# Compass for Simple Western

## User Guide

*Compatible with Leo, Jess and Abby*

Compass for Simple Western User Guide



P/N 031-109

Revision J, March 2025

For research use only. Not for use in diagnostic procedures

## Patents and Trademarks

Bio-Techne® Simple Western™ Technology is covered by issued and pending patents in the U.S. and other countries. For more information, please see **https://www.bio-techne.com/support/instrument-support/intellectual-property**.

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# Let’s Get Started

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The user guide will provide you with information on using Compass™ for Simple Western Software to perform and monitor Simple Western™ assays on Leo™ sytems, Jess™ systems, and Abby™ systems. It will also take you through Compass for Simple Western features used to view data from a completed run and analyze your data.

**NOTE:** Refer to the **Compass for Simple Western User Guide, Rev H** for information on using Compass Software to operate a Wes system.

# Launching Compass for Simple Western Software

To open Compass Software, double-click the icon on the computer desktop.

**NOTE:**
If you are using a Wes system, Peggy Sue™ system, Sally Sue system, or NanoPro™ 1000, Compass for Simple Western version 7.0 and higher does not support these instruments. You can still use software versions 5.x and lower for Peggy Sue, Sally Sue, or NanoPro 1000 and software versions 6.x and lower for Wes to create, load, and run assays on these systems, and to analyze data. Lower versions of Compass can be downloaded at the **Instrument Software Download Center** on the Bio-Techne website.

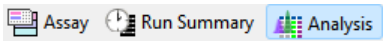
# Compass Software Overview

Compass Software 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.

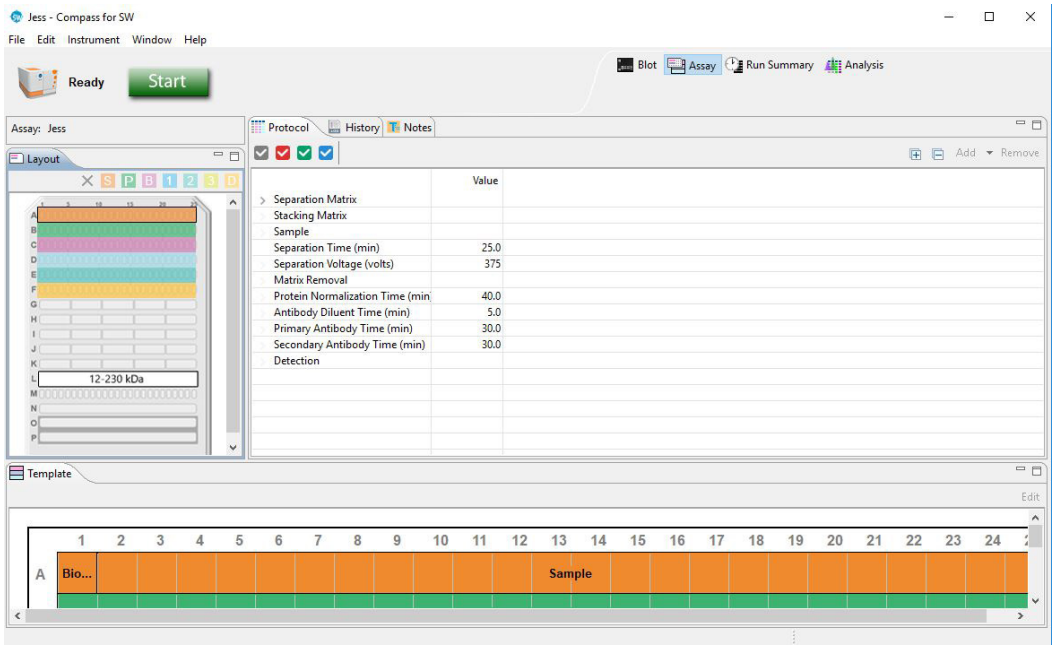
## Changing the Screen View

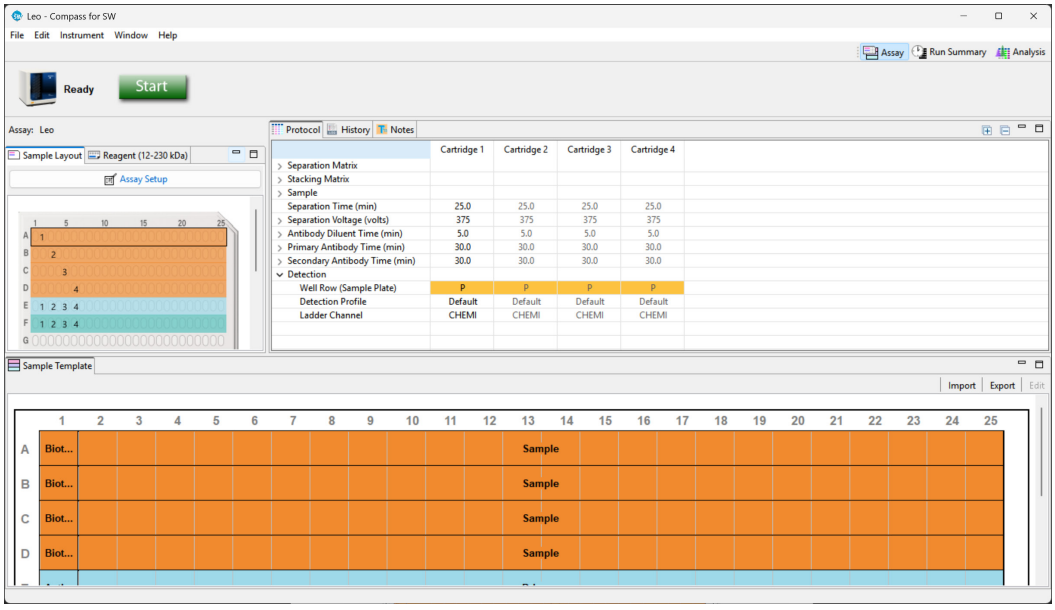
To move between the Assay, Run Summary, and Analysis screens, just click the button in the screen tab located in the upper right corner of the main window.



# Assay Screen

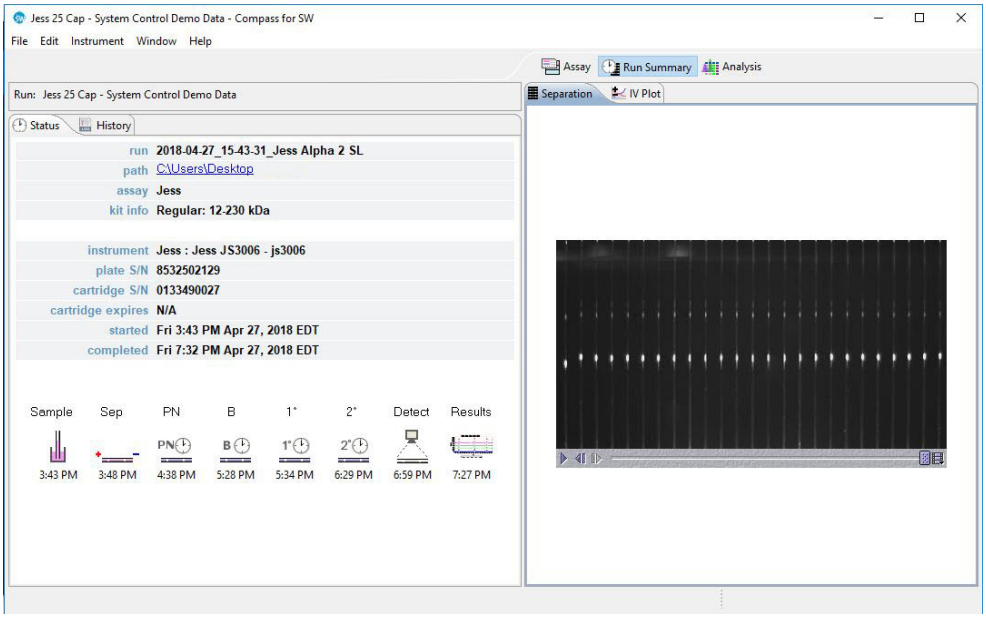
The Assay screen is used to create, view, and edit assays. You can assign well locations for assay plate reagents, modify assay protocol steps, enter assay notes, and add annotations for individual wells on the assay plate.

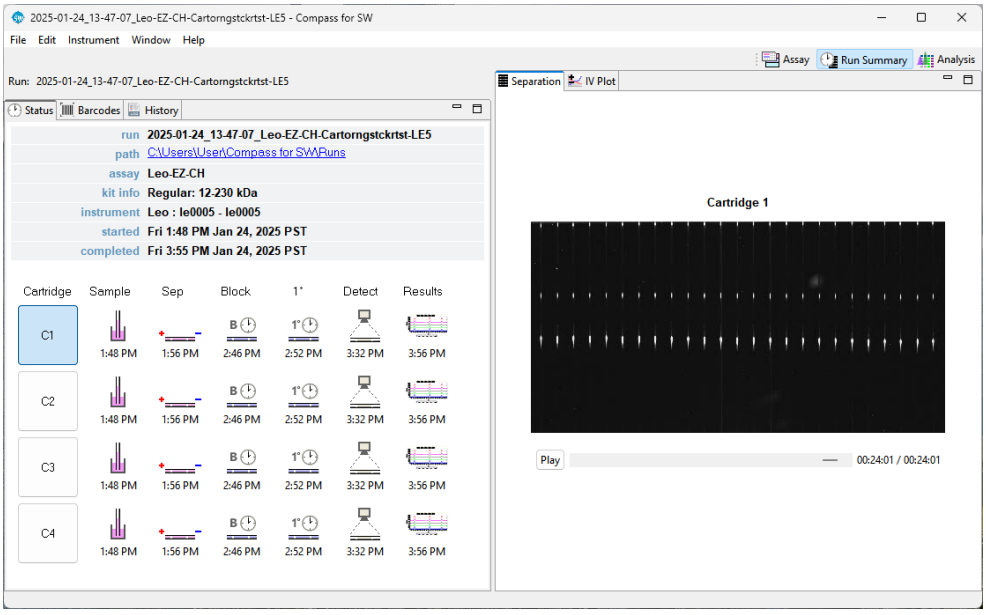


# Run Summary Screen

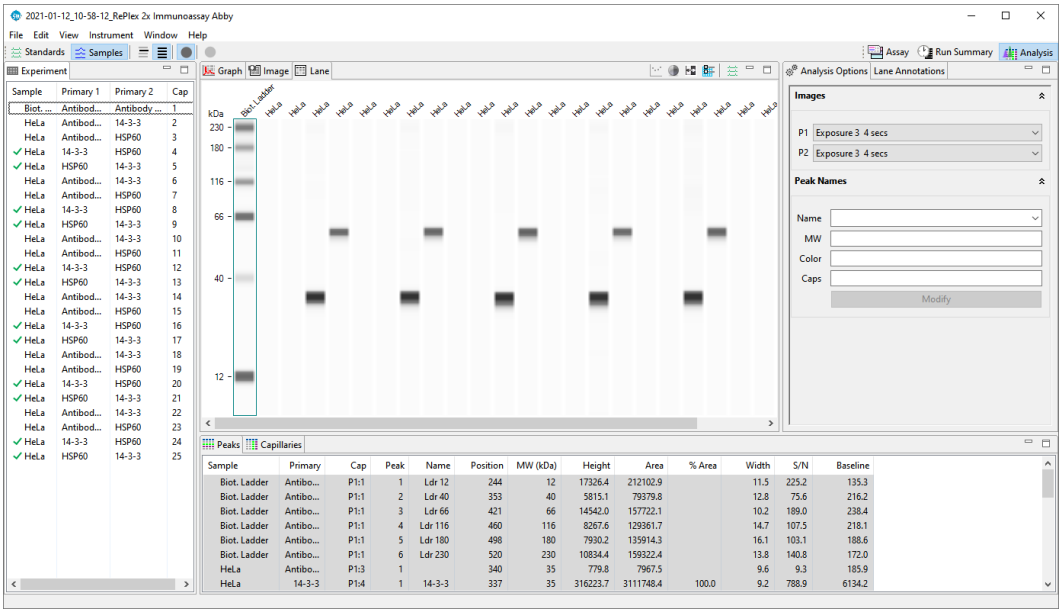
The Run Summary screen is used to monitor the status of a run in progress, watch movies of the separation in the capillaries, and view current and voltage plots for each cartridge can be viewed separately when running multiple cartridges in an experiment on a Leo instrument.



## 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.

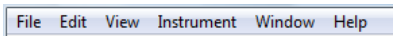
To view a pane, click in the pane or on its tab. The example below shows panes in the Analysis screen, where the Experiment pane is active:


## 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.

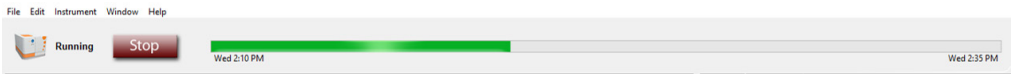
## Main Menu

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 15.




## Instrument Status Bar

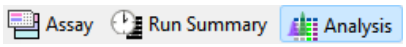
The instrument status bar is used to start runs, relay system status and show run progress. More details on instrument control and status can be found in Chapter 6: "Controlling Jess, Abby, and Leo".




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

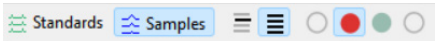
## Screen Tab

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



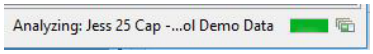

## View Bar

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, data for a single capillary or all capillaries in the run, or grouped capillary data. View bar options are detailed in "Switching Between Sample and Standards Data Views" on page 169 and "Using Groups" on page 199.



## 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.

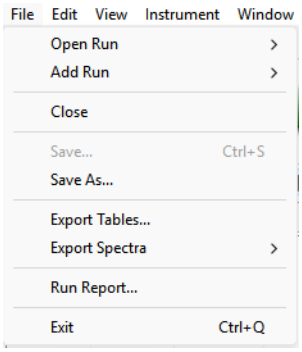



# Software Menus

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.

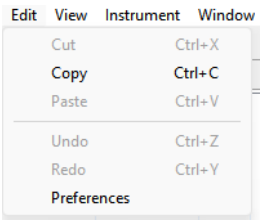
## File Menu

The File menu contains basic file commands.



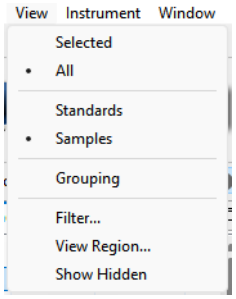

## Edit Menu

The Edit menu contains basic editing commands, analysis, and preferences options. Specific details on preferences are described in Chapter 8: "Setting Your Preferences".



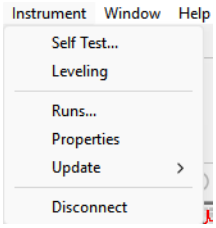
## View Menu

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



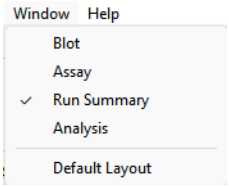

## Instrument Menu

When Compass for Simple Western is not connected to an instrument, the only option that appears will be to connect to a system. The Instrument menu contains instrument control options when the software is connected to an instrument. Instrument control options are explained in Chapter 6, "Controlling Leo, Jess, and Abby".




## Window Menu

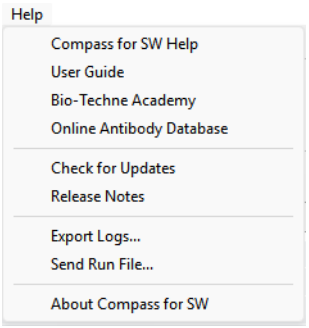
The Window menu allows you to switch between Blot, Assay, Run Summary, or Analysis screens, and restore screens to the Compass for Simple Western default layout.




- **Blot** – Displays the Blot screen where you can view and enhance blotting membrane images acquired on Jess.
- **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 the User Guide for the instrument Compass is connected to. Displays a menu of Simple Western instrument User Guides if Compass is not connected to an instrument.
- **Bio-Techne Academy** – Opens the Bio-Techne Academy where users can view online training videos, register for online workshops, view previous workshops, and view instrument documents.
- **Online Antibody Database** – Opens the Simple Western Antibody Database where you can search for antibodies validated for use on Simple Western.
- **Check for Updates** – Opens the software download center to show the last released software.
- **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 and send it to Technical Support at a later time.
- **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.

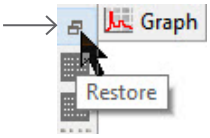
## Resizing Screen Panes

- **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.

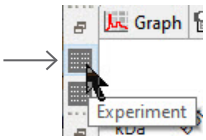

The other panes in the screen will automatically minimize to pane bars in the task area along the window border.


- **To minimize a pane** – Click the minimize button in the upper right corner of the tab. The other panes in the screen will automatically resize and the minimized pane will display as a pane bar in the task area.

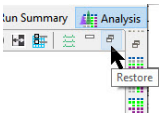

- **To restore all minimized panes** - Click **Restore** on the minimized pane bar.



- **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 click **Restore** in the tab menu bar.



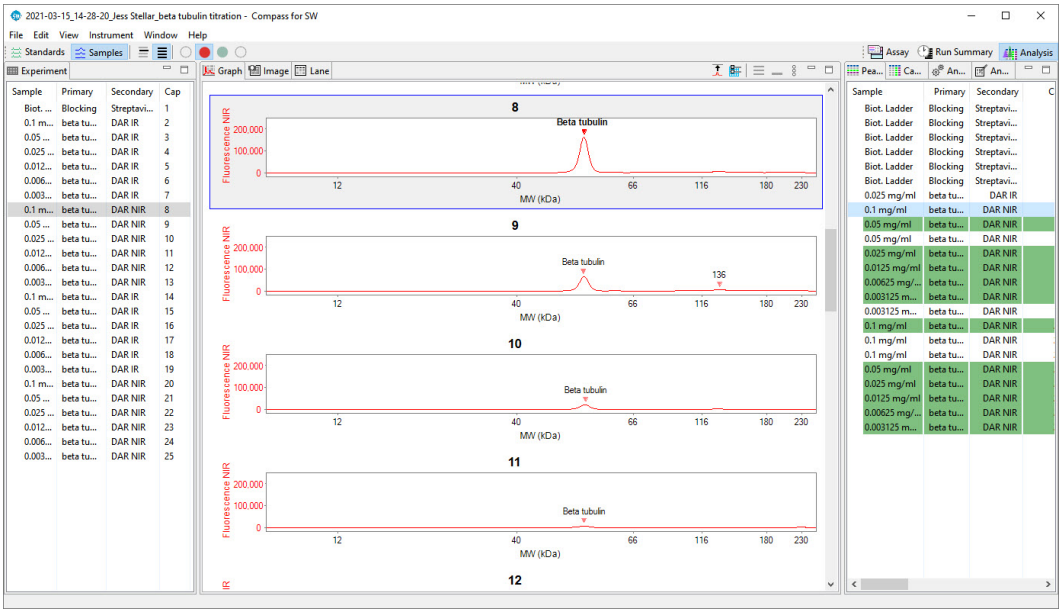

- **To restore all panes to their original sizes** - Select **Window** in the main menu and click **Default Layout**.

## Changing the Location of Screen Panes

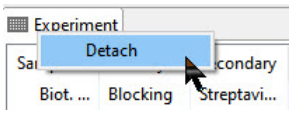
Panes can be moved to different locations within a screen.

- **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.

The following figure shows the Analysis screen after moving the Peaks 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 **Detach**.




- **To move a detached pane back inside the main window** – Select **Window** in the main menu and click **Default Layout**.
- **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 Simple Western User Guide.

