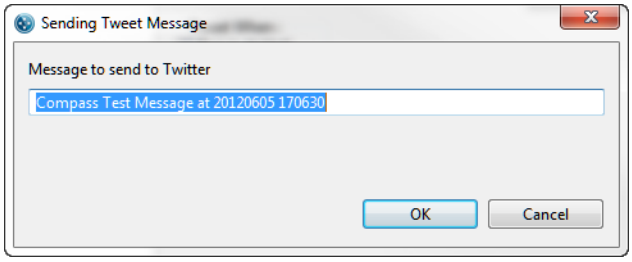
2. Enter a user name or email and password, then click **Authorize app**. A new page will display in the browser with a PIN number.
3. Enter the PIN number in the set account window in Compass for Simple Western and click **OK**:



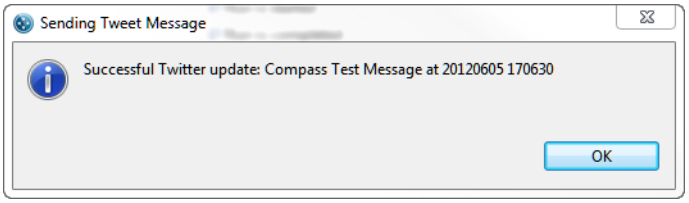
4. The user name will now appear in the Twitter User Name box. Select one or all of the tweet options in the Tweet When box, then click **Apply**.



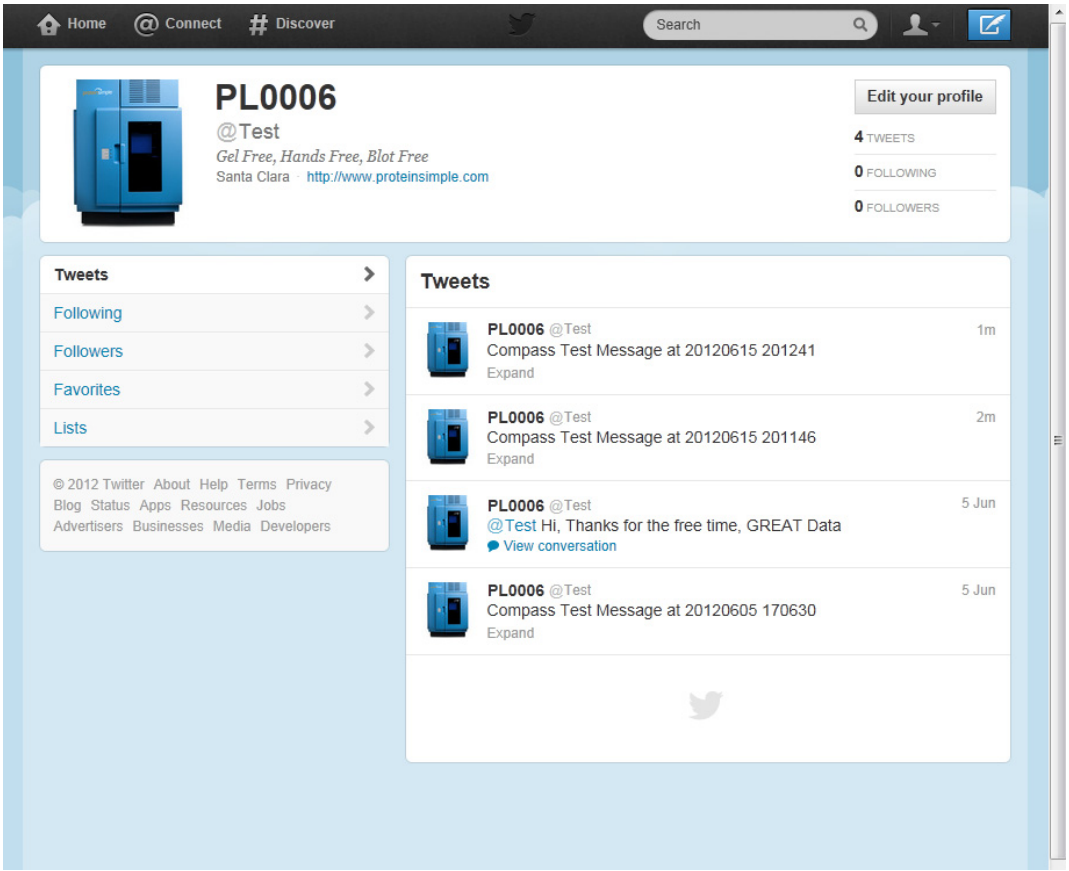
5. To confirm the Twitter account is receiving messages, click **Tweet Message**. Enter a test message and click **OK**.



6. If the test Tweet was successful, Compass for Simple Western will display the following message:



7. Click **OK** to save changes and exit. Jess, Wes, Sally Sue or Peggy Sue will automatically tweet as the selected options occur, as shown below:



Changing the Twitter Account

To change the Twitter account Jess, Wes, Sally Sue or Peggy Sue uses:

1. Select **Edit** in the main menu and click **Preferences**, and click **Twitter** in the preferences list.
2. Click **Clear**.
3. Set up the new account as described in "Setting Up Jess, Wes, Sally Sue and Peggy Sue to Send Tweets" on page 468.

Sending Manual Tweets from Jess, Wes, Sally Sue and Peggy Sue

You can send tweets directly from Jess, Wes, Sally Sue or Peggy Sue. For example, you may want to notify other users that the instrument is available, being serviced or when an error has been cleared. To do this:

1. Select **Edit** in the main menu and click **Preferences** and click **Twitter** in the preferences list.
2. Click **Tweet Message**.
3. Enter a test message and click **OK**. The tweet will be received by any users following the Twitter account Jess, Wes, Sally Sue or Peggy Sue uses.

Chapter 12:

Compass Access Control and 21 CFR Part 11 Compliance

Chapter Overview

- Overview
- Enabling Access Control
- Logging In to Compass for Simple Western
- Saving Changes
- Signing Files
- Instrument Command Log
- Run File History
- Troubleshooting Problems and Suggested Solutions
- Authorization Server

Overview

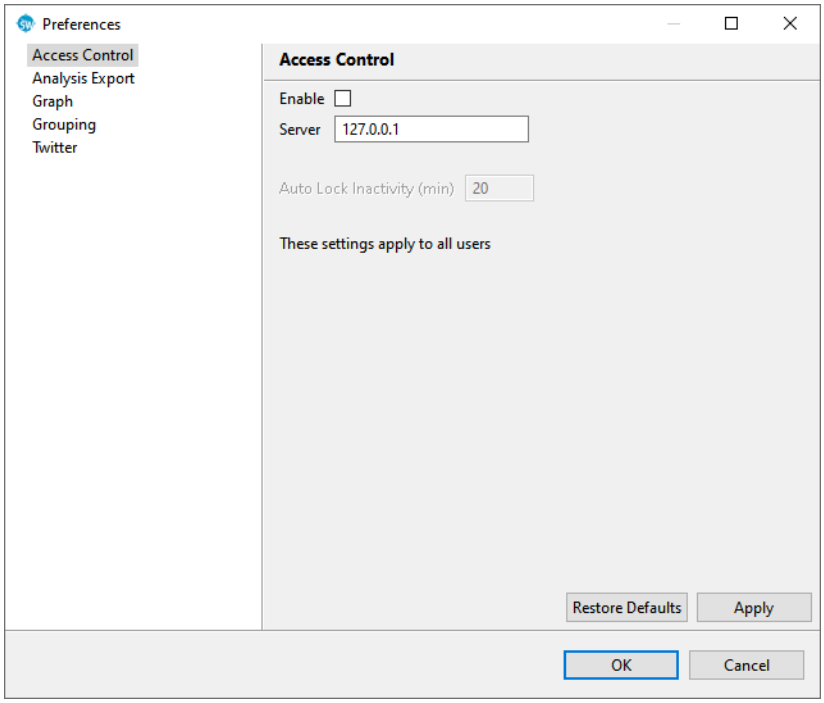
The Compass for Simple Western Access Control feature can be used to help satisfy the 21CFR Part 11 data security requirements when using Simple Western instruments. When Access Control is enabled and the Authorization Server has been installed (see "Authorization Server" on page 485):

- Users are required to log in to Compass for Simple Western when the software is launched
- A history of all actions is maintained
- Data files are signed and encrypted to prevent unauthorized changes (*e.g.*, all files are controlled)
- Each instrument maintains a history of user commands
- Each assay and data file includes a history of signed changes to the file

Compass for Simple Western can be run with or without Access Control enabled. When Access Control is disabled, no user log in is required and files are not encrypted or signed. The instrument history and file history are still maintained but the entries are not signed.