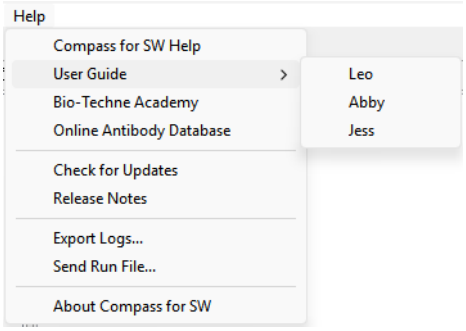
- If the computer you're using has an internet connection, the latest online version of the User Guide PDF will display.
- If the computer you're using does not have an internet connection, the User Guide PDF will only be available if you had previously downloaded the PDF, either through the Compass for SW help menu or from **bio-techne.com**, and saved it in the **Program Files** > **Compass for SW** folder on your computer.

The latest versions of User Guides are also available online at the Bio-Techne **Instrument Software Download Center**.

## Viewing Instrument User Guides

**If Compass is connected to an instrument:**
Select **Help** and click **User Guide** to view the guide for the system Compass is connected to.

**If Compass is not connected to an instrument:**
Select **Help** and click **User Guide** to display a menu of Simple Western Instrument User Guides.



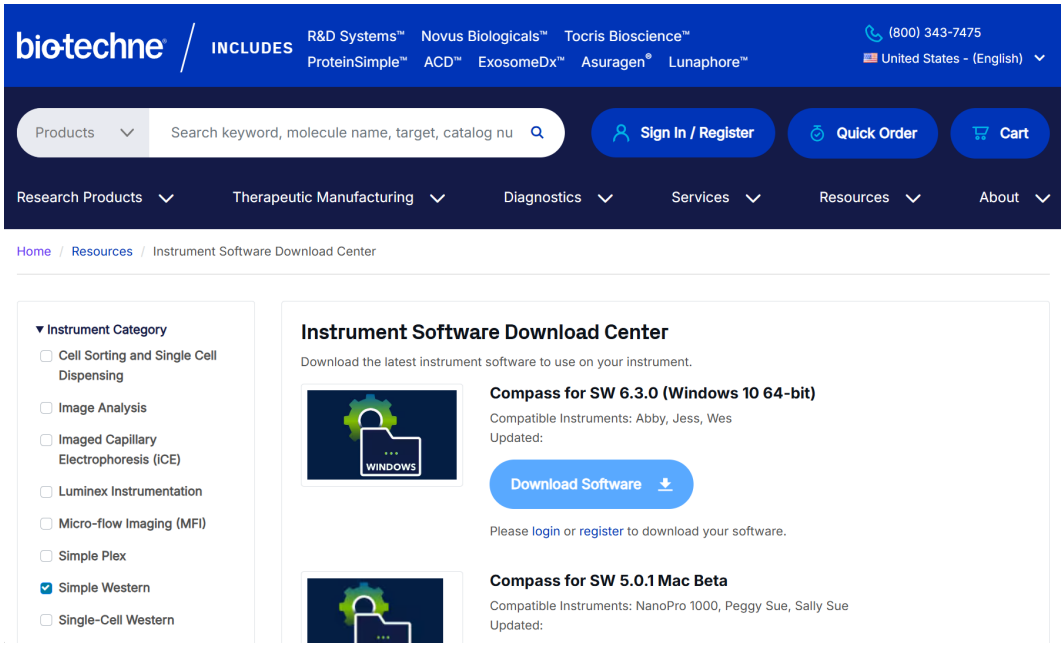
- If the computer you're using has an internet connection, the latest online version of the User Guide PDF will display.
- When an internet connection is not available, the User Guide PDF will only be available if you had previously downloaded the PDF, either through the Compass for SW help menu or from **bio-techne.com**, and saved it in the **Program Files** > **Compass for SW** folder on your computer.

The latest versions of User Guides are also available online at Bio-Techne **Instrument Software Download Center** or on the specific Bio-Techne website instrument page.

# 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 the software is available. To do this:

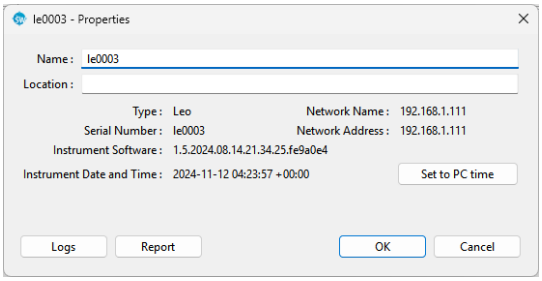
1. Make sure the computer being used has an active internet connection.
2. Select **Help** and click **Check for Updates**. The following web page will load:




3. Under Instrument Category, select **Simple Western**.
4. Under File Type, select **Software**.
5. Download the appropriate version and run the executable file to install.

# Instrument Software (Embedded) Updates

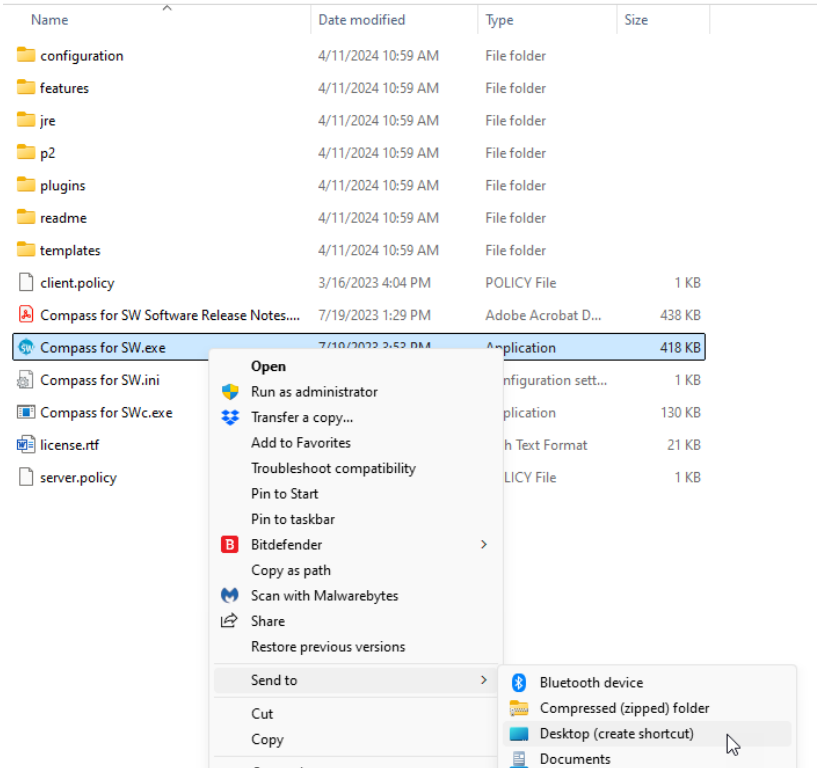
To view the version installed, select **Instrument** in the main menu, then click **Properties**.




To check for embedded updates, select **Instrument** in the main menu, then click **Update** and select **Network**. If the computer connected to the instrument does not have an internet connection, contact ProteinSimple Technical Support for assistance on how to obtain the latest update.

# Keeping Multiple Versions of the Software

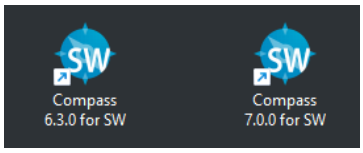
1. Rename the currently installed Compass for SW folder. For example, rename C:\Program Files\Compass for SW to C:\Program Files\Compass for SW_6.3.0.
2. Open the renamed folder, right-click on **Compass for SW.exe**, select **Send to**, and then **Desktop (create shortcut)**.



3. Rename the shortcut per the version number, for example: Compass 6.3.0 for SW.


**IMPORTANT:** Uninstall Compass for SW through the Windows Control Panel.


4. Install the latest (or newer) version of Compass for Simple Western.
5. The new desktop shortcut will point to the last version of Compass for Simple Western that was installed. Rename it to the version that was just installed, for example: Compass 7.0.0. for SW.




# 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 the Bio-Techne **Instrument Software Download Center**.

1. Under Instrument Category, select **Simple Western**.
2. Under File Type, select **Release Note.**

3. Download the notes for the appropriate software version.

Release Notes – Compass for SW software

Version 7.0.0

P/N 805-0003, Revision E

*Released March 2025*

### Recommended PC requirements for Leo/Jess/Abby

| | |
|---|---|
| Operating System | Windows 10 or Win 11 Pro/Enterprise 64 bit |
| Memory | 8 GB |
| Free Disk Space | 100GB |
| Ethernet Ports | 2 (1st port for Instrument, 2nd port for network access) |

### Installation

Open the installer file to start the installation.

**NOTE:** 'Check for Updates' feature in Compass software will not update from version 3.x to version 4.0.0 and higher. User will need to download installer from Bio-Techne website (https://www.bio-techne.com/resources/instrument-software-download-center) and perform a full installation.

**NOTE:** If you are using Peggy Sue, Sally Sue or NP1000, Compass for Simple Western version 6.0 and higher does not support these instruments. You can still use software versions 5.x and lower to create,

# Exporting the Software Log

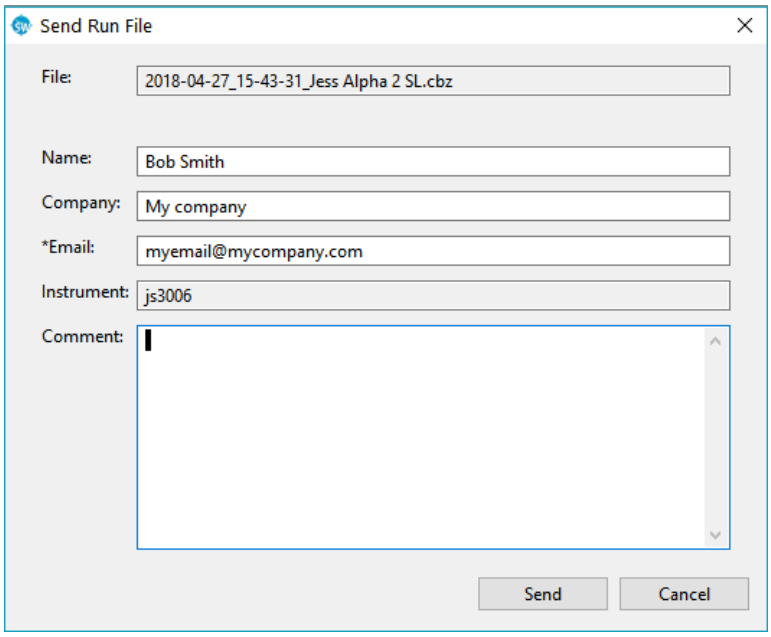
Select **Help** and click **Export Logs** to view the software log file. This will export a zip file containing both the Compass and embedded logs (self test, error, temperature, command logs, etc.) when Compass is connected to an instrument. This information is used for troubleshooting purposes.

# Sending Run Files to Technical Support

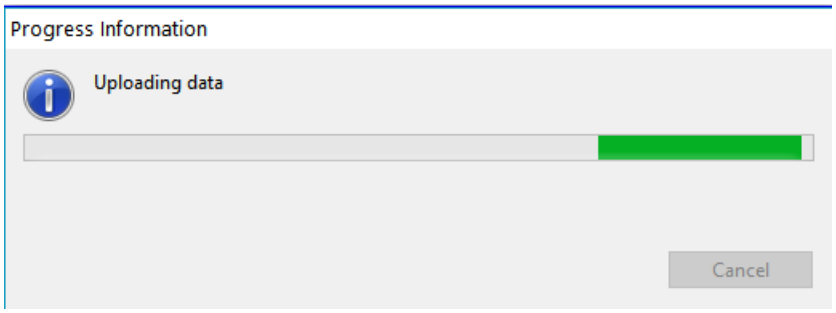
If the computer you are using has an internet connection, Compass for Simple Western can zip and send a run file directly to ProteinSimple Technical Support.

1. Open the run file you'd like to send in the Analysis screen.
2. Select Help and click **Send Run File…**.

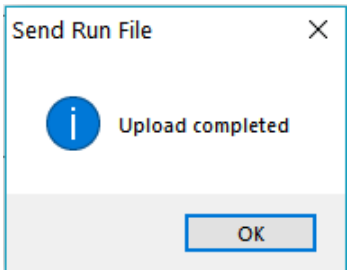
3. Enter your Name, Company, and E-mail address, and any details in the Comments section:




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




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



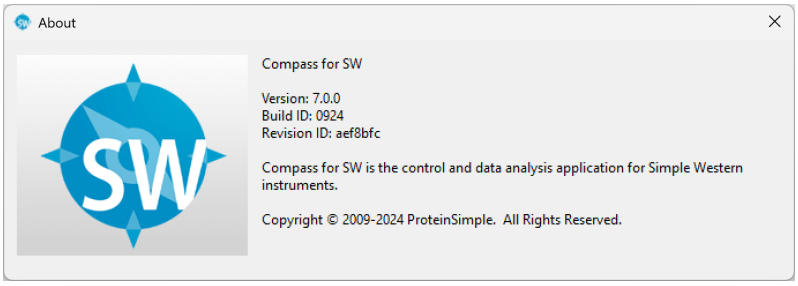
# Basic Troubleshooting

| Problem | Solution |
|---|---|
| The instrument won't connect to the computer | • Confirm the static IP address for the ethernet port is set to 172.30.1.2.<br>• Use a direct ethernet to ethernet connection. USB to ethernet adapters can be unreliable.<br>• Refer to the installation guide for more detailed instructions or contact Technical Support at **support@bio-techne.com** or **Instrument.Support.EMEA@bio-techne.com** (European customers). |
| I can't access my data | • By default, run files save to C:\Users\Username\Documents\Compass for SW\Runs.<br>• If you don't see your file in the default folder, connect to the instrument's embedded drive. From the Compass for Simple Western main menu, select **Instrument** > **Runs...**<br>**NOTE:** Run files are organized chronologically |

For additional instrument, software, and Simple Western assay troubleshooting support, contact ProteinSimple Technical Support by phone at (888) 607-9692 or by email at **support@bio-techne.com**. For customers in Europe, please contact Technical Support at +44 1235 529449, or **Instrument.Support.EMEA@bio-techne.com**. You can also visit the **Bio-Techne Academy** or visit **https://www.bio-techne.com/resources/literature**. You can also contact your local Field Application Scientist for help.

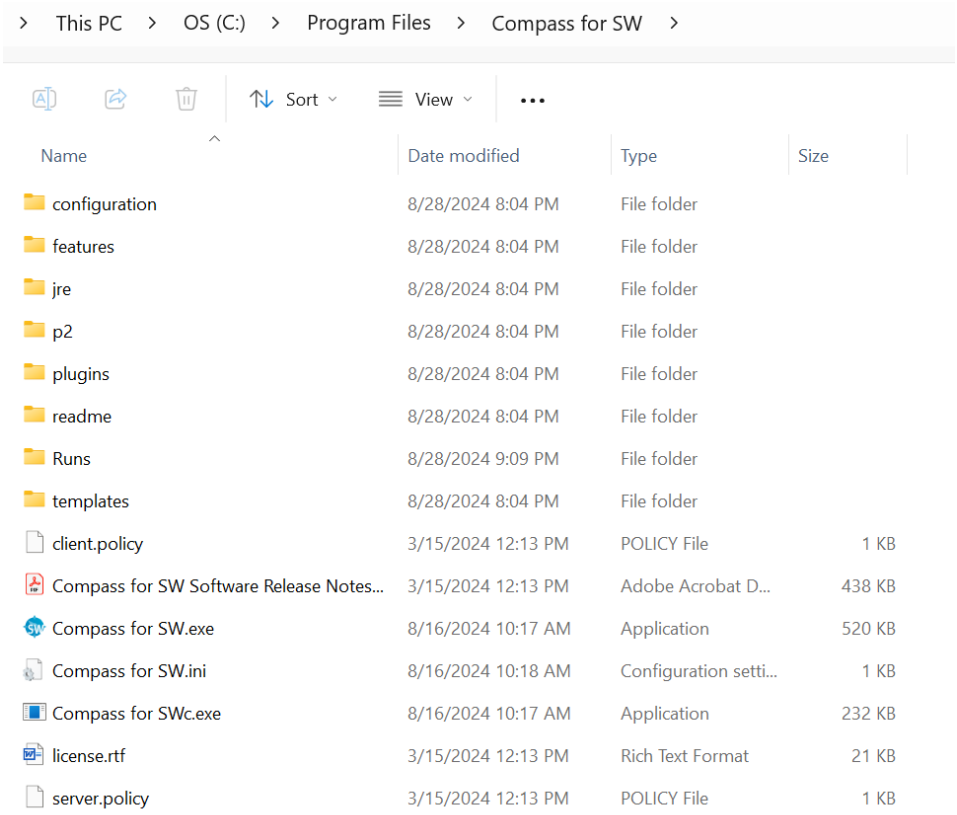
# Compass for Simple Western Version Information

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

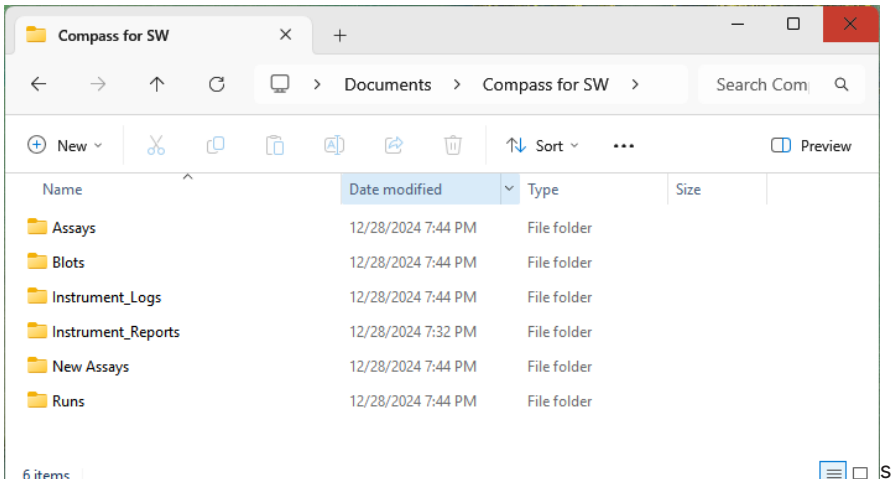


# Directory and File Information

The main Compass for Simple Western directory is located in the Program Files folder.



Compass for Simple Western assay and run files are located in the Documents/Compass for SW folder on your computer.




- **Assays Folder** – Contains all assay files that you've saved.
- **Blots Folder** – Contains all Western blot image files acquired using Jess that you've saved.
- **Instrument_Logs** - Contains saved instrument log files.
- **Instrument_Reports -** Contains saved instrument reports. The folder will only appear after you save your first report.
- **New Assays Folder** – Contains Simple Western default assay template files.
- **Runs Folder** – Contains all run files. Run data is automatically written to this folder.

**NOTE:** When a Compass for Simple Western software update is performed, the template assays in the New Assays folder are overwritten.

## 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 (Jess only).
- **Protocol Files** – Exported protocol files use a *.protocol file extension.
- **Template Files** – Exported template files use a *.csv or *.txt file extension.
- **Analysis Settings Files** – Exported analysis settings files use a *.settings file extension.

Chapter 2:

# Size Assays

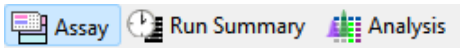
## Chapter Overview

- Assay Screen Overview
- Reagent Color Coding
- Opening an Assay
- Immunoassays on Jess and Abby: Creating a New Assay
- Stellar Assays on Jess: Creating a New Assay
- Protein Normalization on Jess: Creating a New Assay
- RePlex Assays on Jess and Abby: Creating a New Assay
- Immunoassays on Leo: Creating a New Assay
- RePlex Assays on Leo: 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

**NOTE:** If you are using a Wes, Peggy Sue, Sally Sue, or NanoPro 1000, Compass for Simple Western version 7.0 and higher does not support these instruments. You can still use software versions 5.x and lower for Peggy Sue, Sally Sue, or NanoPro 1000 and software versions 6.x and lower for Wes to create, load, and run assays on these systems, and to analyze data. Lower versions of Compass can be downloaded at the **Instrument Software Download Center** on the Bio-Techne website.

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

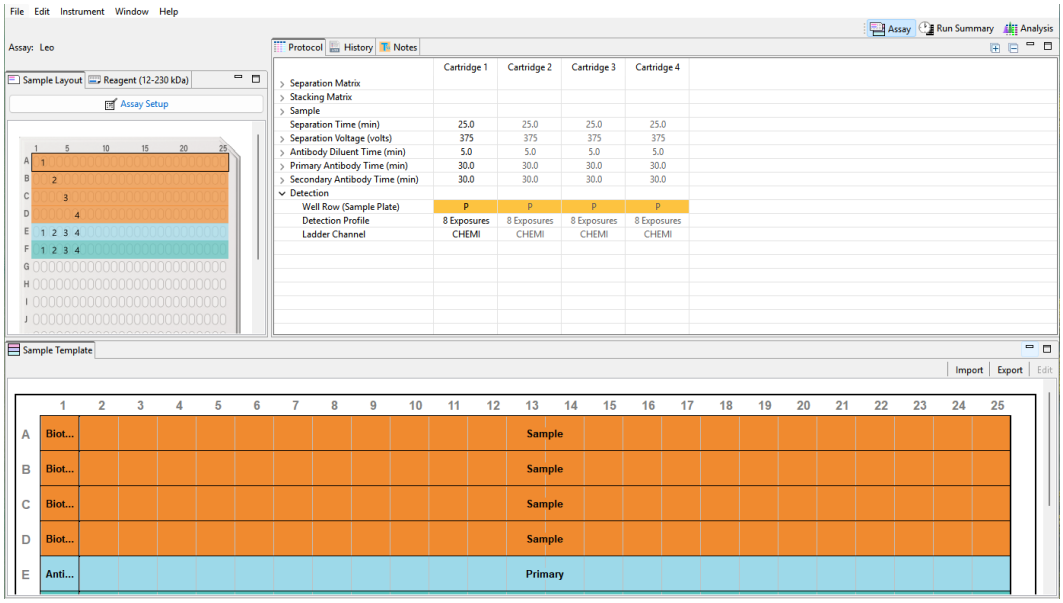



## Assay Screen Panes

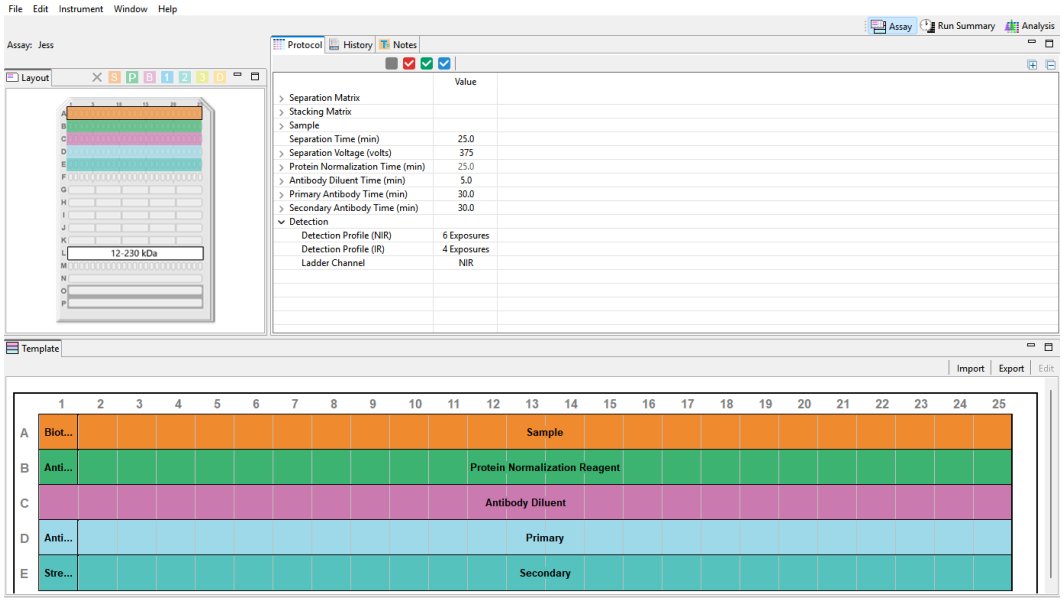
The Assay screen has multiple panes:

- **Layout (Jess and Abby)** – Displays a map of the assay plate and shows where assay reagents will be located.
- **Sample Layout (Leo)** – Displays a map of the Bio-Techne® Simple Western™ Leo™ Sample Plate where assay reagents like the samples and antibodies will be located.
- **Reagent (Leo)** – Displays the number of Bio-Techne® Simple Western™ Pre-filled Reagent Plates that will be used in the assay. The Pre-filled Reagent Plate contains assay reagents like size range-specific matrices and wash buffers.
- **Protocol** – Lists individual assay protocol steps and parameters that Leo, Jess, or Abby will execute.
- **History** – View changes made to the assay tab.
- **Notes** – Lets you enter specific assay information that is saved with the assay and can be used for future reference.
- **Template** – Enter names and attributes for the individual well and row reagents in the Assay Plate (Jess and Abby) or Sample Plate (Leo).

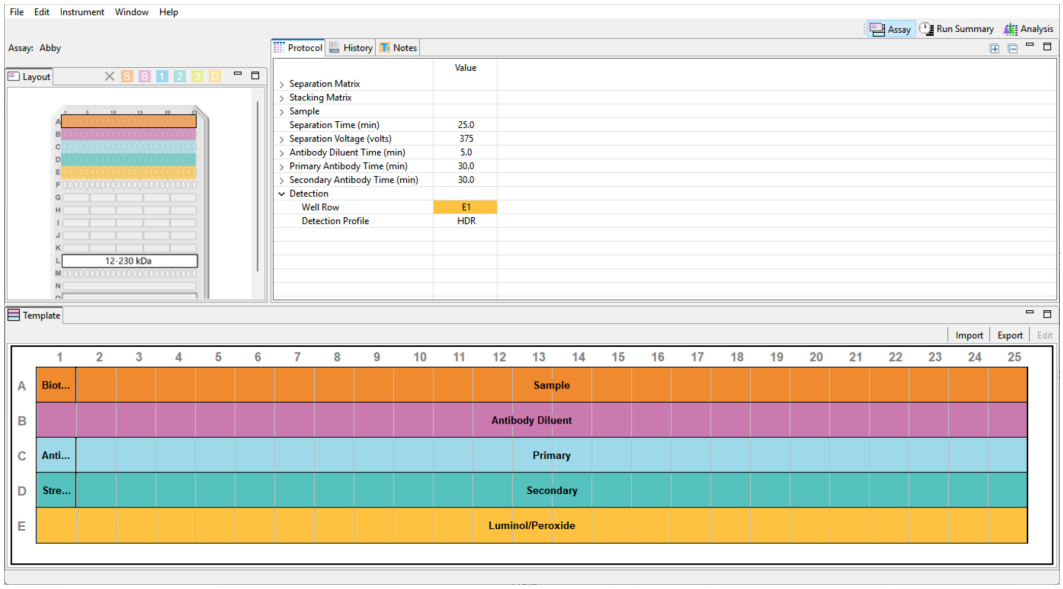
## LEO ASSAY SCREEN PANE




## JESS ASSAY SCREEN PANE



#### ABBY ASSAY SCREEN PANE




**NOTE:** Template files (*.csv, *.txt) may be imported, exported, and edited by clicking the tabs on the top right side of the Template pane or in the File Menu (see below).

## Software Menus in the Assay Screen

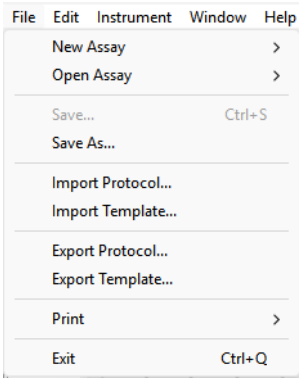
You can use the following software menus:

- File
- Edit
- Instrument (when Compass for Simple Western is connected to Leo, Jess, or Abby)
- Window
- Help

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

## File Menu

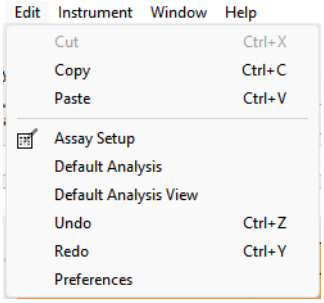
The following options can be found in the File menu:




- **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 options can be found in the Edit menu:



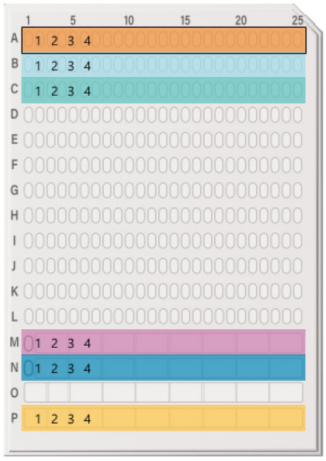

- **Copy** – Copies the information in the Protocol or Template panes into other documents.
- **Paste** – Pastes information from other documents into the Protocol or Template panes.
- **Assay Setup** (Only for Leo Assays) – Opens the Assay Setup wizard that is used to specify the number of cartridges and reagent rows that will be used in the Leo run, and assign Sample Plate reagent locations.
- **Default Analysis** – Displays the default settings that will be used to analyze the run data generated with an assay.
- **Default Analysis View** – Displays the default Graph, Lane, and Table view options that will be used to display run data generated with an assay.
- **Undo** – Reverses the last action performed when setting up your assay.
- **Redo** – Reverts previous undo actions.
- **Preferences** - Set and save your preferences for data export and plot colors in the graph. See Chapter 8: “Setting Your Preferences” for more information.

# Reagent Color Coding

## Immunoassays

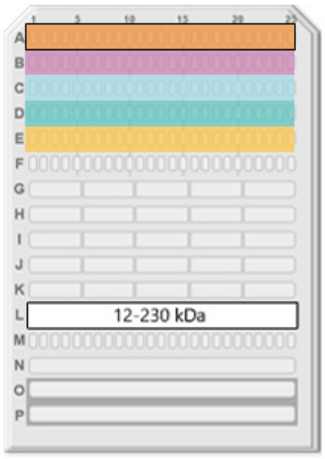
The Assay screen uses color coding to identify various assay reagents in all panes. A few examples are shown below.

### LEO CHEMILUMINESCENCE IMMUNOASSAY



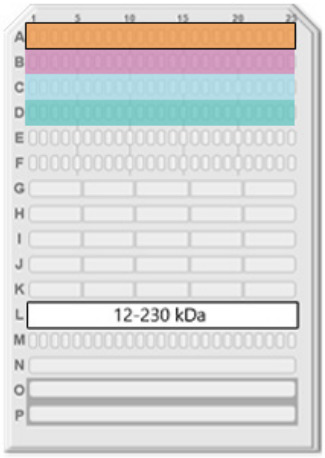
- **Orange** – Samples and ladder
- **Light Teal** – Primary antibody
- **Teal** – Secondary HRP conjugate
- **Magenta** – Antibody Diluent (2 or Milk-Free)
- **Blue** – Wash Buffer
- **Gold** – Luminol/Peroxide mix

### JESS/ABBY CHEMILUMINESCENCE IMMUNOASSAY




- **Orange** – Samples and ladder
- **Magenta** – Antibody Diluent (2 or Milk-Free)
- **Light Teal** – Primary antibody
- **Teal** – Secondary HRP conjugate
- **Gold** – Luminol/Peroxide mix

### JESS FLUORESCENCE IMMUNOASSAY

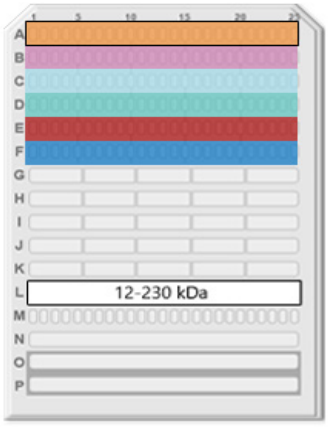



- **Orange** – Samples and ladder
- **Magenta** – Antibody Diluent (2 or Milk-Free)
- **Light Teal** – Primary antibody
- **Teal** – Secondary fluorescent conjugate

# Stellar Assays on Jess

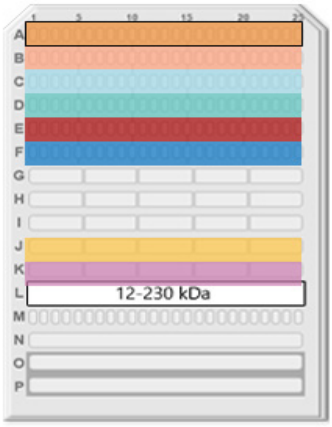
The Assay screen uses color coding to identify various assay reagents in all panes.

### STELLAR FLUORESCENCE NIR/IR IMMUNOASSAY




- **Orange** – Samples and ladder
- **Magenta** – Antibody Diluent (2 or Milk-Free)
- **Light Teal** – Primary antibody
- **Teal** – Stellar secondary antibody
- **Red** – Stellar IR or NIR Anneal
- **Dark Blue** – Stellar IR or NIR Label

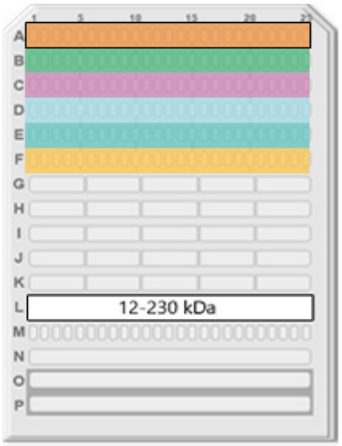
### STELLAR FLUORESCENCE NIR/IR IMMUNOASSAY WITH CHEMILUMINESCENCE TOTAL PROTEIN NORMALIZATION




- **Orange** – Samples and ladder
- **Peach** – Total Protein Biotin Labeling Reagent
- **Light Teal** – Primary antibody
- **Teal** – Stellar secondary antibody + Stellar Total Protein Streptavidin-HRP
- **Red** – Stellar IR or NIR Anneal
- **Dark Blue** – Stellar IR or NIR Label
- **Gold** – Luminol/Peroxide mix
- **Magenta** – Antibody Diluent (2 or Milk-Free)

## Protein Normalization (Fluorescence) 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
- **Teal** – Secondary antibody
- **Gold** – Luminol/Peroxide mix (chemiluminescence only)

## RePlex™ Assays on Leo, Jess, and Abby

Leo, Jess, and Abby let 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 Leo, Jess, and Abby:

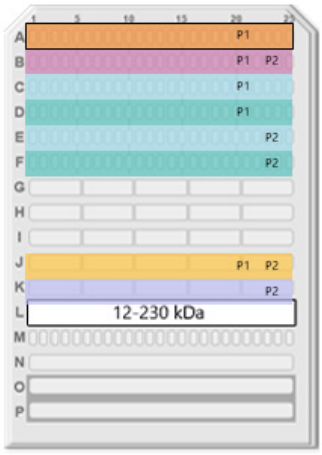
- **Chemiluminescence Immunoassay + Chemiluminescence Immunoassay using RePlex:** Performs a chemiluminescence immunoassay followed by another chemiluminescence immunoassay.
- **Chemiluminescence Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection:** Performs a chemiluminescence immunoassay followed by total protein detection.
- **Chemiluminescence Immunoassay/Fluorescence NIR Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection (Jess only):** Performs a chemiluminescence or fluorescence NIR immunoassay followed by total protein detection.
- **Fluorescence NIR Immunoassay + Fluorescence NIR Immunoassay using RePlex (Jess only):** Performs a fluorescence NIR immunoassay followed by another fluorescence NIR immunoassay.
- **Fluorescence NIR Immunoassay + Chemiluminescence Immunoassay using RePlex (Jess only):** Performs a fluorescence NIR immunoassay followed by a chemiluminescence immunoassay.

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

**IMPORTANT:** The Leo Sample Plate layout is slightly different from Jess and Abby, but the reagent color coding is the same for all three systems.

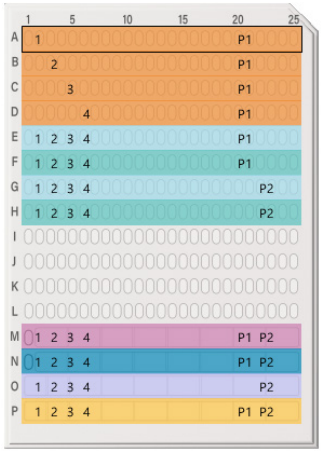
### Chemiluminescence Immunoassay + Chemiluminescence Immunoassay using RePlex

**JESS/ABBY**




- **Orange** – Samples and ladder
- **Magenta** – Antibody Diluent (2 or Milk-Free)
- **Light Teal** – Primary antibody for Probe 1
- **Teal** – Secondary HRP conjugate for Probe 1
- **Light Teal** – Primary antibody for Probe 2
- **Teal** – Secondary HRP conjugate for Probe 2
- **Gold** – Luminol/Peroxide mix
- **Purple** – RePlex reagent mix

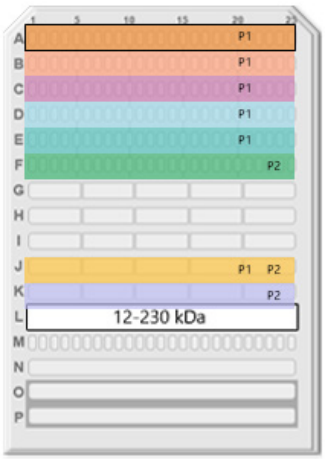
**LEO**



- **Orange** – Samples and ladder
- **Light Teal** – Primary antibody for Probe 1
- **Teal** – Secondary HRP conjugate for Probe 1
- **Light Teal** – Primary antibody for Probe 2
- **Teal** – Secondary HRP conjugate for Probe 2
- **Magenta** – Antibody Diluent (2 or Milk-Free)
- **Dark Blue** – Wash Buffer
- **Purple** – RePlex reagent mix
- **Gold** – Luminol/Peroxide mix

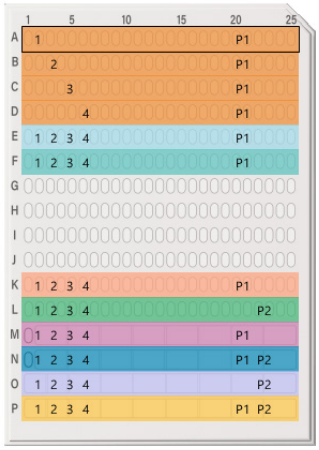
### Chemiluminescence Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection

#### JESS/ABBY



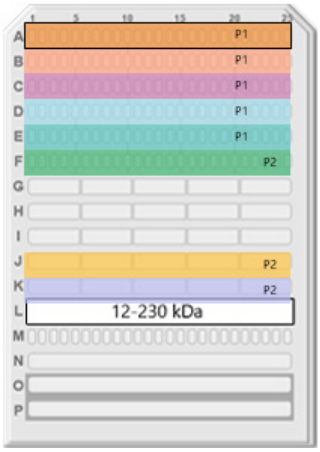

- **Orange** – Samples and ladder
- **Peach** – Total Protein Biotin Labeling Reagent
- **Magenta** – Antibody Diluent (2 or Milk-Free)
- **Light Teal** – Primary antibody for Probe 1
- **Teal** – Secondary HRP conjugate for Probe 1
- **Green** – Total Protein Streptavidin-HRP for Probe 2
- **Gold** – Luminol/Peroxide mix
- **Purple** – RePlex reagent mix