Enabling Access Control

Access Control is enabled in **Preferences**. Select **Edit** in the main menu, click **Preferences**, then select **Access Control**.



To enable Access Control:

1. Check the **Enable** box.
2. Enter the IP address of the Authorization server. Use format X.X.X.X or LocalHost if installing the server on the local machine.
3. Enter the time (in minutes) of inactivity that the software should Auto Lock after.

NOTE: The Auto Lock Inactivity (min) setting will not be enabled until after the user has closed the software and logged into the Authorization Server.

---

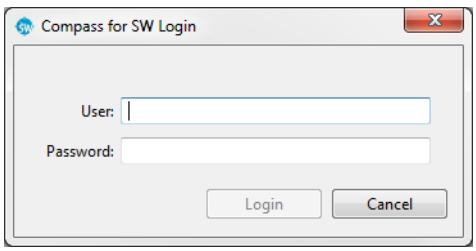
4. Close Compass for Simple Western. The next time the software is launched, a user log in will be required.

---

*NOTE: Access Control can only be disabled by logging into Compass for Simple Western and deselecting the **Enable** box in the Access Control page of Preferences.*

Logging In to Compass for Simple Western

With Access Control enabled, all users must log in to Compass for Simple Western whenever the software is launched.

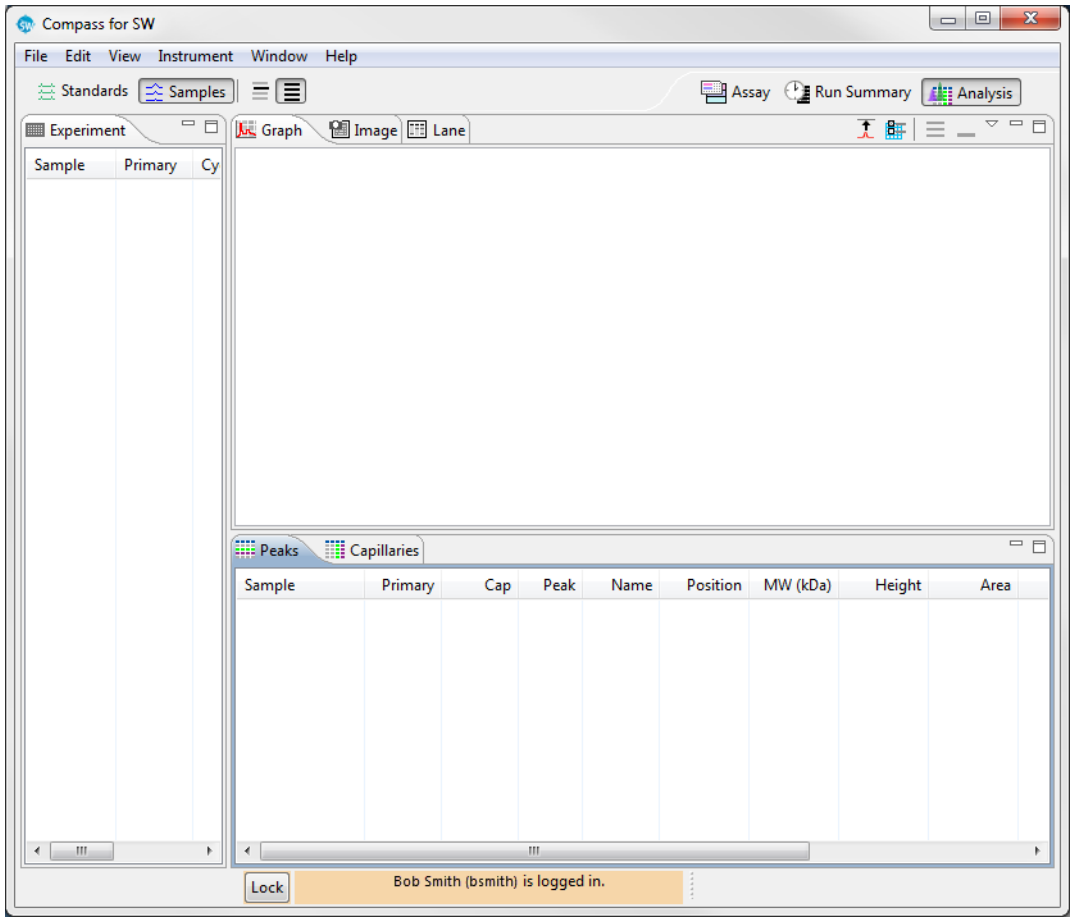


Enter your user name and password previously setup by your Compass for Simple Western Administrator.

NOTES:
Your account will be blocked after a certain number of login failures. If this happens, contact your administrator to unblock the account.

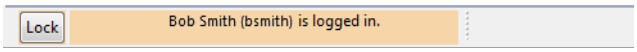
Administrators can have full Compass login permissions. By default the admin/admin user has full Compass login permissions, but it's possible to remove login permissions from any administrator.

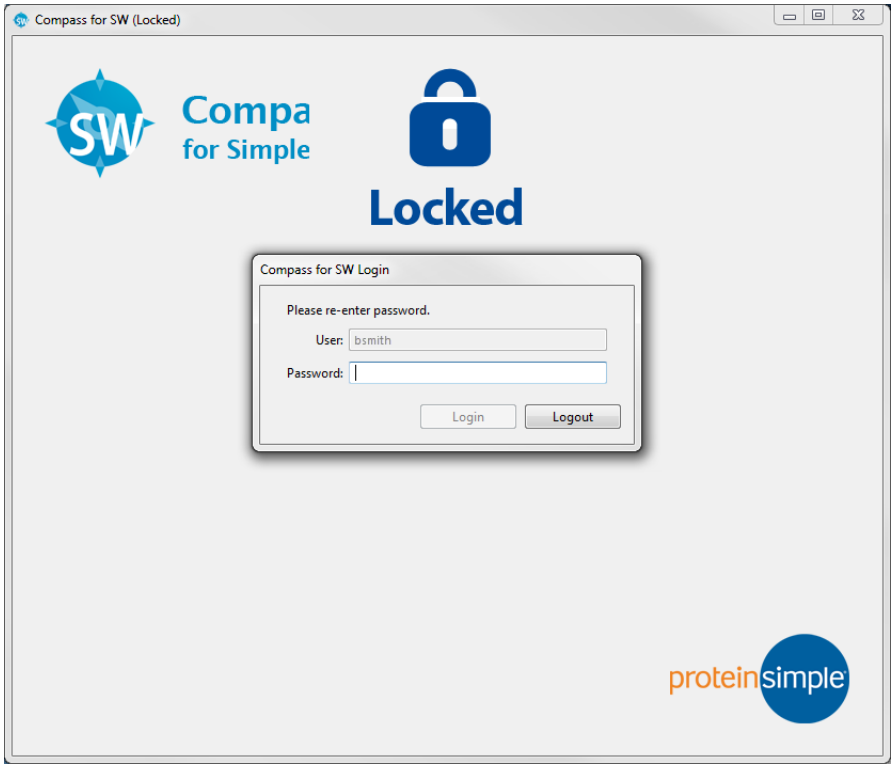
A successful log in will display the Compass for Simple Western main window with the user information in the lower status bar. The full user name is displayed with the unique user ID in parenthesis:



Locking and Unlocking the Application

You can click the **Lock** button to lock Compass for Simple Western and prevent access by other users. To unlock the application, users must re-enter their password.



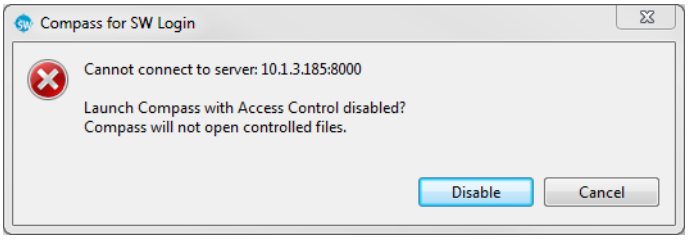


Resolving Log In Issues

Log in failures may occur when:

- The server is temporarily unavailable
- Compass is using the wrong IP address

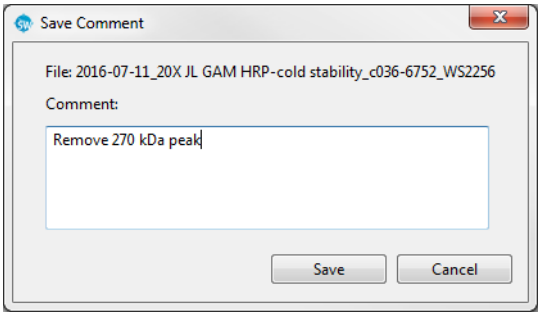
When this happens, the following message displays:



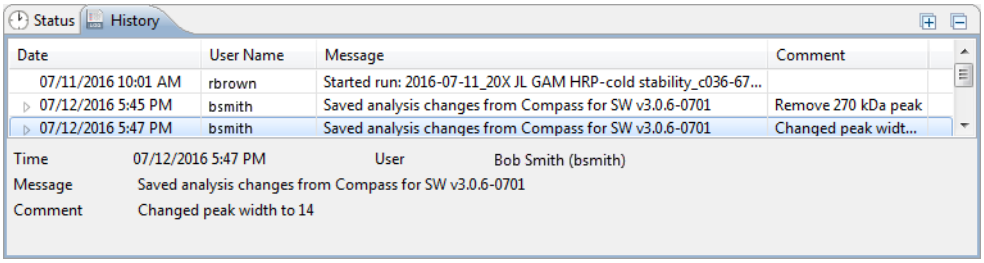
Click **Disable** to restart Compass for Simple Western with Access Control disabled. Verify or correct the server IP address then close and restart Compass to log in with Access Control enabled.

Saving Changes

When **Save** is selected from the **File** menu, a dialog box will display to allow you to enter a comment before saving the signed file:

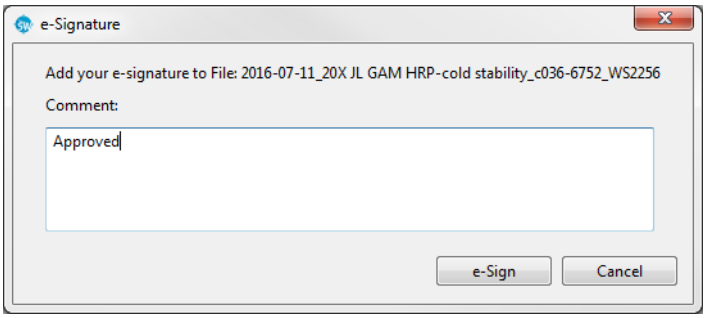


The comment is added to the signature entry in the file History:

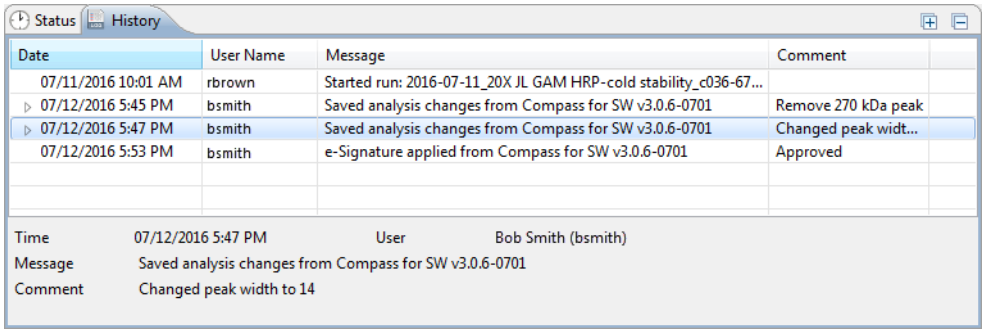


Signing Files

Select **e-Signature** from the **File** menu to add an electronic signature to a file.

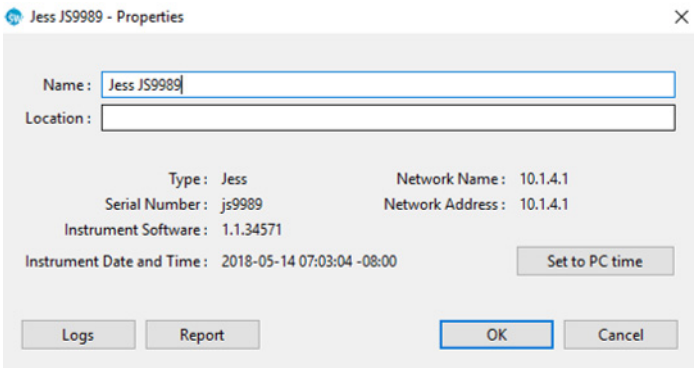


The signed entry will be added to the file History with the meaning of the signature entered in the comment, such as *Approved* or *Verified*.

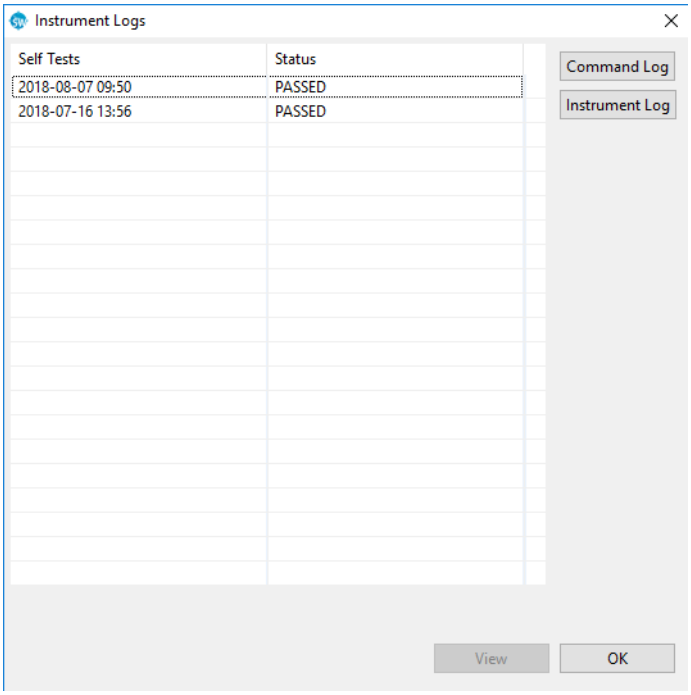


Instrument Command Log

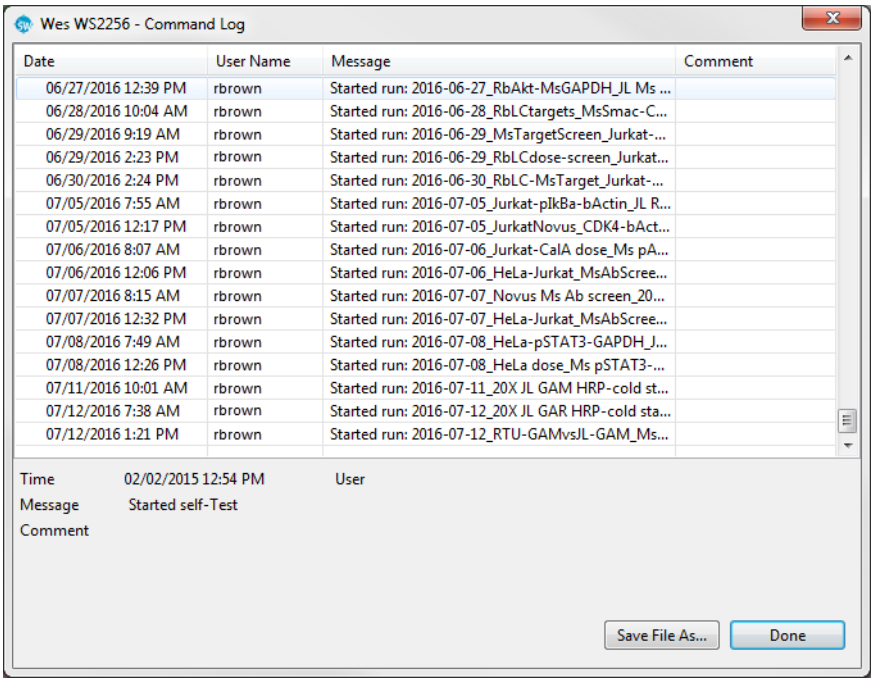
The Instrument Command Log can be viewed at any time by selecting the **Instrument** menu and clicking **Properties**, and then clicking the **Logs** button:



In the Logs dialog box, select **Command Logs**:

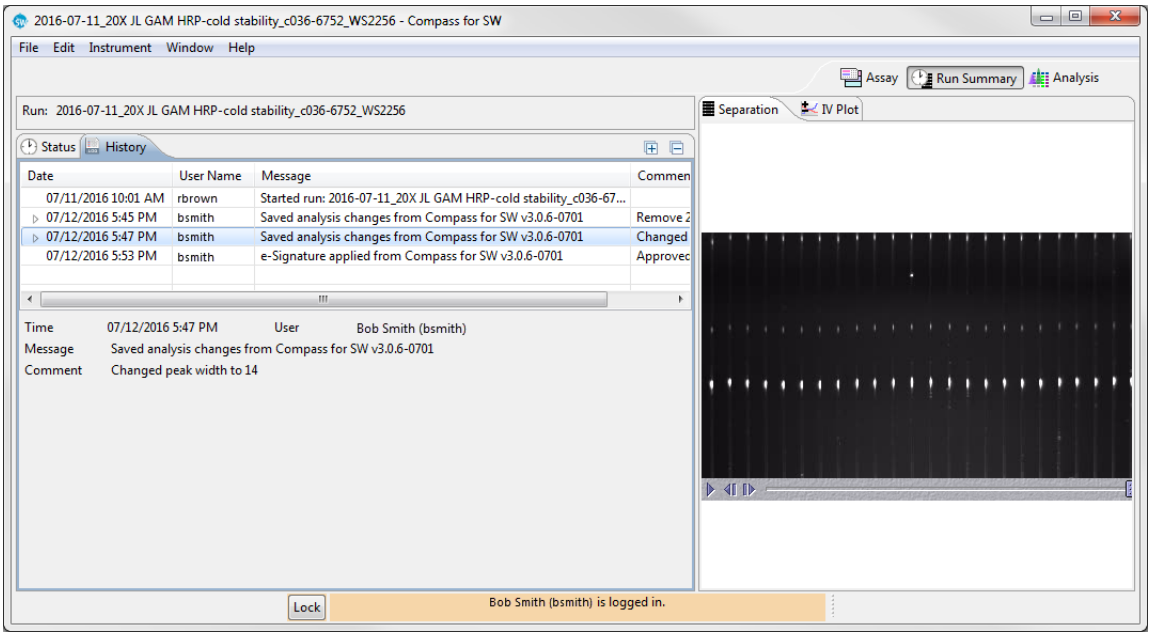


The Command Log lists all the commands sent to the instrument that were signed by the user who sent the command. If you want to copy the Command Log at any time, right click in the table and select **Copy**, then paste into another document.



Run File History

Select the **Run Summary** screen tab and then the **History** tab to see the file History. To copy the file History, right click in the table and select **Copy**, then paste into another document.



Troubleshooting Problems and Suggested Solutions

If any of the following error messages are encountered, follow the recommended steps below to resolve the issue.

- **Unknown user name or password**.
  - Check if the Caps Lock is on, user name and password are case sensitive.
  - Ask a Compass for Simple Western administrator to confirm your user name. If your password is unknown then the administrator can reset your password (see "Resetting User Passwords" on page 493 for more information).
- **Server not available**. From the **Edit** menu, click **Preferences** and then **Access Control** to confirm the server address is set to the correct Authorization server address. Compass must be able to reach the server on the network.
- **Controlled file cannot be opened without log in.** To open a controlled Run file, enable Access Control by clicking **Edit**, then **Preferences** and **Access Control.** Select **Enable**, close Compass, then re-launch Compass with a valid log in.
- **Uncontrolled file cannot be opened when logged in.** To open an uncontrolled Run file, disable Access Control by clicking **Edit**, then **Preferences** and **Access Control.** Deselect **Enable**, close Compass then re-launch the software.

NOTE: Uncontrolled Assay files can be opened when Compass for Simple Western Access Control is enabled (controlled mode).

- **Command disabled.** Certain commands are only available when a user with the correct permissions is logged in. To change user permissions, use a web browser to log in to the Authorization server web interface at the address shown on the **Access Control** page in **Preferences**, such as: 10.1.3.231:8000.
- **Compass for Simple Western does not prompt for log in.** Compass for Simple Western will only prompt for a log in on launch when Access Control is enabled in Preferences. Enable Access Control by clicking **Edit**, then **Preferences** and **Access Control.** Select **Enable**, close Compass then re-launch the software. You should now be prompted for a log in.

Authorization Server

The Authorization Server controls the log in access to Compass for Simple Western. In the simplest configuration, the server is run on the same computer as Compass for Simple Western and only that copy of Compass is controlled. A single server can also be used to control access to multiple copies of Compass for Simple Western running on different computers, so long as they have network access to the server. Multiple copies of the server may be run on the same network, and each server will have its own user database.

To enable Compass for Simple Western to use a particular Authorization Server, click **Edit**, then **Preferences** and **Access Control** and enter the server IP address using format X.X.X.X.

NOTE: If the server is installed on the same computer as Compass for Simple Western (e.g., the local machine), enter LocalHost instead of the IP address. Contact your local IT Administrator to assist with installing the Authorization Server in your preferred format.

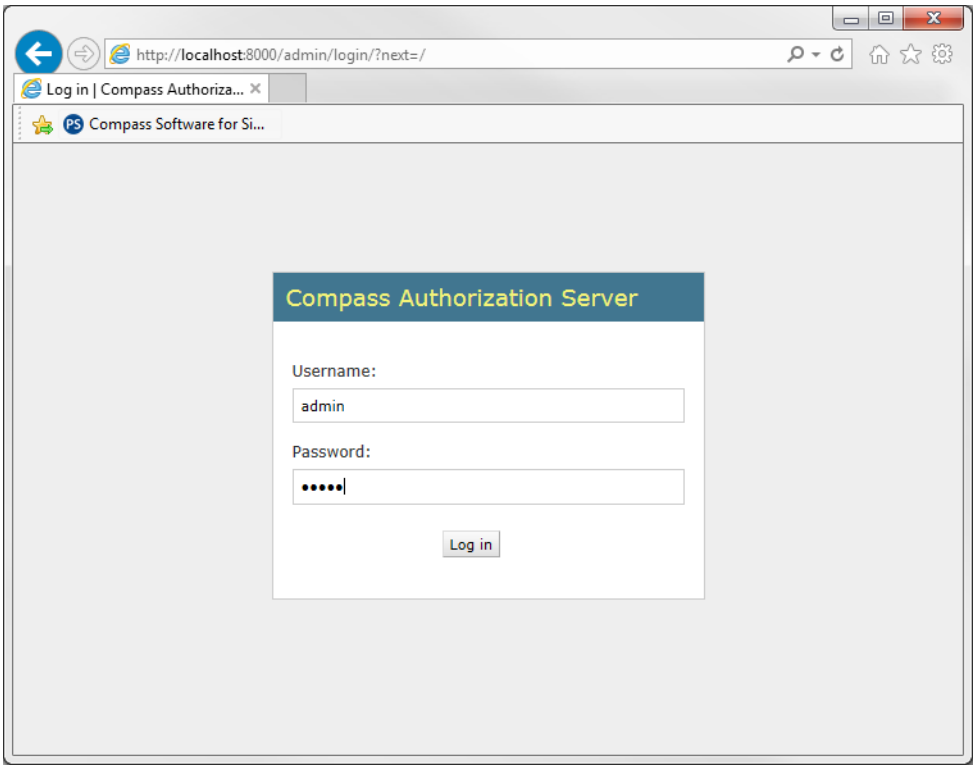
Server Administration

The Authorization Server is configured through a web interface at the IP address of the server on port 8000. To access the Server home page, open any browser and type the IP address on port 8000 in a X.X.X.X:8000 or http://X.X.X.X:8000 format. Use LocalHost instead of the IP address if the Server is installed on the local machine.