#### LEO



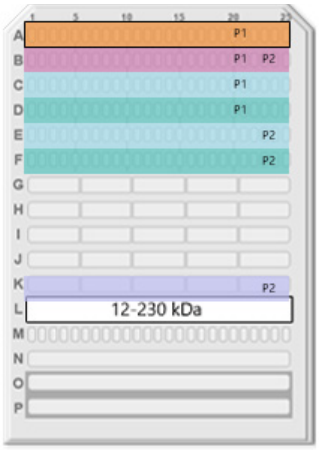
- **Orange** – Samples and ladder
- **Light Teal** – Primary antibody for Probe 1
- **Teal** – Secondary HRP conjugate for Probe 1
- **Peach** – Total Protein Biotin Labeling Reagent
- **Green** – Total Protein Streptavidin-HRP for Probe 2
- **Magenta** – Antibody Diluent (2 or Milk-Free)
- **Dark Blue** – Wash Buffer
- **Purple** – RePlex reagent mix
- **Gold** – Luminol/Peroxide mix

**Chemiluminescence or Fluorescence NIR Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection (Jess only)**




- **Orange** – Samples and ladder
- **Peach** – Total Protein Biotin Labeling Reagent
- **Magenta** – Antibody Diluent (2 or Milk-Free)
- **Light Teal** – Primary antibody for Probe 1
- **Teal** – Secondary antibody for Probe 1
- **Green** – Total Protein Streptavidin-HRP for Probe 2
- **Gold** – Luminol/Peroxide mix
- **Purple** – RePlex reagent mix

**Fluorescence NIR Immunoassay + Fluorescence NIR Immunoassay using RePlex (Jess only)**



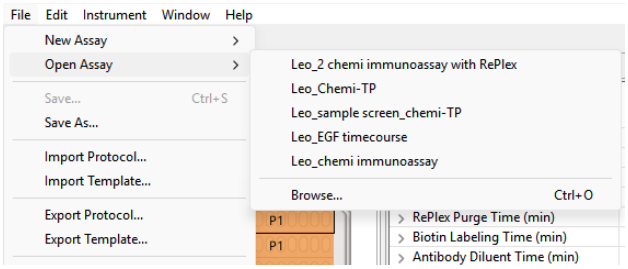

- **Orange** – Samples and ladder
- **Magenta** – Antibody Diluent (2 or Milk-Free)
- **Light Teal** – Primary antibody for Probe 1
- **Teal** – Secondary antibody for Probe 1
- **Light Teal** – Primary antibody for Probe 2
- **Teal** – Secondary antibody for Probe 2
- **Purple** – RePlex reagent mix

# Opening an Assay

**NOTE:** If you are using a Wes, Peggy Sue, Sally Sue, or NanoPro 1000, Compass for Simple Western version 7.0 and higher does not support these instruments. You can still use software versions 5.x and lower for Peggy Sue, Sally Sue, or NanoPro 1000 and software versions 6.x and lower for Wes to create, load, and run assays on these systems, and to analyze data. Lower versions of Compass can be downloaded at the **Instrument Software Download Center** on the Bio-Techne website.

To open an existing assay:

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




3. 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.
4. To make changes to assay parameters, follow the instructions for the assay being performed:
   - “Immunoassays on Jess and Abby: Creating a New Assay” on page 41.
   - “Stellar Assays on Jess: Creating a New Assay” on page 59.
   - “Protein Normalization on Jess: Creating a New Assay” on page 65.
   - “RePlex Assays on Jess and Abby: Creating a New Assay” on page 67.
   - “Immunoassays on Leo: Creating a New Assay” on page 78.
   - “RePlex Assays on Leo: Creating a New Assay” on page 87.
5. Select **File** from the main menu and click **Save**.

# Immunoassays on Jess and Abby: Creating a New Assay

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

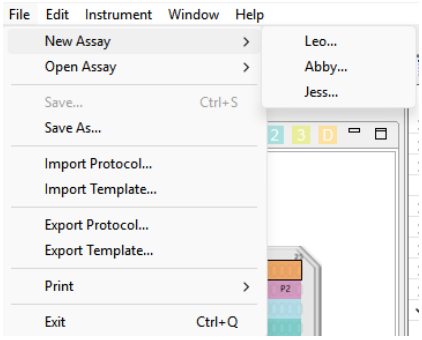
**NOTES:**
This section provides details on how to create an Immunoassay on Jess or Abby. To create an Immunoassay on Leo, see “Immunoassays on Leo: Creating a New Assay” on page 78.

To create a RePlex Assay on Jess and Abby that includes an immunoassay, see “RePlex Assays on Jess and Abby: Creating a New Assay” on page 67.

To create a Stellar Assay on Jess, see “Stellar Assays on Jess: Creating a New Assay” on page 59.

## Step 1 - Open a Template Assay

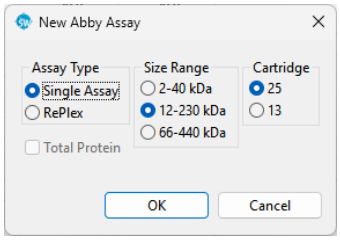
1. Select the **Assay** screen.
2. If your instrument is not connected to Compass for Simple Western, select **File** in the main menu, click **New Assay,** and select your instrument:

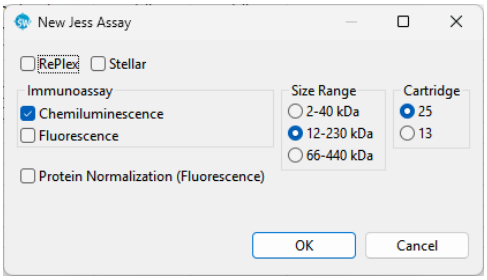



   If your instrument is connected to Compass for Simple Western select **File** in the main menu and click **New Assay**.

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

3. Select the template assay for your instrument by selecting the instrument-appropriate **Assay Type** or **Immunoassay, Size Range**, and **Cartridge Type**.



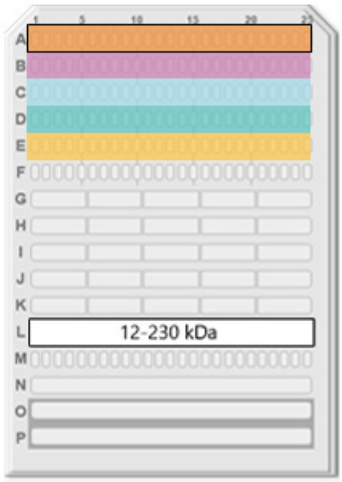

4. Click **OK**.

**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.

**PLATE LAYOUT:**

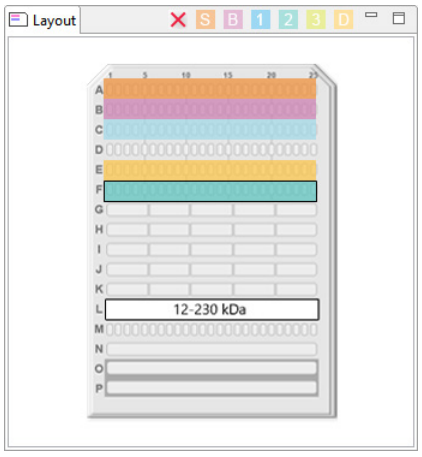



- **Row A** – Biotinylated Ladder (A1) and Sample (A2–25)
- **Row B** – Antibody Diluent
- **Row C** – Antibody Diluent (C1) and Primary antibody (C2–C25)
- **Row D** – Streptavidin-HRP or NIR (D1) and Secondary conjugate (D2–D25)
- **Row E** – Luminol-S/Peroxide mix (chemiluminescence only)
- **Row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

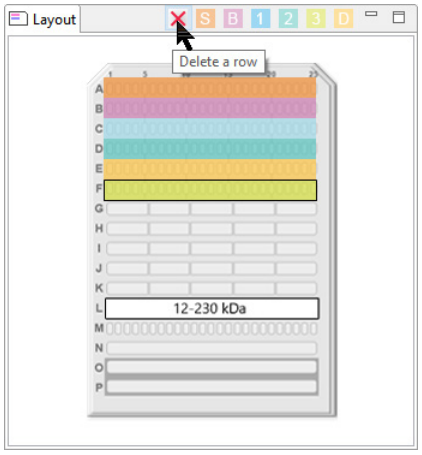
**NOTE:** For details on sample, reagent, and assay plate preparation, please refer to the product insert provided with Abby and Jess Simple Western kits.

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** – Select 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 delete a reagent row** – Select the row to be deleted, then click the red X icon (Delete a row) in the toolbar. Rows for required assay reagents cannot be deleted.



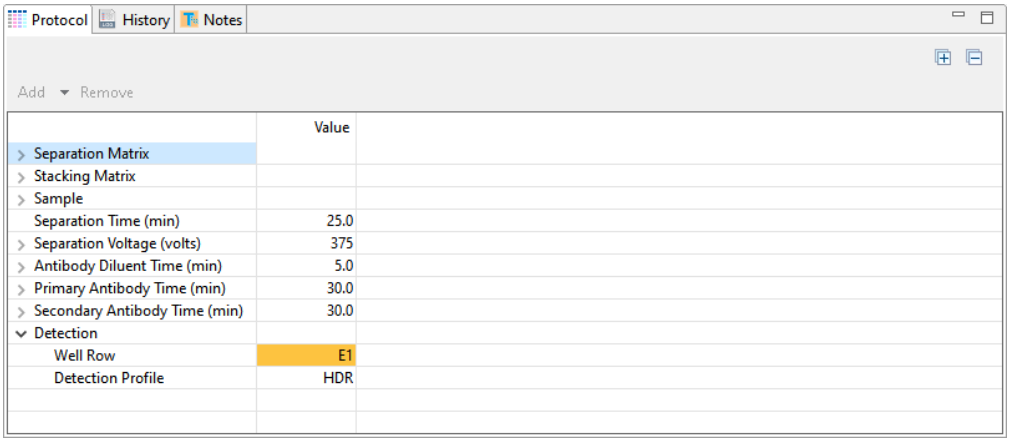

- **To insert a reagent row** – Select an empty row where the new row should be inserted, then click on the icon in the Layout pane toolbar for your reagent. A new reagent row will be added in the empty row.

S Insert a Sample row
P Insert a Protein Normalization Reagent row (Jess only)
1 Insert a Primary row
2 Insert a Secondary row
3 Insert a Tertiary row (optional)
D Insert a Detection row

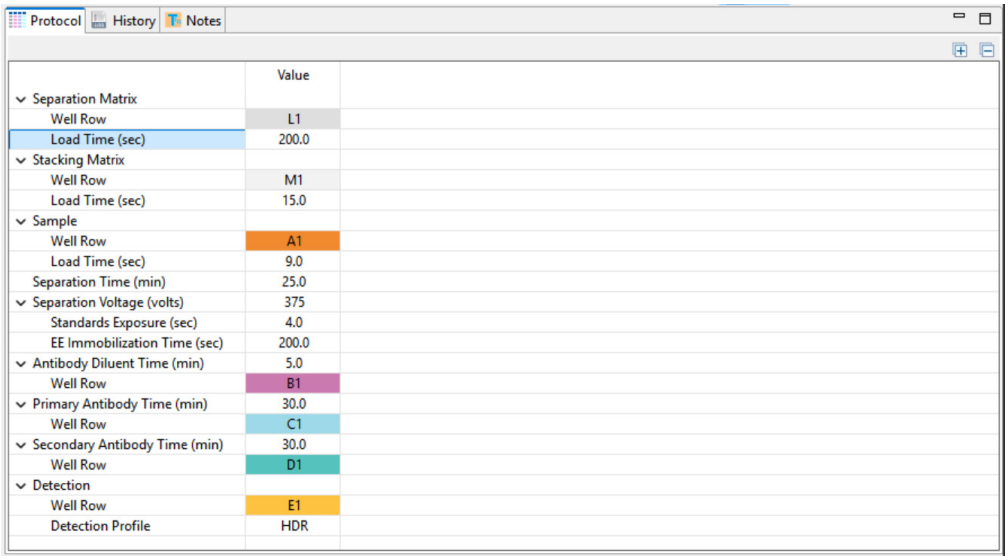
**NOTE:** Row F is the last row assignment that can be used on the assay plate. New sample, incubation, or detection rows cannot be inserted if all rows have an assigned reagent. To insert a row in this case, you must first delete the contents of a row.

## 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. An Abby assay is shown as an example:

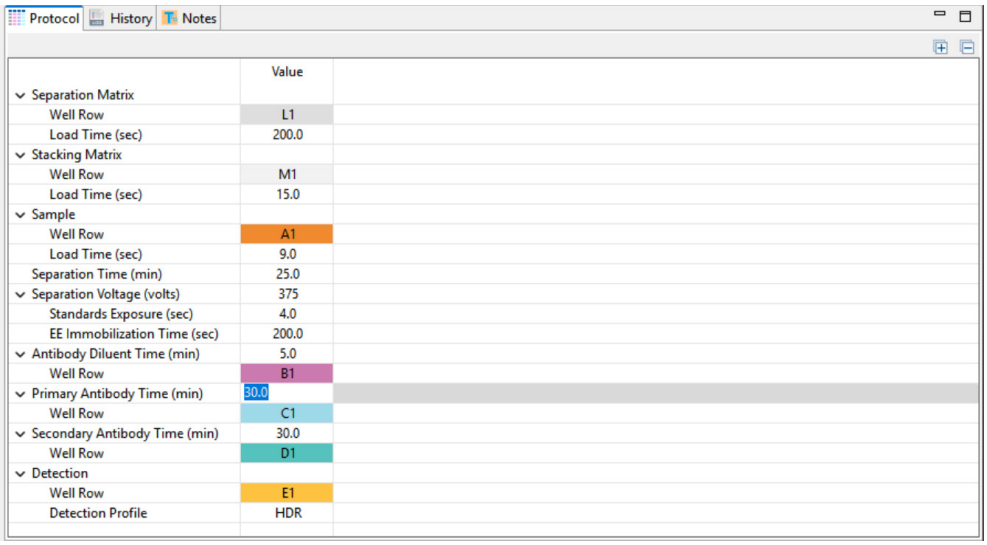



Each row contains an assay protocol step. Each step can contain a reagent assay plate row assignment and one or more parameter settings. To view the details for a step, click on the gray arrow next to the step name. We recommend using the default protocol settings for the assays. An expanded list of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

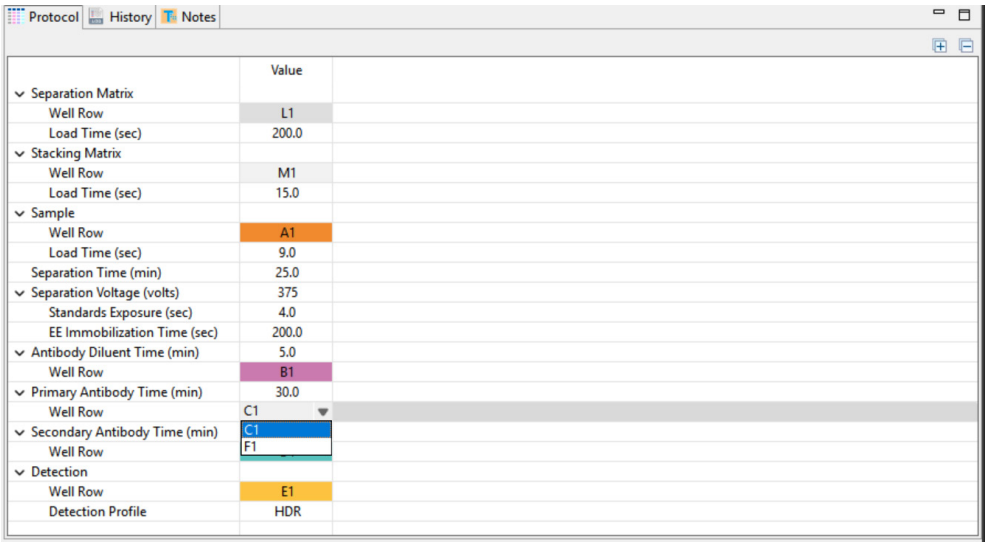



**NOTE:** To expand all parameters, click the ⊞ button at the upper right of the pane. To collapse all parameters, click the ⊟ button at the upper right of the pane.

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.




3. You can change the primary and secondary antibody incubation reagent row location if there is more than one row for each reagent type. Click the cell in the value column next to Well Row and select a different row on the assay plate:



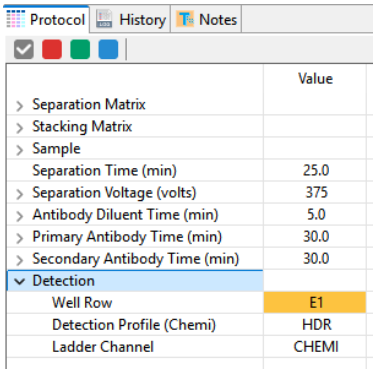

**NOTE:** Only rows you've designated as a primary antibody or secondary antibody in the Layout pane can be selected in the Well Row drop-down menu for Primary Antibody or Secondary Antibody, respectively.

For chemiluminescence assays, the default detection mode is High Dynamic Range (HDR) for chemiluminescence assays (Compass for Simple Western 6.1 and higher). See "High Dynamic Range Detection Profile (Jess and Abby)

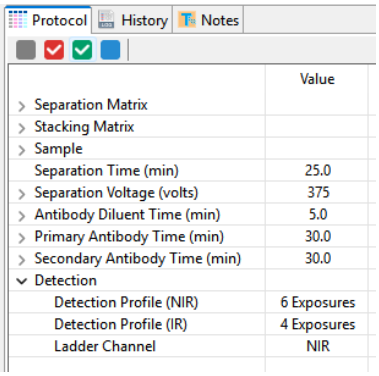
and Default Detection Profile (Leo)" on page 100 and "RePlex Dynamic Range Detection Profile (Jess and Abby) and Default Detection Profile for RePlex assays (Leo)" on page 102 for more information.

For fluorescence assays, the default modes are 6 exposures for NIR and 4 exposures for IR. If you do not want to run a detection channel in your assay, remove all the exposures in the Detection Profile. See "NIR Fluorescent Detection (Jess only)" on page 49 for more information.

**CHEMILUMINESCENCE ASSAYS**




**FLUORESCENCE ASSAYS**

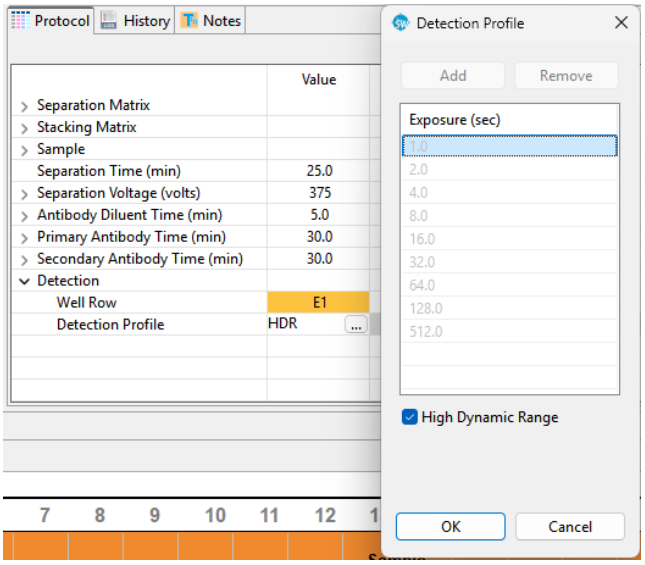



The number of exposures collected in the assay can be adjusted if desired.