The default server administrator is:

- User: admin
- Password: admin

After installing the Authorization Server, the administrator user name and password can be changed.



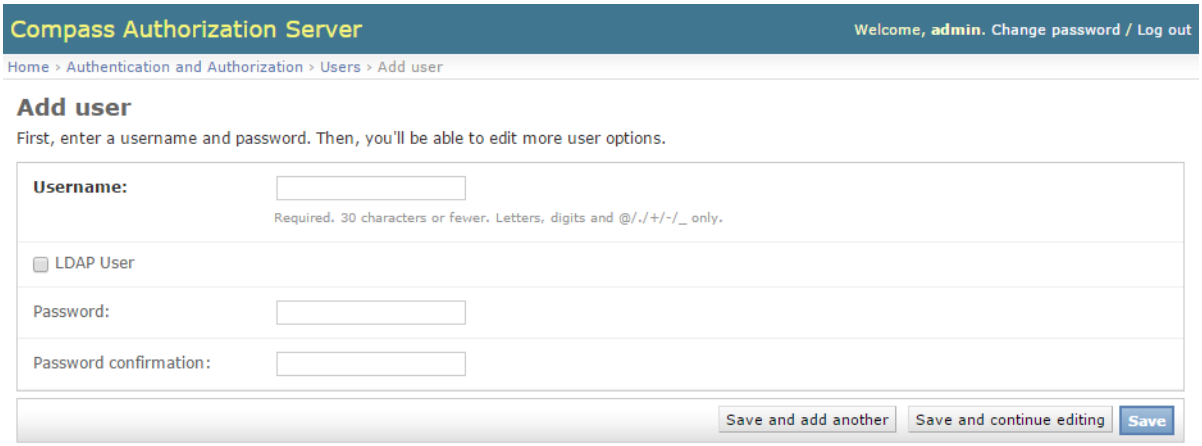
Adding Non-admin Users

Add a user to the server to allow that user to log in to Compass for Simple Western. To do this:

1. Select **Users** from the Site Administration home page:

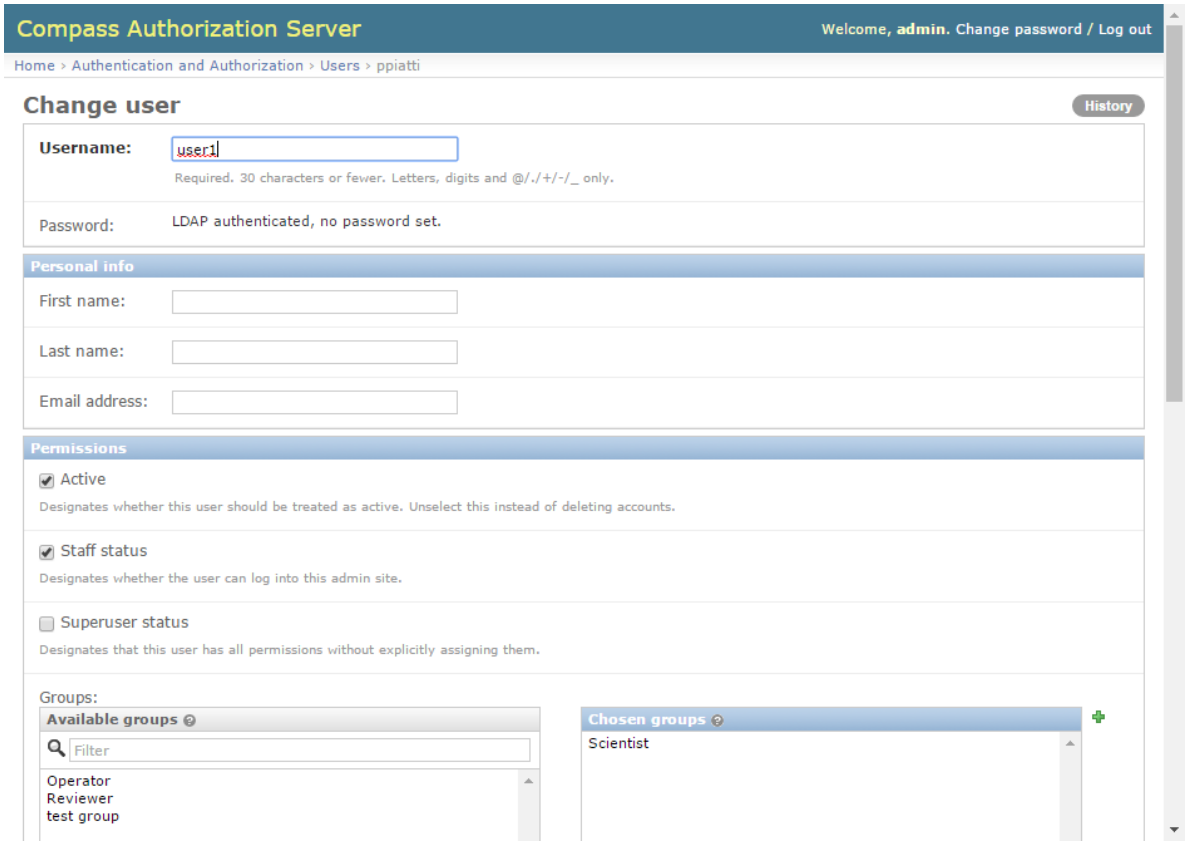
2. From the Users page, select **Add User**:

3. Fill in the fields to create a new user:



After adding a new user more information can be added:

NOTE: You must enter the user's first and last name.

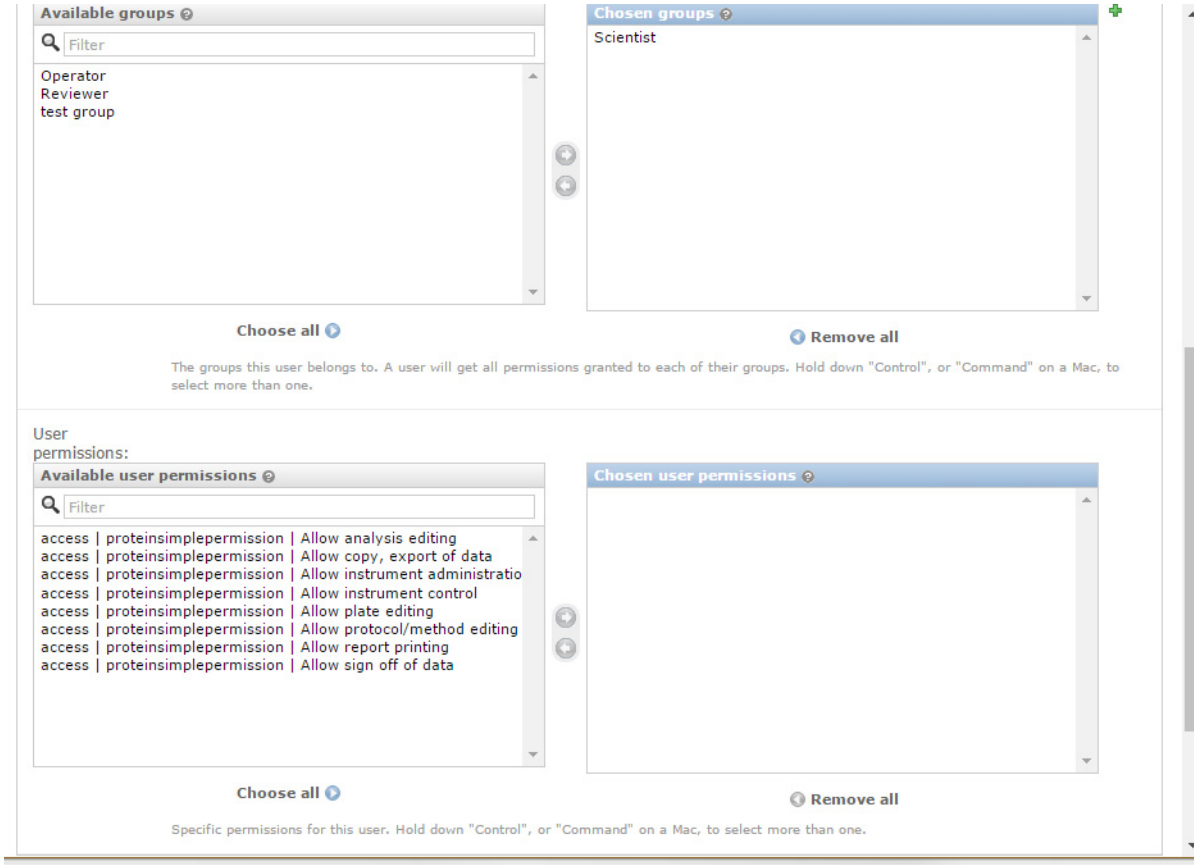


NOTE: Users are blocked after the number of login failures defined in the Password policy setting.

Permissions

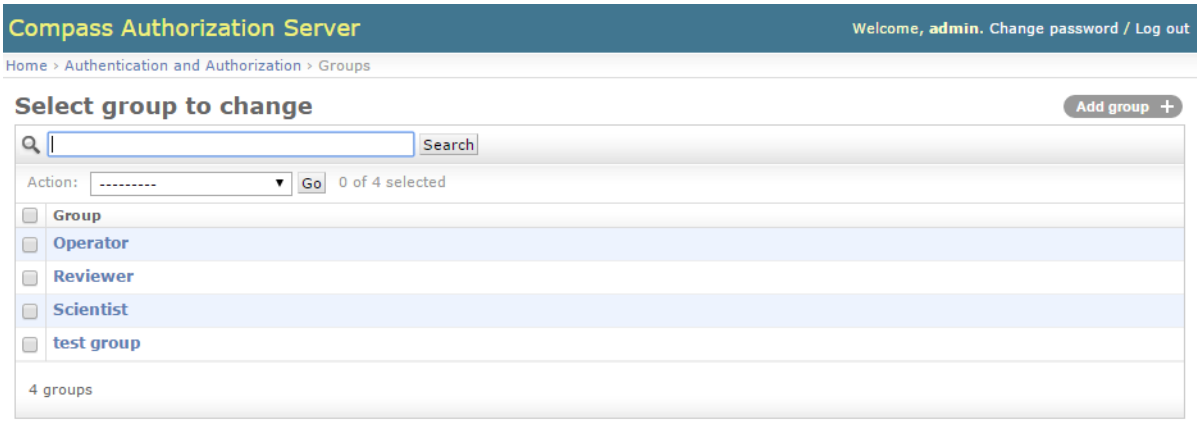
All users can log in to Compass for Simple Western, but the commands available within Compass are controlled by Permission settings. Commands a user does not have permission to use will be disabled. After user permissions have been changed on the server the user must close and re-open Compass to use the new permissions.

Users can belong to groups that have multiple permissions such as Manager or Scientist:

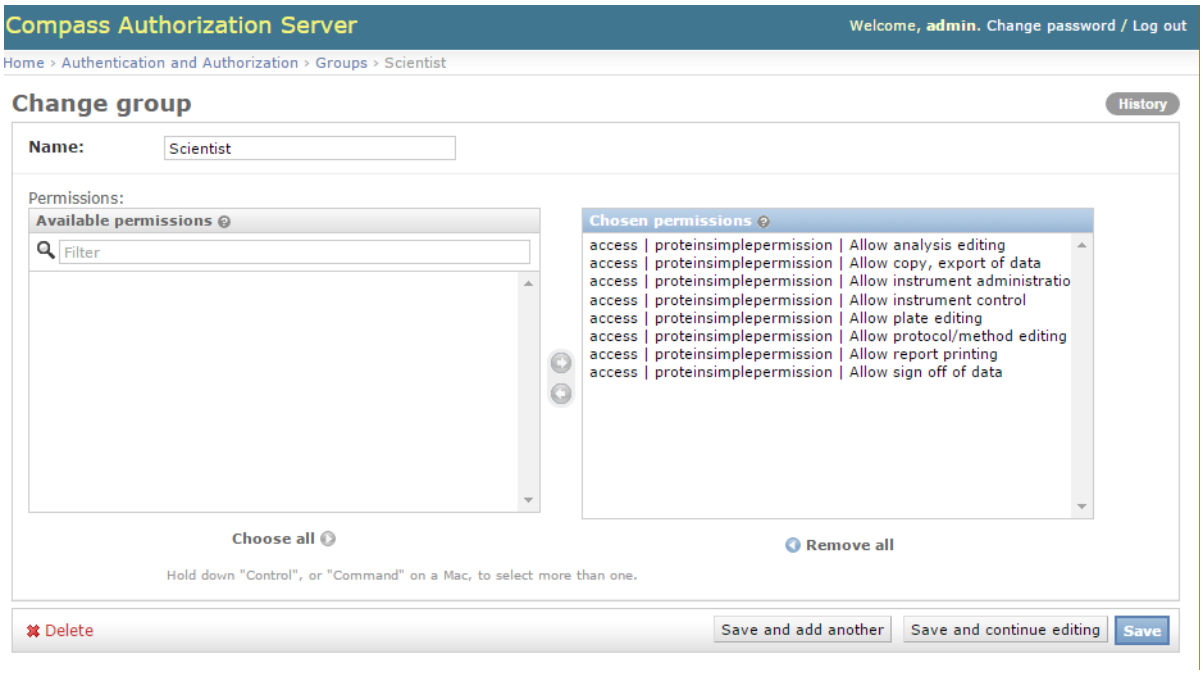

Available groups
Filter
Operator
Reviewer
test group
Chosen groups
Scientist
Choose all
Remove all
The groups this user belongs to. A user will get all permissions granted to each of their groups. Hold down "Control", or "Command" on a Mac, to select more than one.
User permissions:
Available user permissions
Filter
access | proteinsimplepermission | Allow analysis editing
access | proteinsimplepermission | Allow copy, export of data
access | proteinsimplepermission | Allow instrument administratio
access | proteinsimplepermission | Allow instrument control
access | proteinsimplepermission | Allow plate editing
access | proteinsimplepermission | Allow protocol/method editing
access | proteinsimplepermission | Allow report printing
access | proteinsimplepermission | Allow sign off of data
Chosen user permissions
Choose all
Remove all
Specific permissions for this user. Hold down "Control", or "Command" on a Mac, to select more than one.

Use the Groups page to change the permissions in a group or create new groups:

To change permissions for a group click **Change**, then select a group:



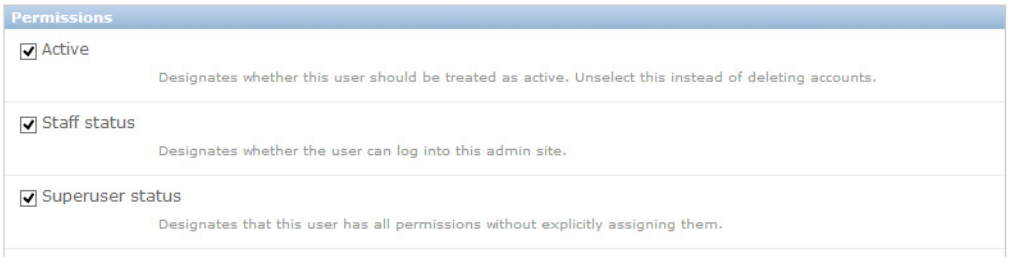
Move individual group permissions in or out of the Available Permissions and Chosen Permissions boxes by selecting a permission in either box. Click the **left** or **right** arrow button to move the permission into the other box.