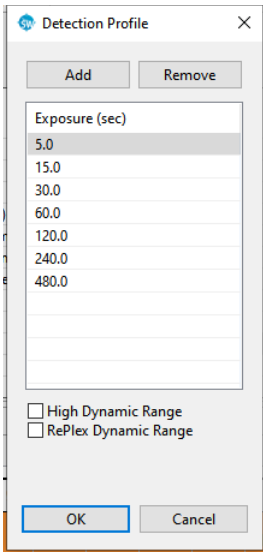
### Chemiluminescent

Click the gray arrow next to Detection to expand the row. Click the HDR cell in the column next to Detection Profile (Chemi) and click the ... button that appears to open the Detection Profile window.

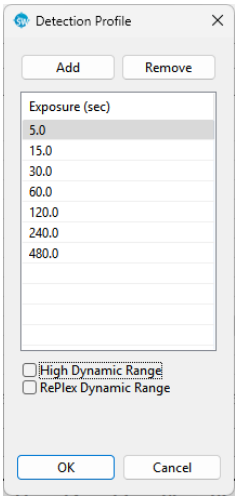
The examples below are from a chemiluminescence assay on Abby.

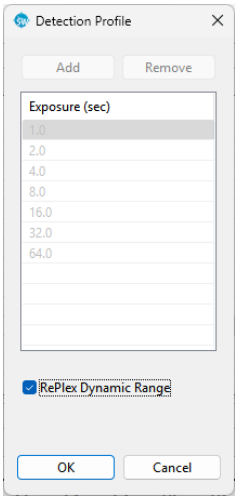


Deselect the High Dynamic Range checkbox. To remove exposure times, click the **Remove** button. To add additional exposure times to the protocol, click the **Add** button and enter the values. Select **OK** when you are done.




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

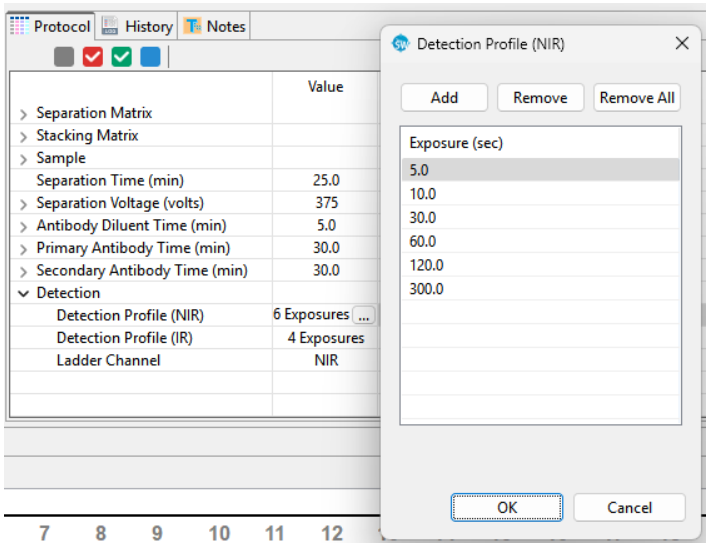


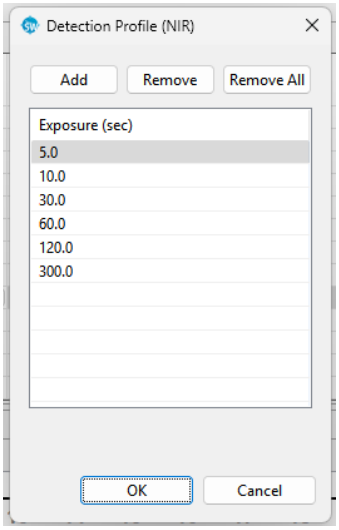

**NOTE:** A Remove All button will appear in the Detection Profile for Jess chemiluminescence assays. You can only use the Remove All button if the ladder was not run in the channel you are modifying. See “Ladder Channel (Jess only)” on page 51 for more information.

### NIR Fluorescent Detection (Jess only)

Click the gray arrow next to Detection to expand the row. Click the exposure number cell in the column next to Detection Profile (NIR) and click the **...** button that appears to open the Detection Profile window.



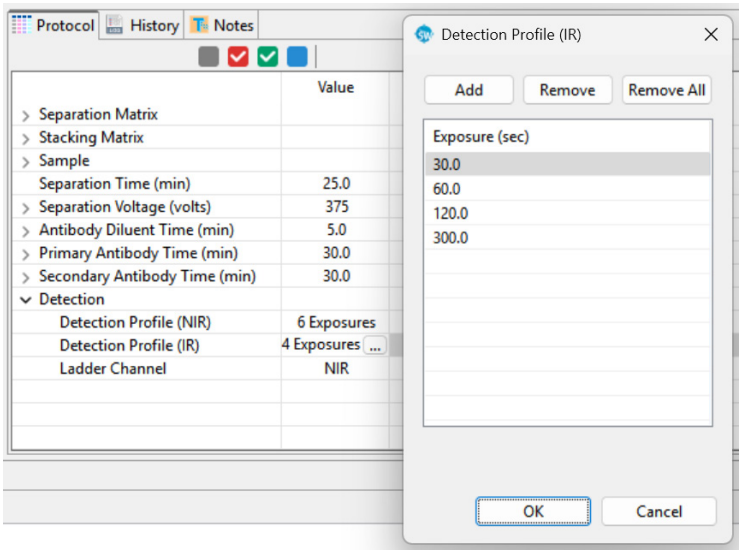

To remove exposure times, click the **Remove** or **Remove All** button. To add additional exposure times to the protocol, click the **Add** button and enter the values. Select **OK** when you are done.



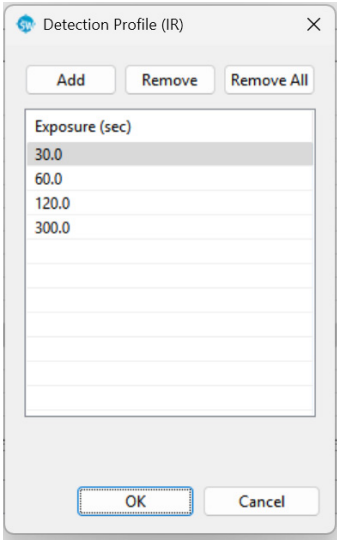

**NOTE:** You can only use the Remove All button to remove exposures if your ladder was not run in the channel you are modifying. See “Ladder Channel (Jess only)” on page 51 for more information.

### IR Fluorescent (Jess only)

Click the gray arrow next to Detection to expand the row. Click the exposure number cell in the column next to Detection Profile (IR) and click the **...** button that appears to open the Detection Profile window.



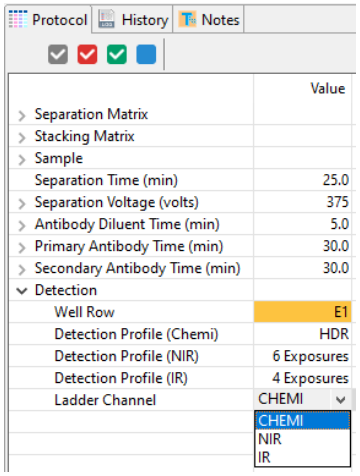

To remove exposure times, click the **Remove** or **Remove All** button. To add additional exposure times to the protocol, click the **Add** button and enter the values. Select **OK** when you are done.




**NOTE:** You can only use the Remove All button to remove exposures if your ladder was not run in the channel you are modifying.

### Ladder Channel (Jess only)

The ladder channel defaults to NIR in assays including fluorescence detection. If you'd like to change the channel for the ladder, select the cell next to Ladder Channel and select the desired channel in the drop-down menu.

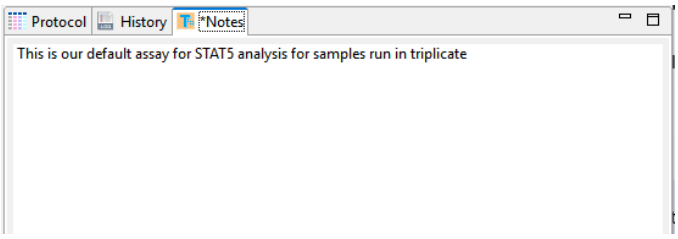



5. You can modify any other protocol parameters as needed.

**NOTE:** For more information on changing protocol step, contact ProteinSimple Technical Support at **support@bio-techne.com** (US/Canada) or **Instrument.Support.EMEA@bio-techne.com** (Europe). You can also contact your local Field Application Specialist.

## Step 4 - Add Assay Notes (Optional)

1. Click on the **Notes** tab.
2. Type any assay or protocol notes in the pane. 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.

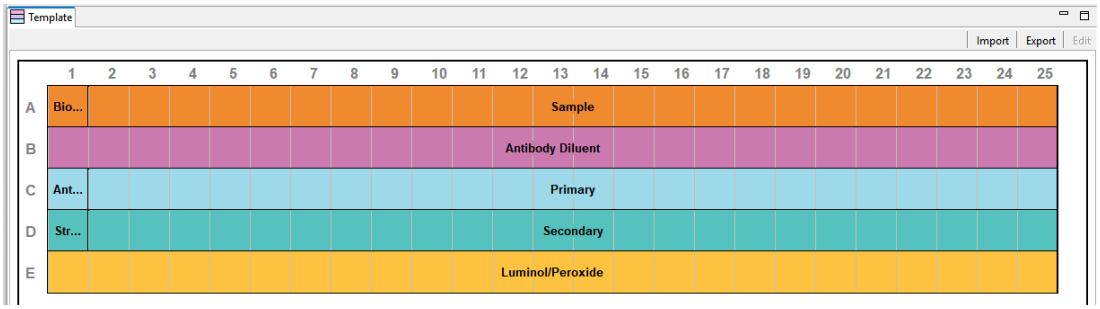
## Step 5 - Add Assay Plate Annotations (Optional)

Custom reagent names, notes and attributes 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 and secondary antibody names, or sample, primary antibody, and secondary antibody attributes. This will be explained in more detail in “Analysis Settings Overview” on page 277.

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

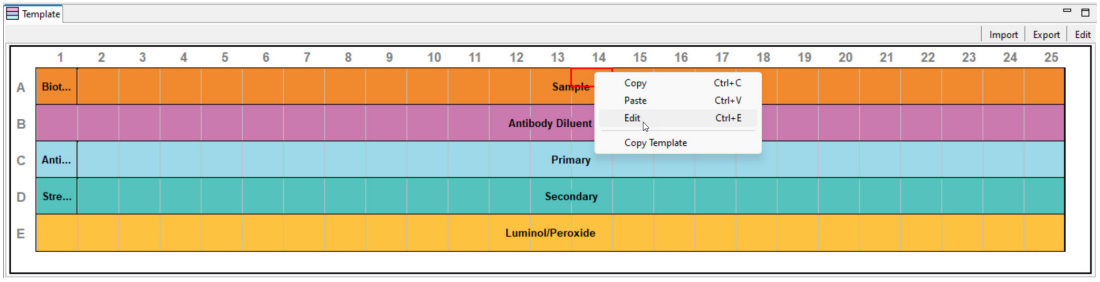
To enter annotations:

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

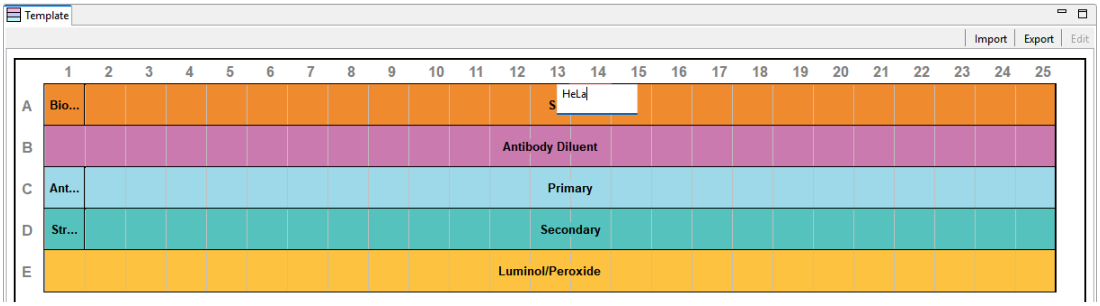



2. Change or add rows and well names and attributes as needed. To do this:

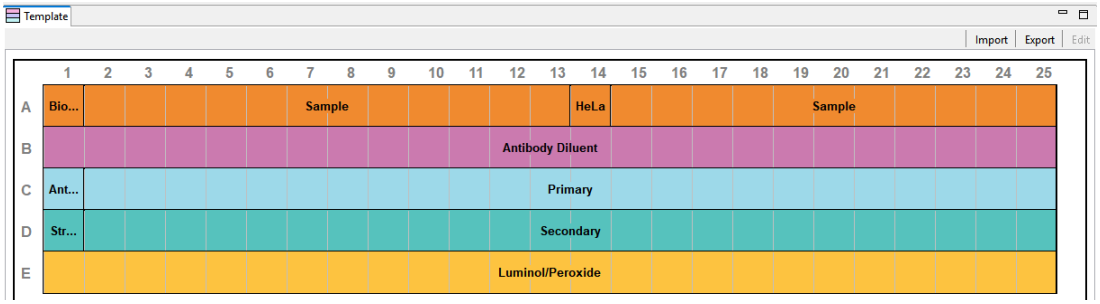
   - **To change the reagent name for a specific well** – Select the top of the well. You can then either right-click and select **Edit**, click **Edit** in the upper right corner of the pane, press **Ctrl+E**, or double-click at the top of the selected well.



Enter the reagent name in the box that appears and click **Enter**.

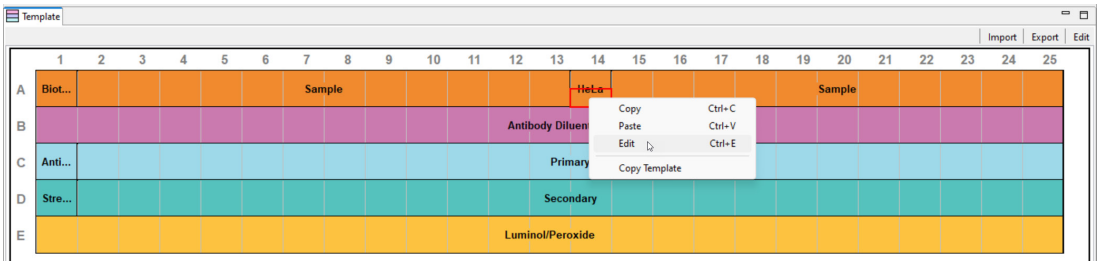



The new information will display in the selected well:

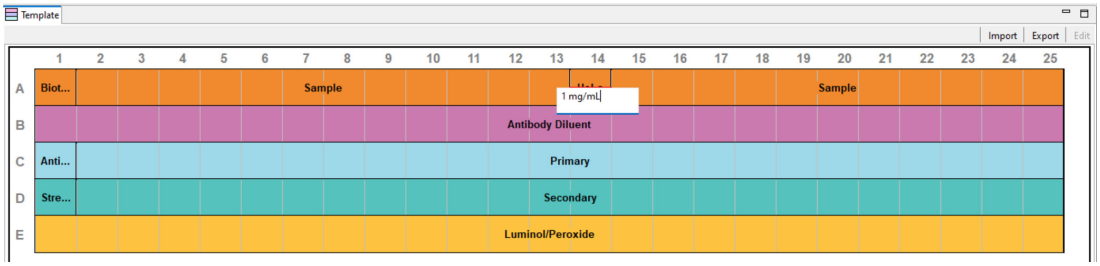



To undo changes, press **Ctrl+Z** or select **Undo** from the Edit menu.

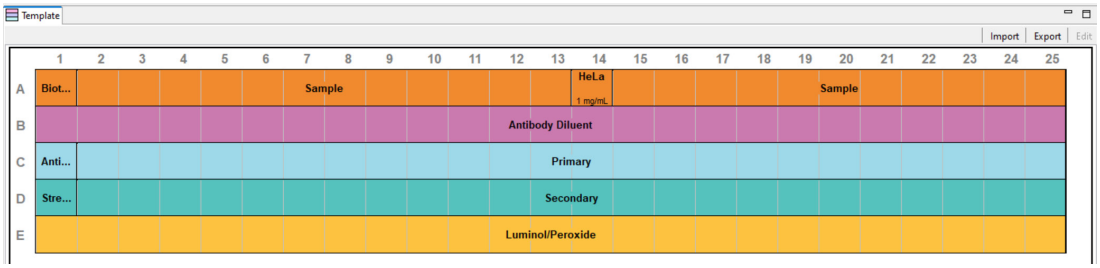
- **To add an attribute to a specific well** – Select the bottom of the well. You can then either right-click and select **Edit**, click **Edit** in the upper right corner of the pane, press **Ctrl+E**, or double-click at the bottom of the selected well.




Type the attribute (for example, sample concentration or dilution ratio) in the box that appears and click **Enter**.

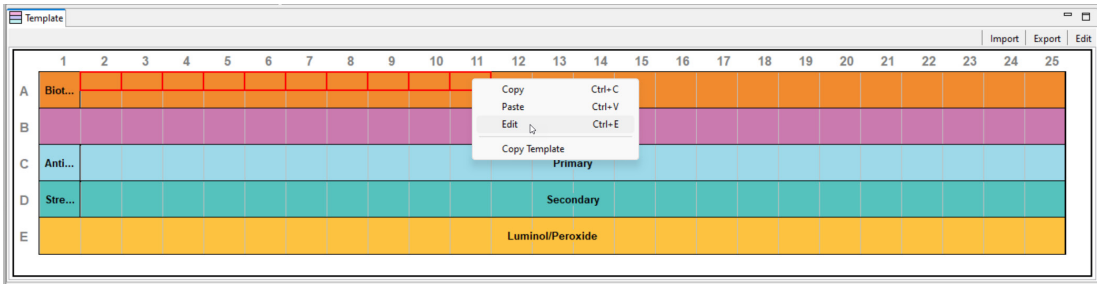


The new information will display in the selected well.

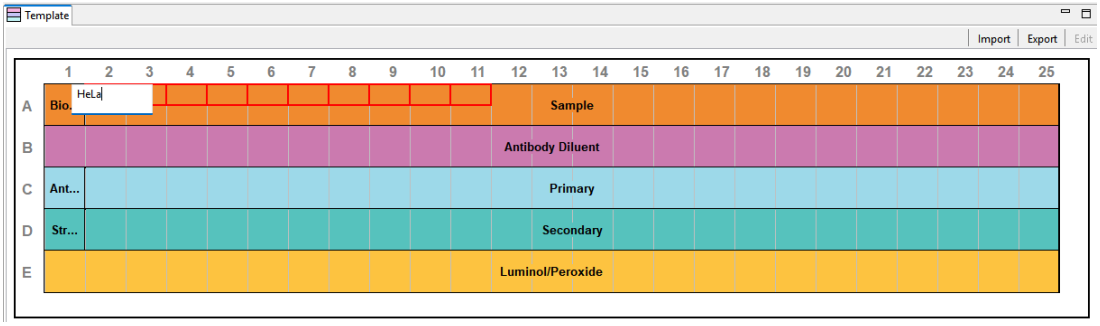



To undo changes, press **Ctrl+Z** or select **Undo** from the Edit menu.