Adding Admin Users

To create a user with administrator permissions:

1. Follow the steps described in "Adding Non-admin Users" on page 486 to create the admin user.
2. Under permissions, select **Staff status** and **Superuser status**:



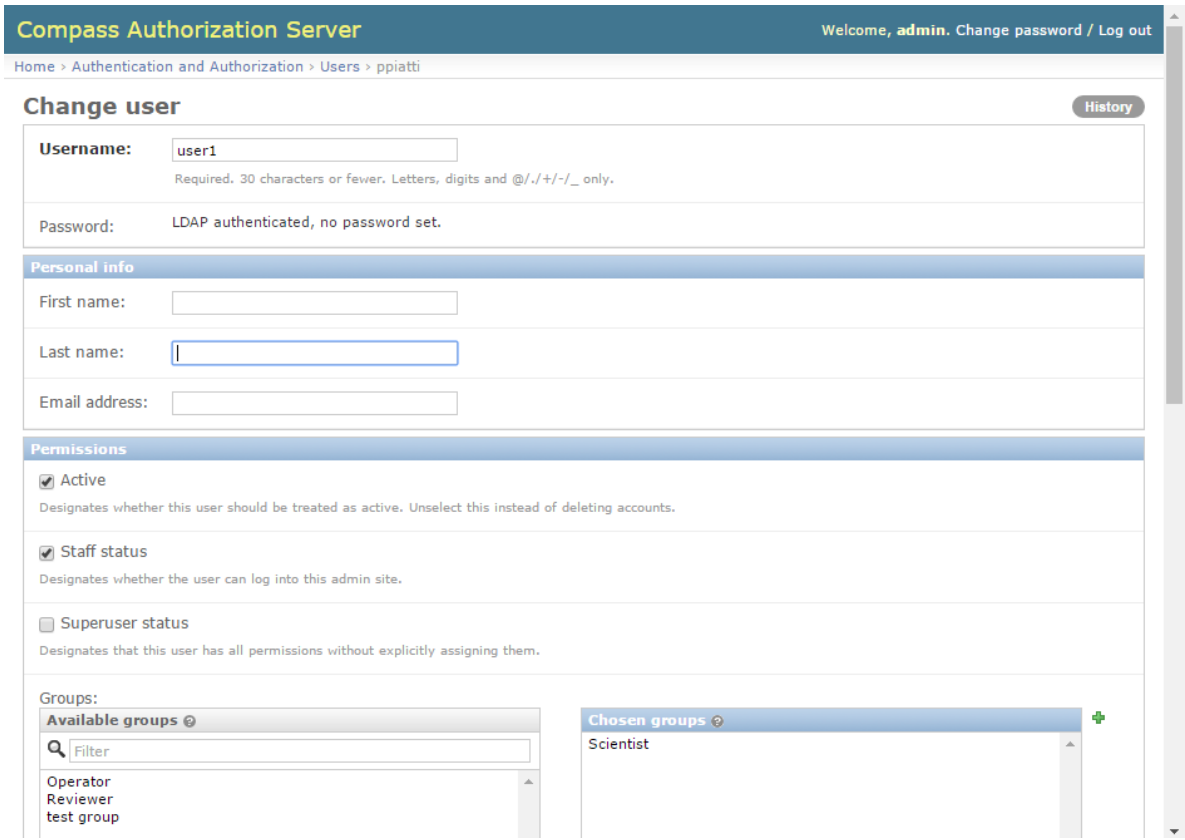
3. Assign the admin user to a group.

NOTE: Selecting Superuser status enables server permissions only. Admin users must be also be assigned to a group to in order to have Compass for Simple Western permissions.

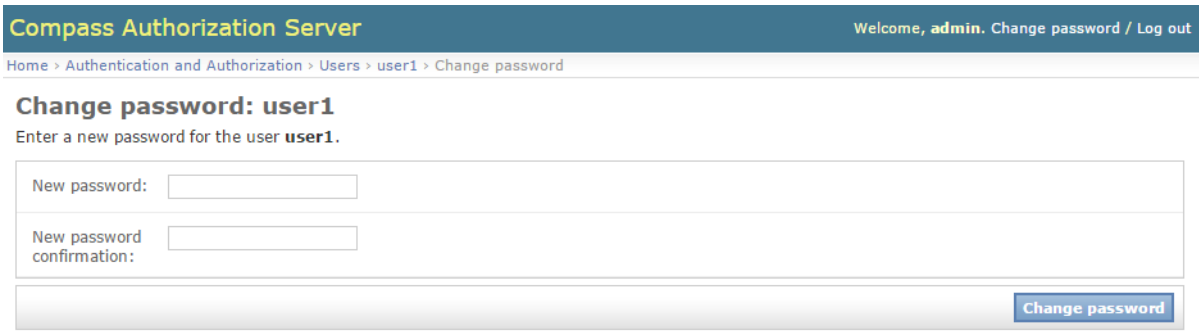
Resetting User Passwords

You need to be an Administrator to reset a user password. To reset a user password:

1. Select **Users** from the Site Administration home page, then select the user to change. The following screen displays:



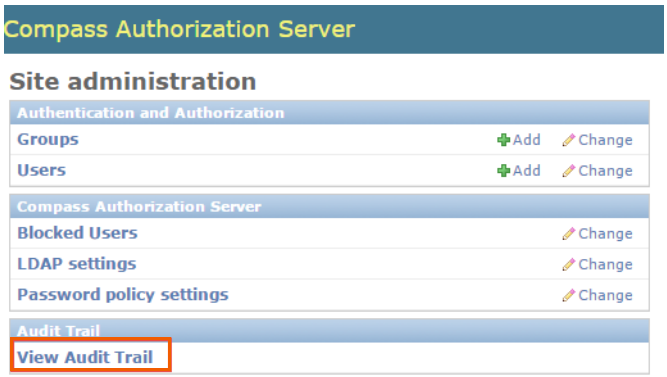
2. Raw passwords are not stored, they must be changed manually. Click the text link to access the password change form:

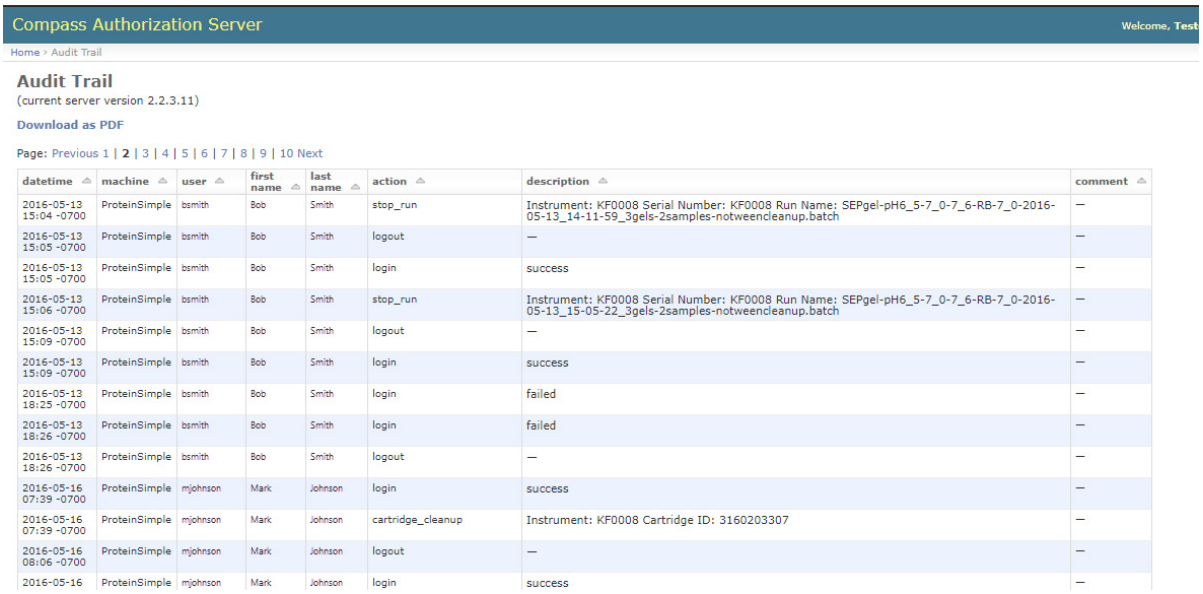


3. Enter the new password, then click **Change password**.

Audit Trail

Admin users with Staff Status can view, print and download the Audit Trail. Select **View Audit Trail** from the Site Administration home page to access it.





Compass Authorization Server

Welcome, Test

Home › Audit Trail

Audit Trail

(current server version 2.2.3.11)

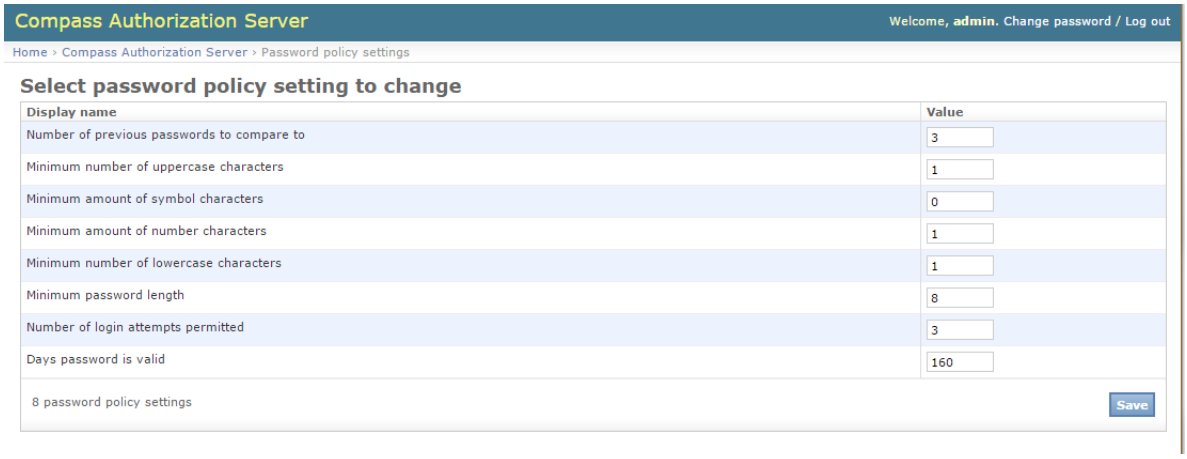
Download as PDF

Page: Previous 1 | **2** | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 Next

datetime	machine	user	first name	last name	action	description	comment
2016-05-13 15:04 -0700	ProteinSimple	bsmith	Bob	Smith	stop_run	Instrument: KF0008 Serial Number: KF0008 Run Name: SEPgel-pH6_5-7_0-7_6-RB-7_0-2016-05-13_14-11-59_3gels-2samples-notweencleanup.batch	—
2016-05-13 15:05 -0700	ProteinSimple	bsmith	Bob	Smith	logout	—	—
2016-05-13 15:05 -0700	ProteinSimple	bsmith	Bob	Smith	login	success	—
2016-05-13 15:06 -0700	ProteinSimple	bsmith	Bob	Smith	stop_run	Instrument: KF0008 Serial Number: KF0008 Run Name: SEPgel-pH6_5-7_0-7_6-RB-7_0-2016-05-13_15-05-22_3gels-2samples-notweencleanup.batch	—
2016-05-13 15:09 -0700	ProteinSimple	bsmith	Bob	Smith	logout	—	—
2016-05-13 15:09 -0700	ProteinSimple	bsmith	Bob	Smith	login	success	—
2016-05-13 18:25 -0700	ProteinSimple	bsmith	Bob	Smith	login	failed	—
2016-05-13 18:26 -0700	ProteinSimple	bsmith	Bob	Smith	login	failed	—
2016-05-13 18:26 -0700	ProteinSimple	bsmith	Bob	Smith	logout	—	—
2016-05-16 07:39 -0700	ProteinSimple	mjohnson	Mark	Johnson	login	success	—
2016-05-16 07:39 -0700	ProteinSimple	mjohnson	Mark	Johnson	cartridge_cleanup	Instrument: KF0008 Cartridge ID: 3160203307	—
2016-05-16 08:06 -0700	ProteinSimple	mjohnson	Mark	Johnson	logout	—	—
2016-05-16	ProteinSimple	mjohnson	Mark	Johnson	login	success	—

Password Policy Settings

These settings let administrators set password policies. Select **Password policy settings** from the Site Administration home page to make changes.



Compass Authorization Server

Welcome, **admin**. Change password / Log out

Home › Compass Authorization Server › Password policy settings

Select password policy setting to change

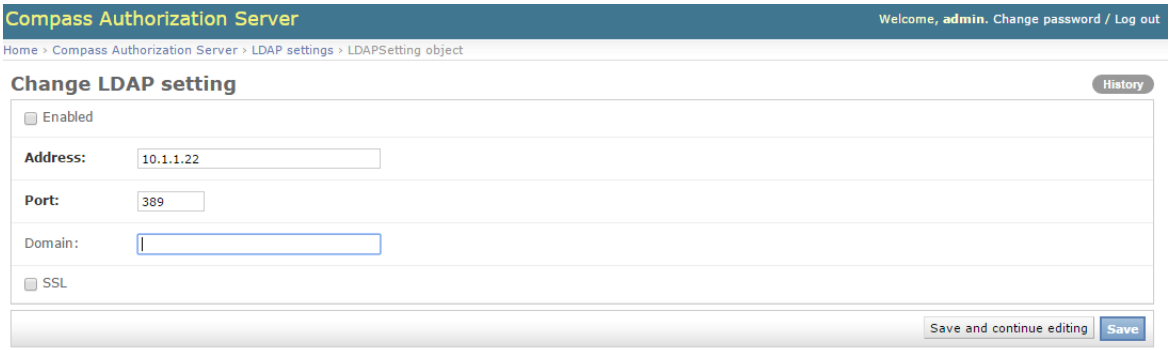
Display name	Value
Number of previous passwords to compare to	3
Minimum number of uppercase characters	1
Minimum amount of symbol characters	0
Minimum amount of number characters	1
Minimum number of lowercase characters	1
Minimum password length	8
Number of login attempts permitted	3
Days password is valid	160

8 password policy settings

Save

Lightweight Directory Access Protocol (LDAP) Settings

LDAP settings allow you to connect the Compass Authorization Server to your own network's domain controller, so users can log on with their existing network password. With LDAP, passwords are not maintained by the Compass Authorization Server, they are administered by the network admin.

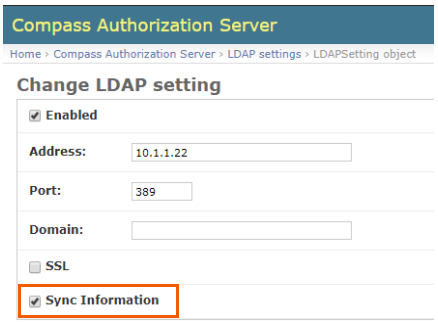


First select **LDAP settings** from the Site Administration page and set your LDAP settings.

Next, add users as described in "Adding Non-admin Users" on page 486 and select the **LDAP User** checkbox.

Passwords aren't required for LDAP users.

To synchronize the Compass Authorization Sever with the LDAP user information on your server, select the **Sync Information** box.



Encryption Details

Compass for Simple Western uses the SHA1 hash algorithm to generate a 160 bit hash code that is unique for all files. All files saved by Compass are encrypted with a digital key. This key along with the hash codes guarantees the file history is correct and no other edits were made. All changes saved to a file have the electronic signature of the user who saved the file. The **e-Signature** command allows a user to sign off on a state such as approved or verified.

There is no individual ownership of files, all users who log into Compass can open any file.