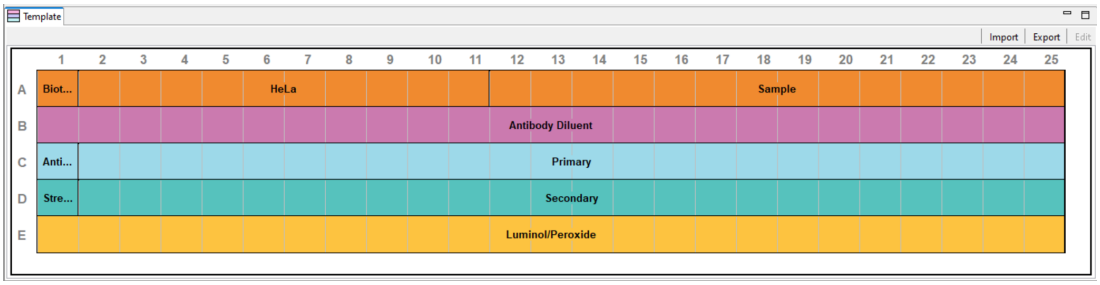
- **To change the reagent name for multiple wells or a row** – To select individual wells, click on the top of individual wells while holding down the **Ctrl** key. To select a sequential set of wells or a full row, select the top of the first well and the last well while holding down the **Shift** key. Next, right-click and select **Edit**, click **Edit** in the upper right corner of the pane, press **Ctrl+E**, or double-click while continuing to hold down the **Ctrl** or **Shift** key.




Type the new reagent name in the box that appears and click **Enter**.

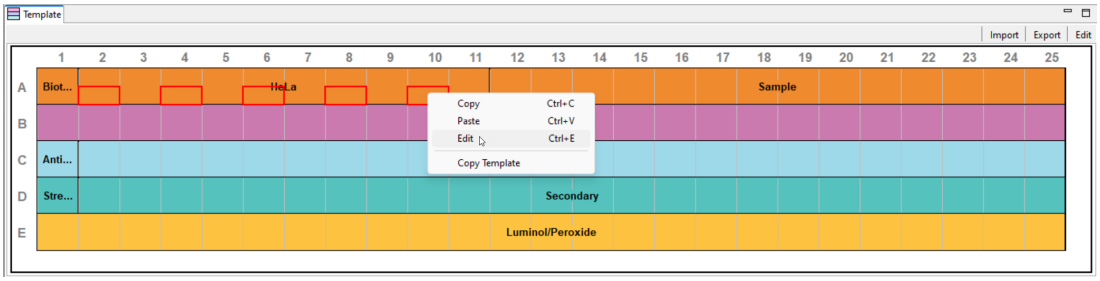


The new information will be displayed in the selected wells.

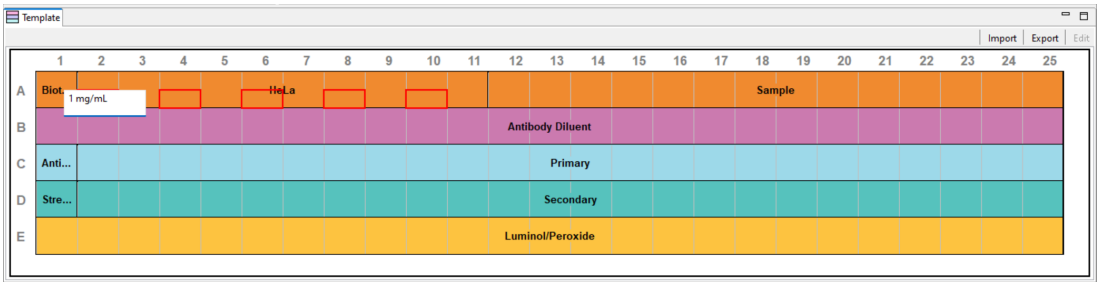



To undo changes, press **Ctrl+Z** or select **Undo** from the Edit menu.

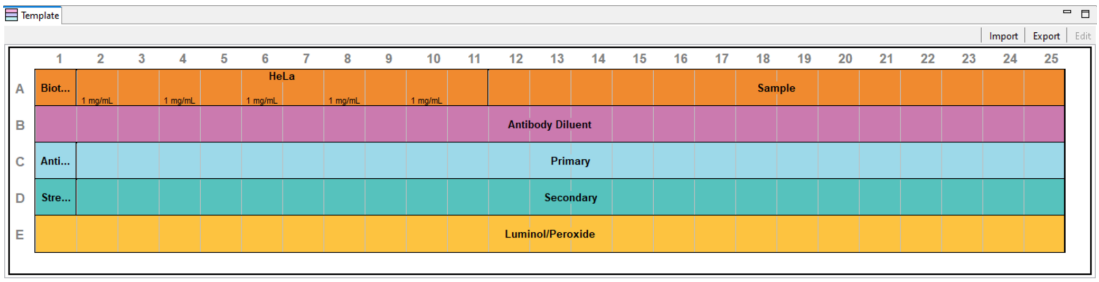
- **To add an attribute to multiple wells** – To select individual wells, click on the bottom of individual wells while holding down the **Ctrl** key. To select a sequential set of wells or a full row, select the bottom of the first well and the last well while holding down **Shift** key. Next, right-click and select **Edit**, click **Edit** in the upper right corner of the pane, press **Ctrl+E**, or double-click while continuing to hold down the **Ctrl** or **Shift** key.




Type the attribute (for example, sample concentration or dilution ratio) in the box that appears and click **Enter**.



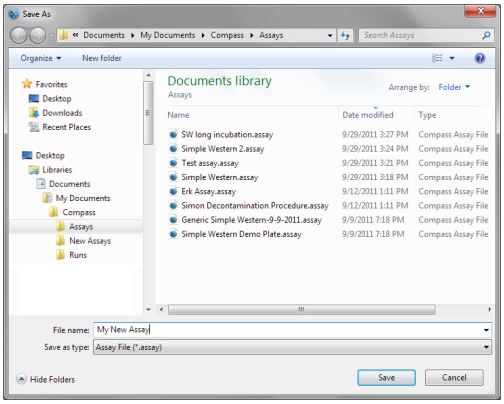
The attribute information will appear in the selected wells.




To undo changes, press **Ctrl+Z** or select **Undo** from the Edit menu.

## Step 6 - Save the Assay

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



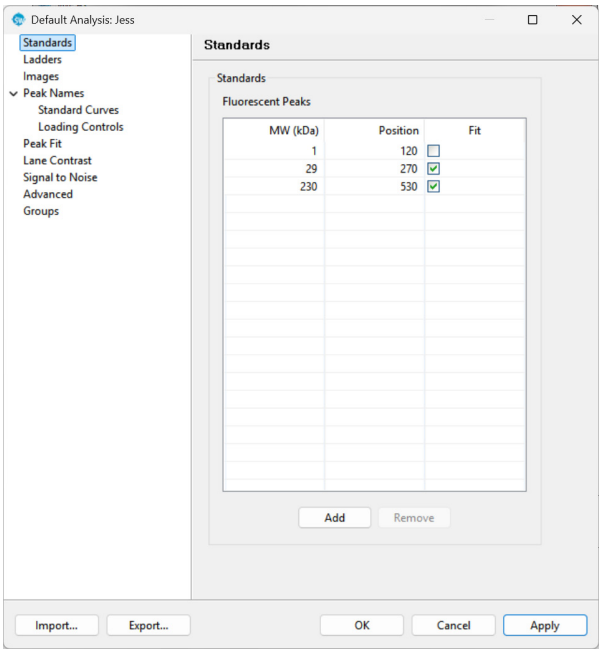

**NOTE:** New assays are saved in the Compass Assays directory.

## Step 7 - Modify Default Analysis Parameters (Optional)

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

1. Select the **Assay** screen.

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

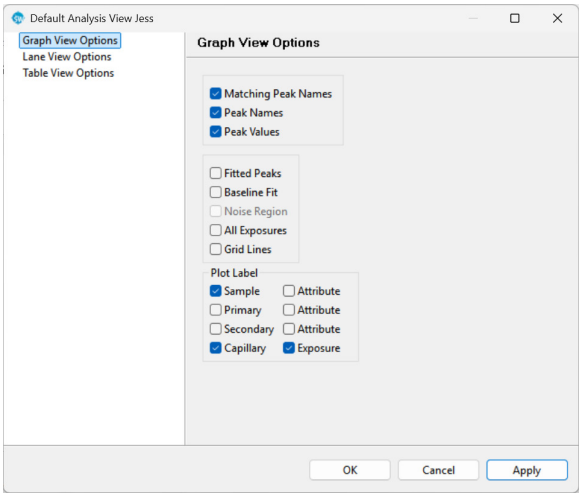



3. We recommend using the default parameters for Simple Western assays. However, you can modify any you want, then click **OK**. For detailed information on analysis parameters, please refer to “Analysis Settings Overview” on page 277.

## Step 8 - Modify Default Analysis View (Optional)

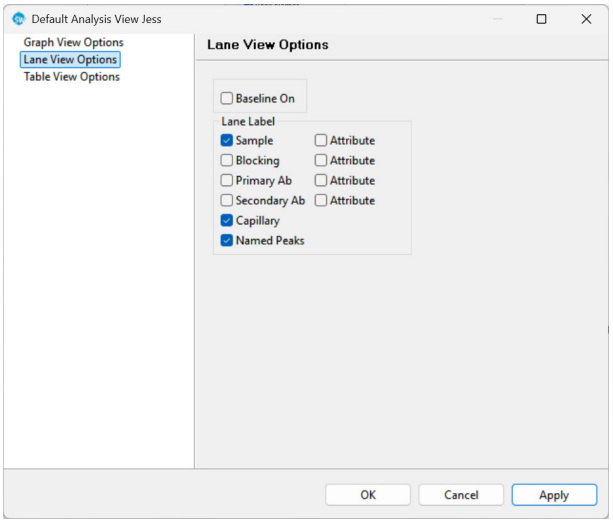
You can preset the Graph, Lane, and Table view options used to display run data generated with the assay.

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

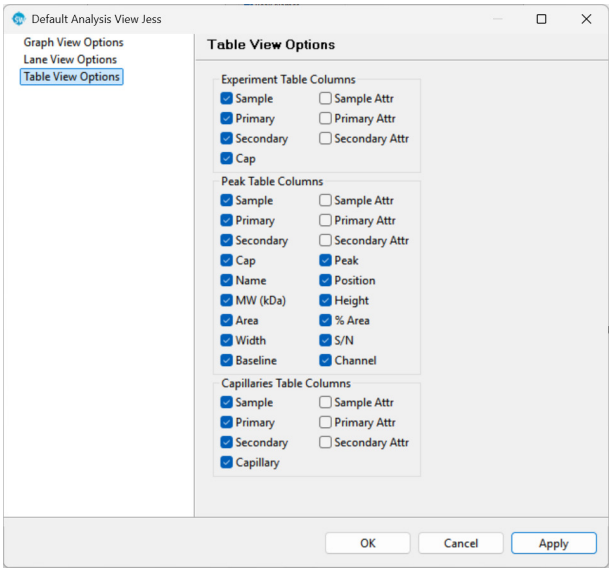


3. Select the items you want to use as default Graph display options for your run data, then click **Apply**. When you are done selecting items, click **OK**. For detailed information on these options, please refer to “Graph Options: Customizing the Data Display” on page 259.

4. Click **Lane View Options.** Select the items you want to use as default Lane display options for your run data, then click **Apply**. When you are done selecting items, click **OK**. For detailed information on these options, please refer to “Lane Options” on page 224.




5. Click **Table View Options**. Select the items you want to use as default Table display options for your run data, then click **Apply.** When you are done select items, click **OK**.




Check one or multiple label boxes, and uncheck those you don’t want to display. You can also adjust the columns that display in each table in the Analysis Screen. See “Experiment Pane: Assay and Capillary Information” on page 155, “Peaks Pane: Calculated Results” on page 161, and “Viewing the Capillaries Pane” on page 165 for more information.

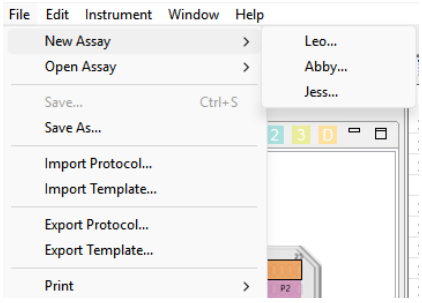
**NOTE:** If you save the run file after updating Graph, Lane, and Table options in the Analysis screen, those selections will override the Default Analysis settings and will automatically display the next time you open the run file.

# Stellar Assays on Jess: Creating a New Assay

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

## Step 1 - Open a Template Assay

1. Select the **Assay** screen.
2. If Jess is not connected to Compass for Simple Western, select **File** in the main menu, click **New Assay**, and choose **Jess**:

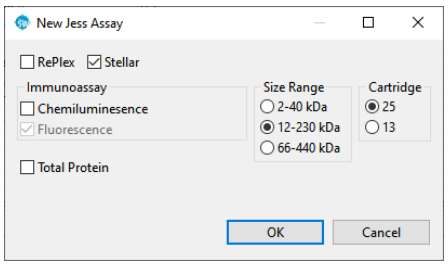



   If Jess is connected to Compass for Simple Western select **File** in the main menu and click **New Assay**.

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

**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.

3. Select **Stellar**.



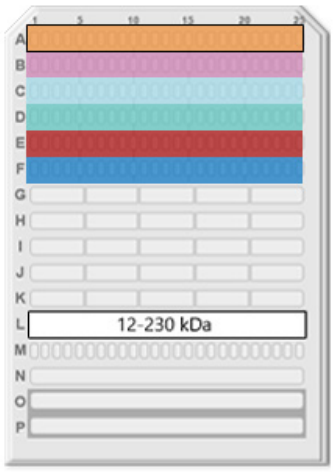
4. Select **Chemiluminescence** or **Total Protein** if desired.
5. Select **Size Range** and **Cartridge Type**.
6. Click **OK**.

## Step 2 - Review 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.

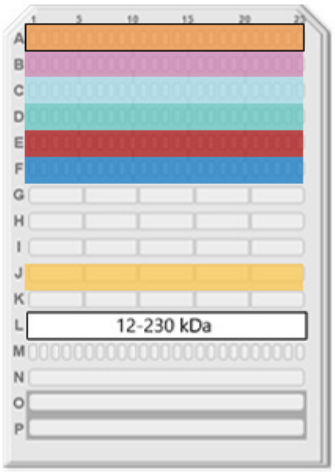
**PLATE LAYOUT:**

**STELLAR FLUORESCENCE NIR/IR IMMUNOASSAY**



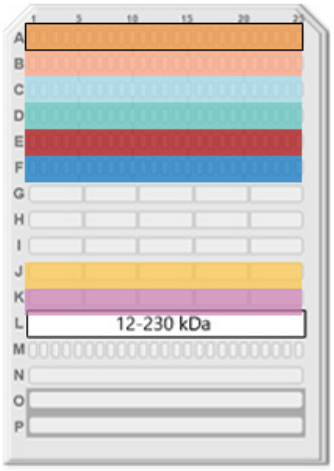

- **Row A** – Biotinylated ladder (A1) and sample (A2–25)
- **Row B** – Antibody Diluent
- **Row C** – Antibody Diluent (C1) and Primary antibody (C2–C25)
- **Row D** – Streptavidin-NIR (D1) and Stellar secondary antibody (D2-D25)
- **Row E** – Stellar IR or NIR Anneal
- **Row F** – Stellar IR or NIR Label

**STELLAR NIR/IR IMMUNOASSAY + CHEMILUMINESCENCE IMMUNOASSAY**




- **Row A** – Biotinylated ladder (A1) and sample (A2–25)
- **Row B** – Antibody Diluent
- **Row C** – Antibody Diluent (C1) and Primary antibody (C2–C25)
- **Row D** – Streptavidin-NIR (D1) and Stellar and HRP-conjugated secondary antibody (D2–D25)
- **Row E** – Stellar IR or NIR Anneal
- **Row F** – Stellar IR or NIR Label
- **Row J** – Luminol-S/Peroxide mix

#### STELLAR NIR/IR IMMUNOASSAY + TOTAL PROTEIN NORMALIZATION USING CHEMILUMINESCENCE DETECTION



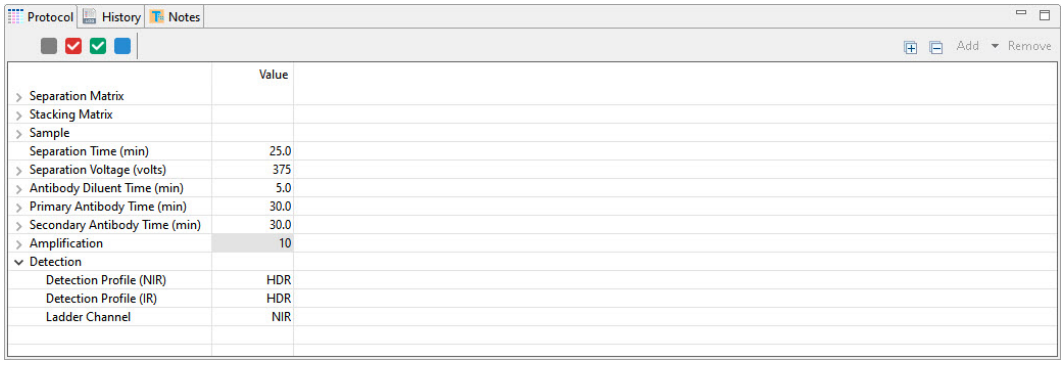

- **Row A** – Biotinylated ladder (A1) and sample (A2–25)
- **Row B** – Total Protein Biotin Labeling Reagent
- **Row C** – Antibody Diluent (C1) and primary antibody (C2–C25)
- **Row D** – Streptavidin-NIR (D1) and Stellar secondary antibody + Stellar Total Protein Streptavidin-HRP (D2–D25)
- **Row E** – Stellar IR or NIR Anneal
- **Row F** – Stellar IR or NIR Label
- **Row J** – Luminol-S/Peroxide mix
- **Row K** – Antibody Diluent (2 or Milk-Free)

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

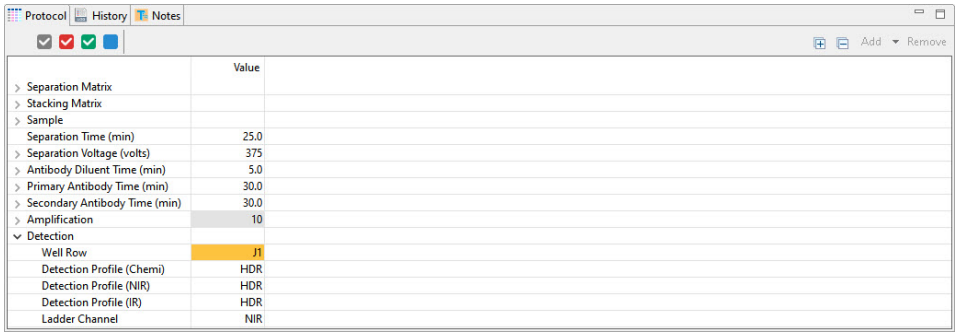
## Step 3 - Modifying the Assay Protocol (Optional)

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. Some examples are shown below:

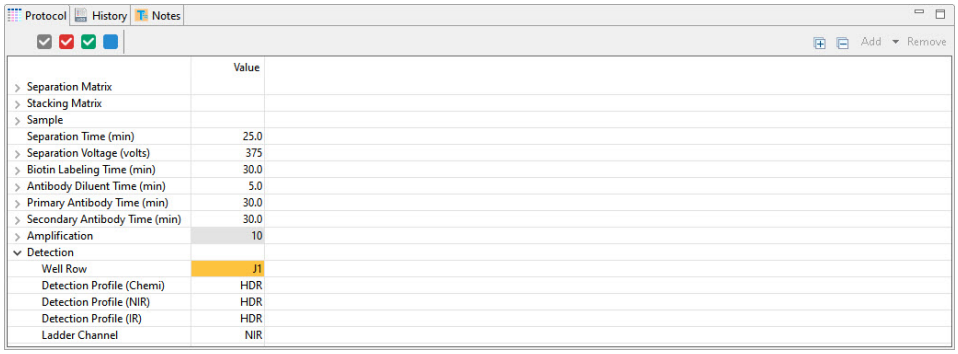
#### STELLAR FLUORESCENCE IMMUNOASSAY



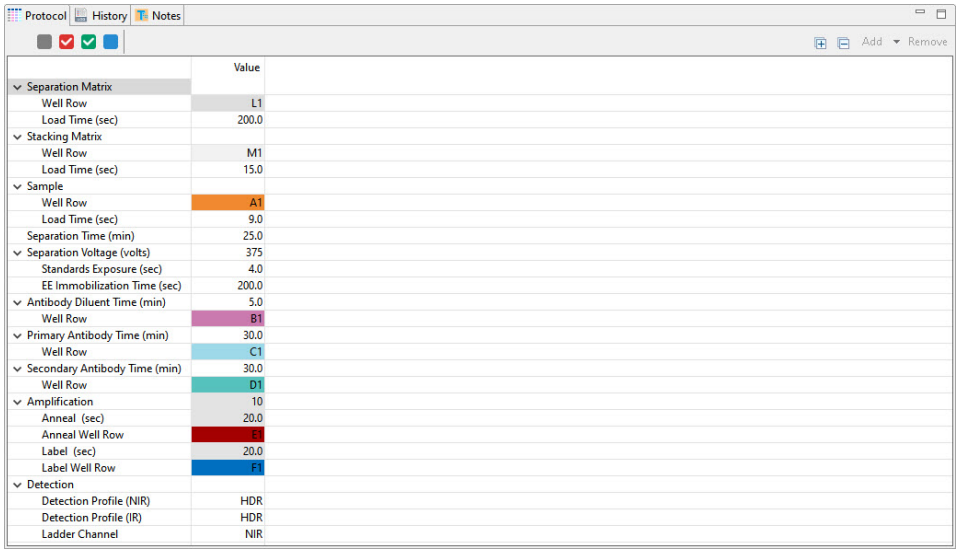
### STELLAR NIR/IR FLUORESCENCE IMMUNOASSAY + CHEMILUMINESCENCE IMMUNOASSAY




### STELLAR FLUORESCENCE IMMUNOASSAY + TOTAL PROTEIN NORMALIZATION USING CHEMILUMINESCENCE DETECTION

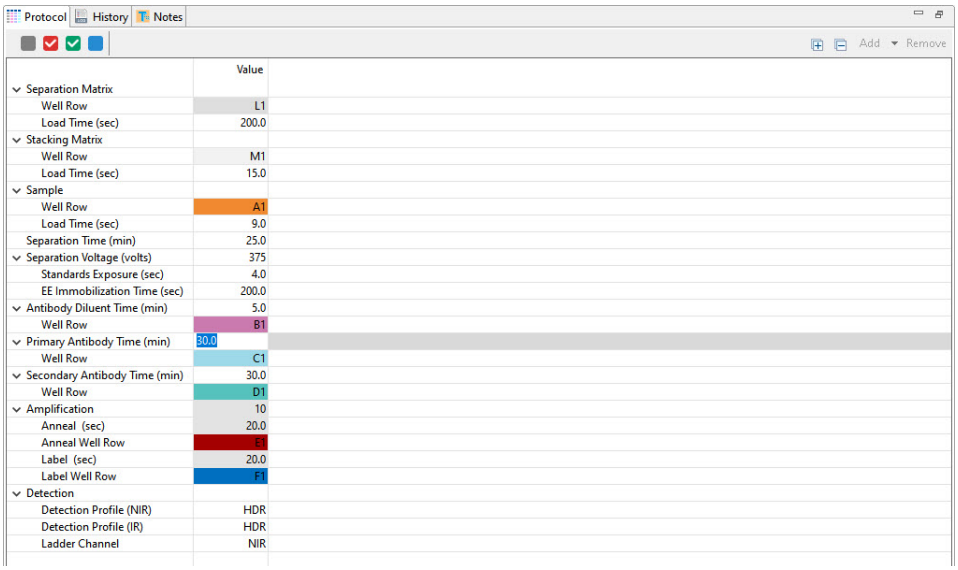



Each row contains an assay protocol step. Each step can contain a reagent assay plate row assignment and one or more parameter settings. To view the details for a step, click on the gray arrow next to the step name. We recommend using the default protocol settings for the assays. An expanded list of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

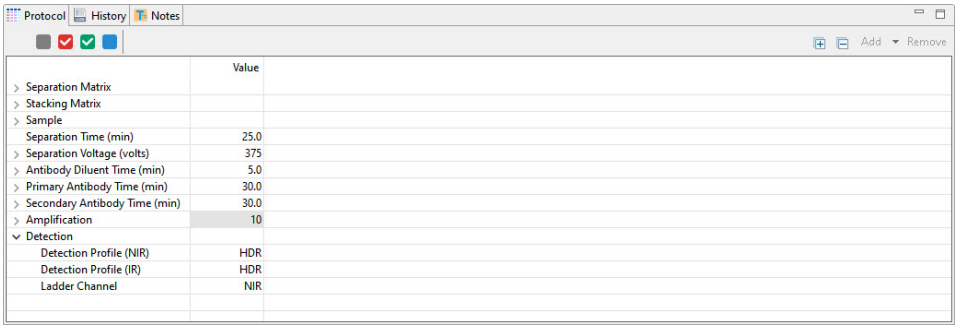


**NOTE:** To expand all parameters, click the ⊞ button at the upper right of the pane. To collapse all parameters, click the ⊟ button at the upper right of the pane.

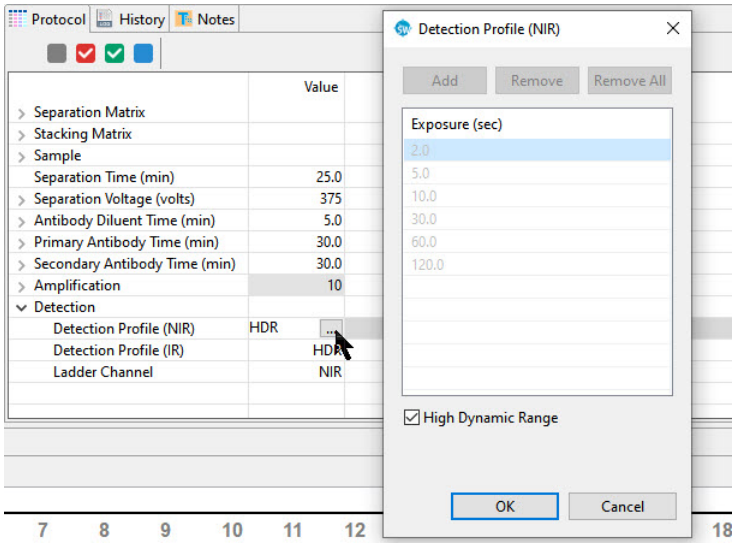
2. You can change the primary or secondary antibody incubation time. Click the cell in the value column next to Primary Antibody Time (min) or Secondary Antibody Time (min) and enter a new value in minutes.




3. The default detection mode is High Dynamic Range (HDR). See “High Dynamic Range Detection Profile (Jess and Abby) and Default Detection Profile (Leo)” on page 100 for more information.

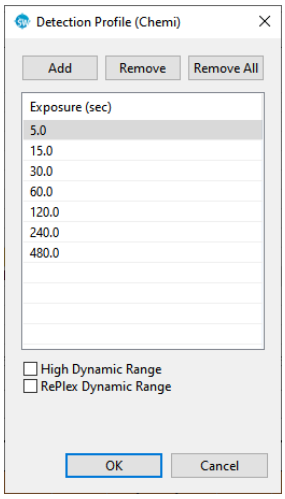


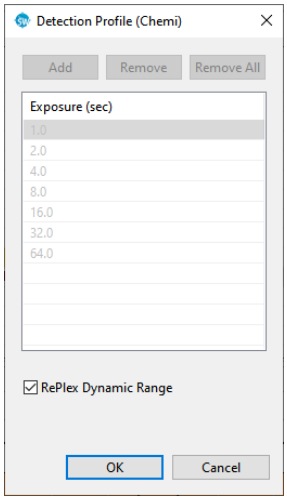
Additional exposures can be collected in the assay if desired. To do this, click the gray arrow next to Detection to expand the row. Click the HDR cell in the column next to Detection Profile (NIR) or (IR) and click the **...** button that appears to open the Detection Profile window.




Deselect the High Dynamic Range checkbox. To remove exposure times, click the **Remove** button or click **Remove All** if the ladder was not run in the channel you are modifying. To add additional exposure times to the protocol, click the **Add** button and enter the values. Select **OK** when you are done.

For Stellar Assays with Chemiluminescence or Total Protein, when the High Dynamic Range box is unchecked in Detection Profile (Chemi), you also have the option of selecting the RePlex Dynamic Range. See “High Dynamic Range Detection Profile (Jess and Abby) and Default Detection Profile (Leo)” on page 100 and “RePlex Dynamic Range Detection Profile (Jess and Abby) and Default Detection Profile for RePlex assays (Leo)” on page 102 for more information.



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

**NOTE:** For more information on changing protocol step parameters, contact ProteinSimple Technical Support at **support@bio-techne.com** (US/Canada) or **Instrument.Support.EMEA@bio-techne.com** (Europe). You can also contact your local Field Application Scientist for help.

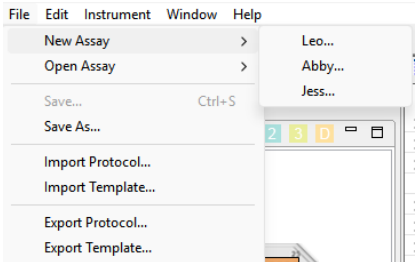
## Steps 4–8

Steps 4 through 8 for creating a Stellar Assay are the same as when you're creating an Immunoassay. Please go to "Step 4 - Add Assay Notes (Optional)" on page 51 to continue.

# Protein Normalization on Jess: Creating a New Assay

## Step 1 - Open a Template Assay

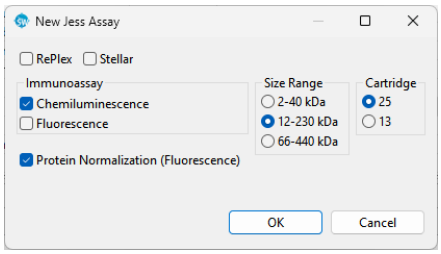
1. Select the **Assay** screen.
2. If Jess is not connected to Compass for Simple Western, select **File** in the main menu, click **New Assay,** and choose **Jess**:




   If Jess is connected to Compass for Simple Western select **File** in the main menu and click **New Assay**.

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

3. Select the template assay by selecting **Protein Normalization** in addition to **Fluorescence** and/or **Chemiluminescence** as your assay type, **Size Range**, and **Cartridge Type**.



4. Or choose **Open Assay** from the File menu to select from the menu of saved assays.
5. Click **OK**.

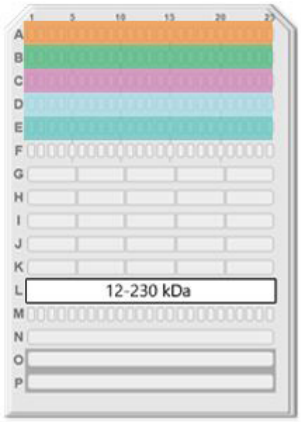
**NOTES:**
Protein Normalization isn't available with Stellar Assays. A Stellar Total Protein Assay can be used instead when normalization is needed.

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** – Antibody Diluent (B1) and Protein Normalization Reagent (B2–B25)
- **Row C** – Antibody Diluent
- **Row D** – Antibody Diluent (D1) and Primary antibody (D2–D25)
- **Row E** – Streptavidin-NIR (E1) and Secondary conjugate (E2–E25)
- **Row F** – Empty for fluorescence only, Luminol-S/Peroxide mix for chemiluminescence

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

## Step 3–8

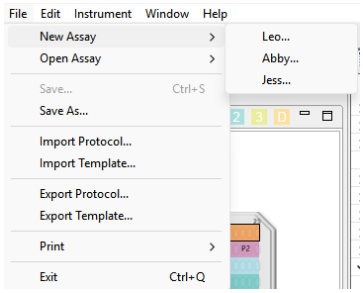
Steps 3 through 8 for creating a Protein Normalization Assay on Jess are the same as when you're creating an Immunoassay. Please go to "Step 3 - Modifying the Assay Protocol (Optional)" on page 45 to continue.

# RePlex Assays on Jess and Abby: Creating a New Assay

**NOTE:** To create a RePlex Assay on Leo, see “RePlex Assays on Leo: Creating a New Assay” on page 87.

## Step 1 – Open a Template Assay

1. Select the **Assay** screen.
2. If your instrument is not connected to Compass for Simple Western, select **File** in the main menu, click **New Assay**, and select your instrument:

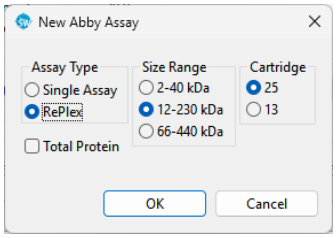


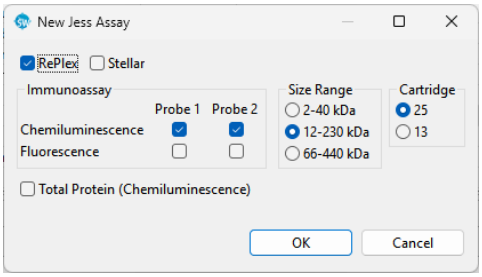

If your instrument is connected to Compass for Simple Western select **File** in the main menu and click **New Assay**.

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

**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.

3. Select **RePlex**.



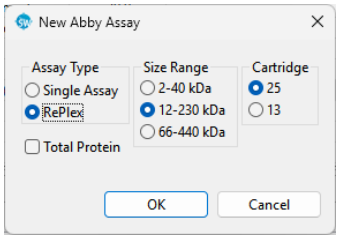
These RePlex Assay combinations are currently validated on Jess and Abby:

- **Chemiluminescence Immunoassay + Chemiluminescence Immunoassay using RePlex:** Performs a chemiluminescence immunoassay followed by another chemiluminescence immunoassay.
- **Chemiluminescence Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection:** Performs a chemiluminescence immunoassay followed by total protein detection.
- **Chemiluminescence Immunoassay/Fluorescence NIR Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection (Jess only):** Performs a chemiluminescence or fluorescence NIR immunoassay followed by total protein detection.
- **Fluorescence NIR Immunoassay + Fluorescence NIR Immunoassay using RePlex (Jess only):** Performs a fluorescence NIR immunoassay followed by another fluorescence NIR immunoassay.
- **Fluorescence NIR Immunoassay + Chemiluminescence Immunoassay using RePlex (Jess only):** Performs a fluorescence NIR immunoassay followed by a chemiluminescence immunoassay.

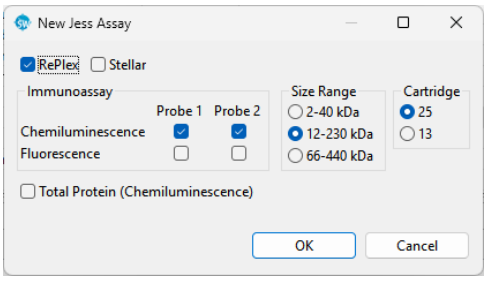
The assay selected for Probe 1 is run first. When Probe 1 is complete, the instrument performs a wash, RePlex purge, and wash step combination prior to beginning the assay selected for Probe 2.

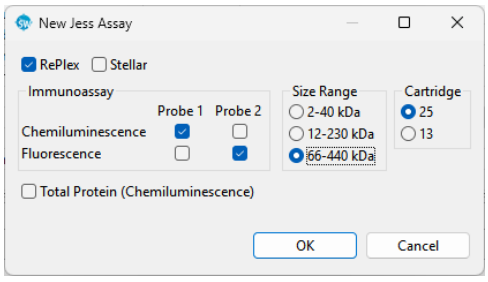
4. Select the type of assays.

**To run an Immunoassay + Immunoassay using RePlex**
**Abby**: When RePlex is selected as the Assay Type, chemiluminescence will be run for both Probe 1 and 2.



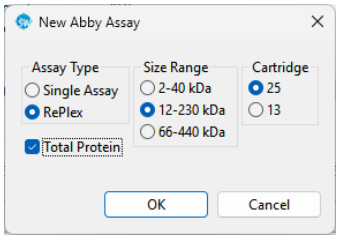

**Jess:** Select any combination of chemiluminescence or fluorescence for Probes 1 and 2. The default selection is chemiluminescence for both. For example:



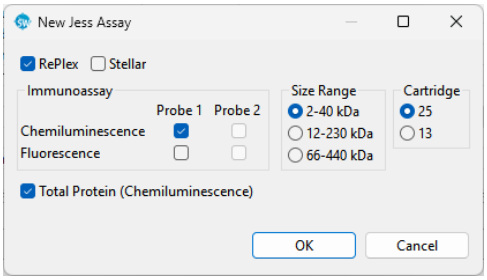



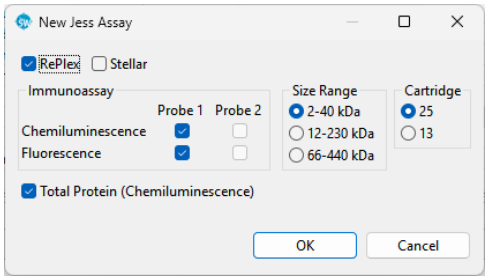
**To run a Chemiluminescence or Fluorescence Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection**

**Abby:** Select **Total Protein**. Probe 1 will be used for chemiluminescence. Probe 2 will be used for the Total Protein assay.




**Jess**: 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:



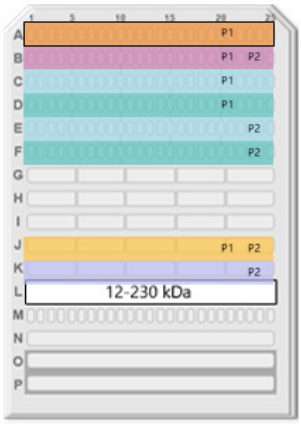




5. Select the **Size Range** and **Cartridge Type**.
6. Click **OK**.

## Step 2 - Assign Assay Plate Reagents

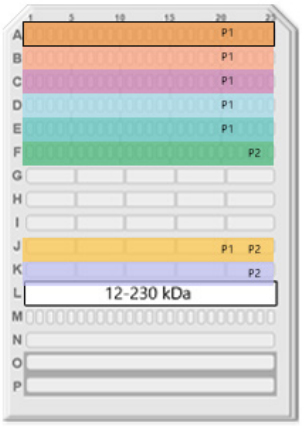
1. Click on the **Layout** tab. Default row locations where each reagent should be placed on the assay plate are displayed. Reagents used in Probe 1 are labeled 'P1' on the plate, those used in Probe 2 are labeled 'P2':

### IMMUNOASSAY + IMMUNOASSAY USING REPLEX




- **Row A** – Biotinylated Ladder (A1) and Sample (A2–A25)
- **Row B** – Antibody Diluent
- **Row C** – Primary Probe 1: 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: Antibody Diluent (E1) and Primary antibody for Probe 2 (E2–E25)
- **Row F** – Secondary Probe 2: 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 NORMALIZATION USING REPLEX AND CHEMILUMINESCENCE DETECTION

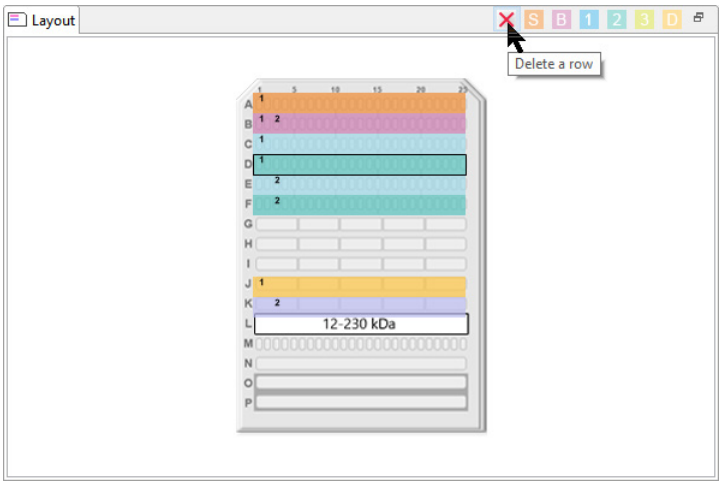



- **Row A** – Biotinylated Ladder (A1) and Sample (A2–A25)
- **Row B** – Antibody Diluent (B1) and Total Protein Biotin Labeling Reagent (B2-B25)
- **Row C** – Antibody Diluent
- **Row D** – Primary Probe 1: Antibody Diluent (D1) and Primary antibody for Probe 1 (D2–D25)
- **Row E** – Secondary Probe 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
- **Row K** – RePlex reagent mix

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

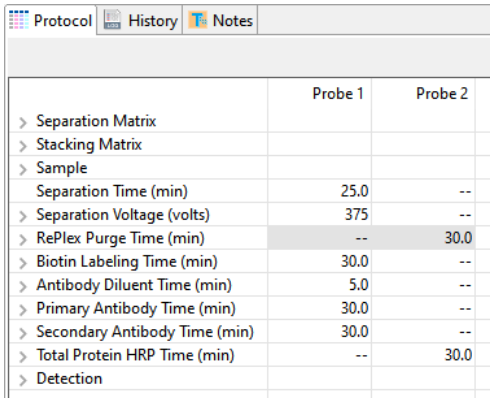
2. If needed, the Secondary antibody row assignments for either Probe 1 or 2 can be deleted for assays where the Primary antibody contains HRP. Any row assignments changed in the Layout pane are updated in the Protocol pane automatically.

   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



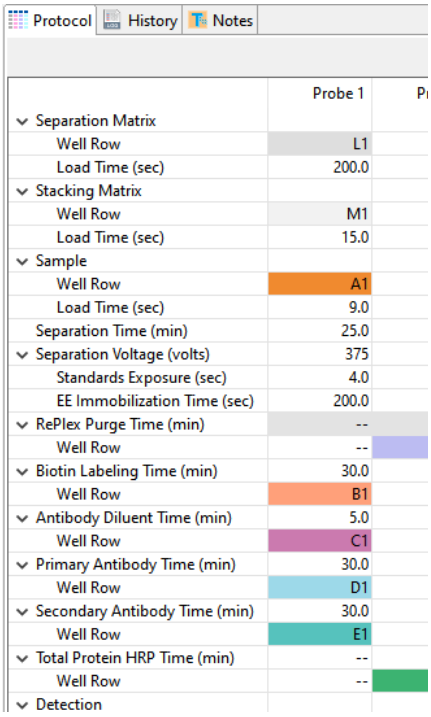

## Step 3 - Modifying the Assay Protocol (Optional)

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 Jess Chemiluminescence/Fluorescence NIR Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection is shown in this example:



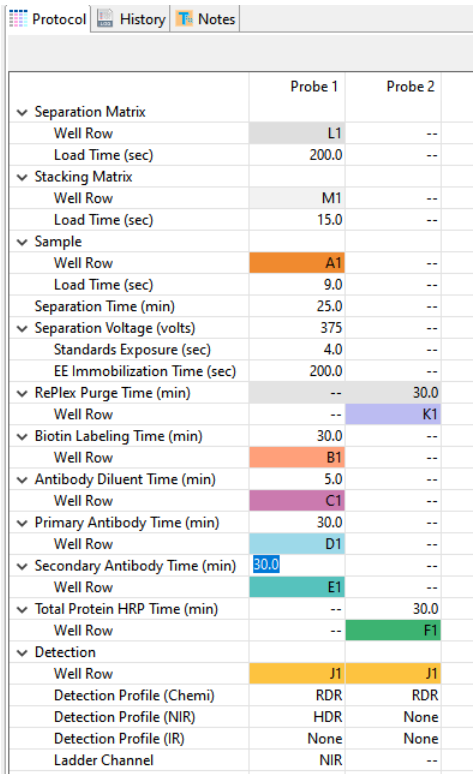

| | Probe 1 | Probe 2 |
|---|---|---|
| Separation Matrix | | |
| Stacking Matrix | | |
| Sample | | |
| Separation Time (min) | 25.0 | -- |
| Separation Voltage (volts) | 375 | -- |
| RePlex Purge Time (min) | -- | 30.0 |
| Biotin Labeling Time (min) | 30.0 | -- |
| Antibody Diluent Time (min) | 5.0 | -- |
| 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 gray 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 for a Jess Chemiluminescence/Fluorescence NIR Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection is shown below:



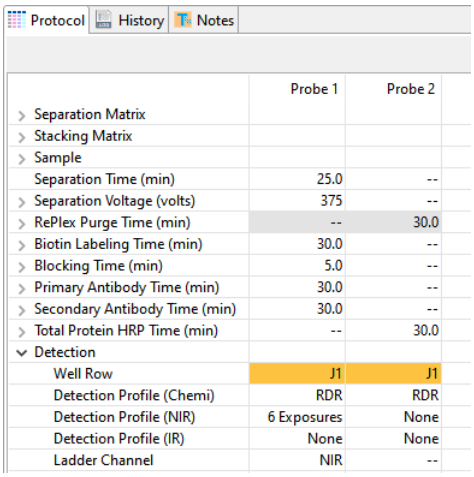

**NOTE:** To expand all parameters, click the ⊞ button at the upper right of the pane. To collapse all parameters, click the ⊟ button at the upper right of the pane.

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:




| | 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 | -- |
| Antibody Diluent 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) | HDR | None |
| Detection Profile (IR) | None | None |
| Ladder Channel | NIR | -- |

3. The default detection mode for chemiluminescent RePlex Assays is RePlex Dynamic Range (RDR). See “RePlex Dynamic Range Detection Profile (Jess and Abby) and Default Detection Profile for RePlex assays (Leo)” on page 102 for more information. The default detection mode for a fluorescence immunoassay using RePlex assay (Jess only) is NIR with 6 standard exposures.

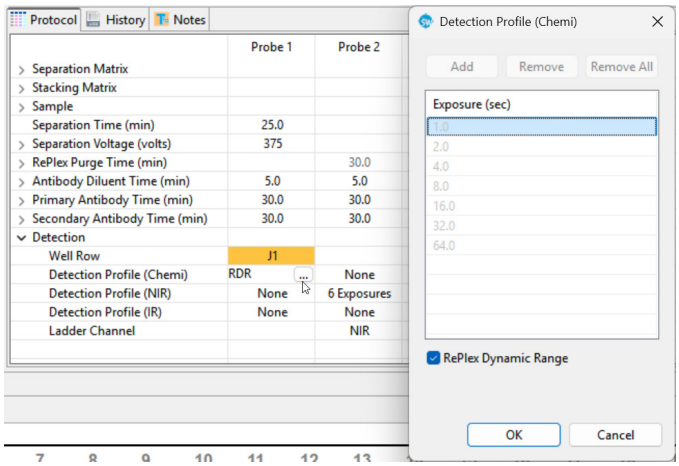



| | Probe 1 | Probe 2 |
|---|---|---|
| Separation Matrix | | |
| Stacking Matrix | | |
| Sample | | |
| Separation Time (min) | 25.0 | -- |
| Separation Voltage (volts) | 375 | -- |
| RePlex Purge Time (min) | -- | 30.0 |
| Biotin Labeling Time (min) | 30.0 | -- |
| Blocking Time (min) | 5.0 | -- |
| Primary Antibody Time (min) | 30.0 | -- |
| Secondary Antibody Time (min) | 30.0 | -- |
| Total Protein HRP Time (min) | -- | 30.0 |
| Detection | | |
| Well Row | J1 | J1 |
| Detection Profile (Chemi) | RDR | RDR |
| Detection Profile (NIR) | 6 Exposures | None |
| Detection Profile (IR) | None | None |
| Ladder Channel | NIR | -- |

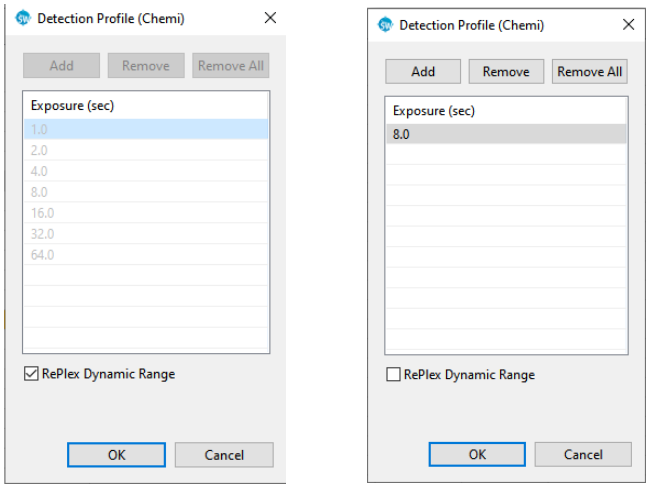
The number of exposures collected in the assay can be adjusted if desired.

### Chemiluminescent

Click the gray arrow next to Detection to expand the row. Click the cell in the RDR column next to Detection Profile (Chemi) in either the Probe 1 or Probe 2 column and click the **...** button that appears to open the Detection Profile window.



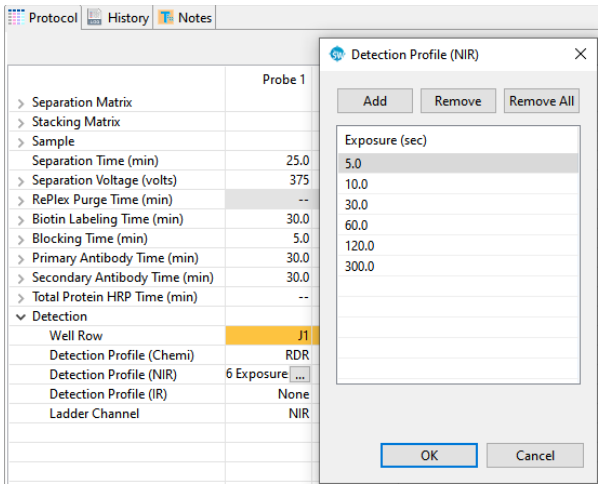

Deselect the RePlex Dynamic Range checkbox. Up to 6 additional exposure times (for a total of 7 exposures) can be added to the protocol. To remove exposure times, click the **Remove** button. To add additional exposure times to the protocol, click the **Add** button and enter the values. Select **OK** when you are done.



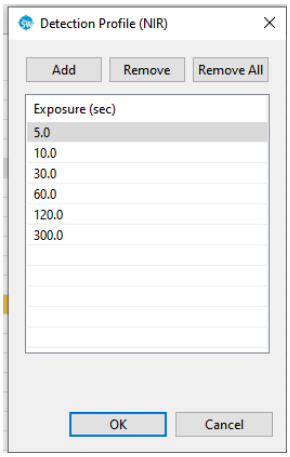

**NOTE:** You can only use the Remove All button to remove exposures if your ladder was not run in the channel you are modifying. See “Ladder Channel (Jess only)” on page 77 for more information.

### NIR Fluorescent (Jess only)

Click the gray arrow next to Detection to expand the row. Click the cell in the exposure cell column next to Detection Profile (NIR) in either the Probe 1 or Probe 2 column and click the **...** button that appears to open the Detection Profile window.




To remove exposure times, click the **Remove** button. To add additional exposure times to the protocol, click the **Add** button and enter the values. Select **OK** when you are done.

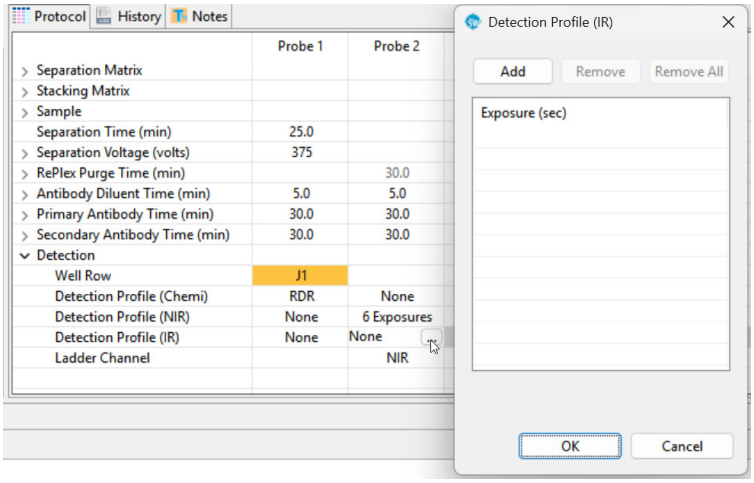



**NOTE:** You can only use the Remove All button to remove exposures if your ladder was not run in the channel you are modifying. See “Ladder Channel (Jess only)” on page 77 for more information.

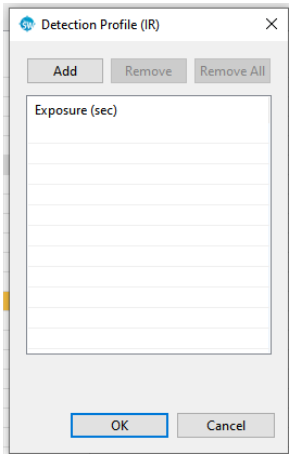
### IR Fluorescent (Jess only, if added to the protocol)

**NOTES:** IR exposures are not selected in a default Jess RePlex assay. Add exposures to add IR fluorescence detection to the assay.

Click the gray arrow next to Detection to expand the row. Click **None** in the exposure cell column next to Detection Profile (IR) in either the Probe 1 or Probe 2 column and click the **...** button that appears to open the Detection Profile window.




To add exposure times to the protocol, click the **Add** button and enter the values. To remove exposure times, click the **Remove** or **Remove All** button. Select **OK** when you are done.

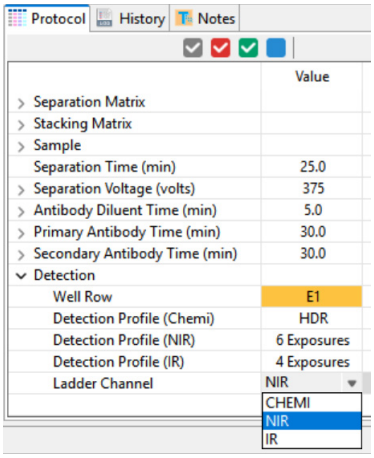



**NOTE:** You can only use the Remove All button to remove exposures if your ladder was not run in the channel you are modifying. See “Ladder Channel (Jess only)” on page 77 for more information.

### Ladder Channel (Jess only)

**NOTE:** The ladder channel defaults to NIR in assays including fluorescence detection.

To change the ladder channel, select the cell next to Ladder Channel and select the desired channel in the drop-down menu.




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

**NOTE:** For more information on changing protocol step parameters, contact ProteinSimple Technical Support at **support@bio-techne.com** (US/Canada) or **Instrument.Support.EMEA@bio-techne.com** (Europe). You can also contact your local Field Application Scientist for help.

## 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)" on page 51 to continue.

# Immunoassays on Leo: Creating a New Assay

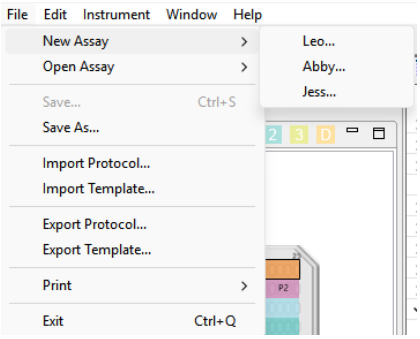
To create a new assay, we recommend using one of the template assays and making any necessary modifications from there.

**NOTES:**
This section provides details on how to create a Chemiluminescence Immunoassay on Leo. To create a Chemiluminescence Immunoassay on Jess or Abby, see “Immunoassays on Jess and Abby: Creating a New Assay” on page 41.

To create a RePlex Assay on Leo, that includes an immunoassay, see “RePlex Assays on Leo: Creating a New Assay” on page 87.

## Step 1: Open a Template Assay

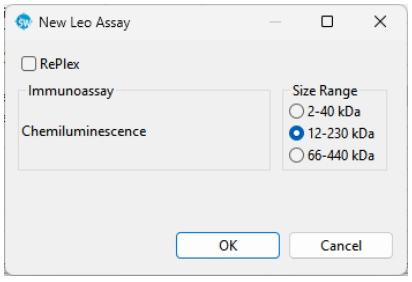
1. Select the **Assay** screen.
2. If Leo is not connected to Compass for Simple Western, select **File** in the main menu, click **New Assay**, and select **Leo**.




   If Leo is connected to Compass for Simple Western, select **File** in the main menu and click **New Assay**.

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

3. Select the appropriate **Size Range**.

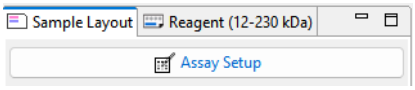


4. Click **OK**.
5. A template assay with default reagent locations will display in the Sample Layout tab of the Assay screen. The numbers on the Sample Plate indicate the cartridge that will load reagents from that row. The Reagent tab will display the size range selected.

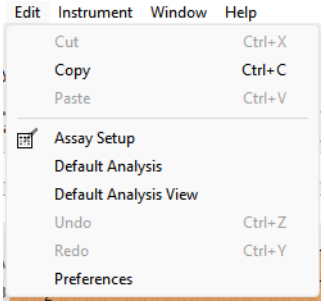
**NOTE:** If you make changes to the assay, such as defining the wells in the plate, be sure to select **File** and **Save** before proceeding.

## Step 2: Assign Sample Plate Reagents

1. Click on the **Layout** tab and click the **Assay Setup** button.

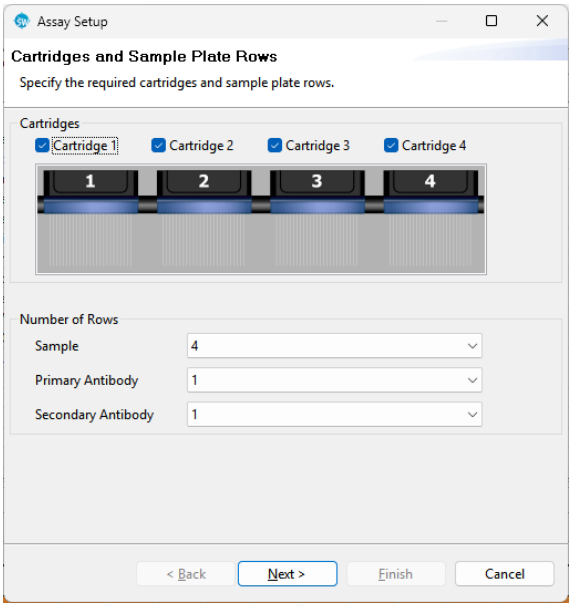



You can also select **Edit** in the main menu when the Assay pane is active and click **Assay Setup**.




2. Define the assay parameters.
   a. Click the boxes next to the cartridge holder to indicate which holders will be loaded with a cartridge.

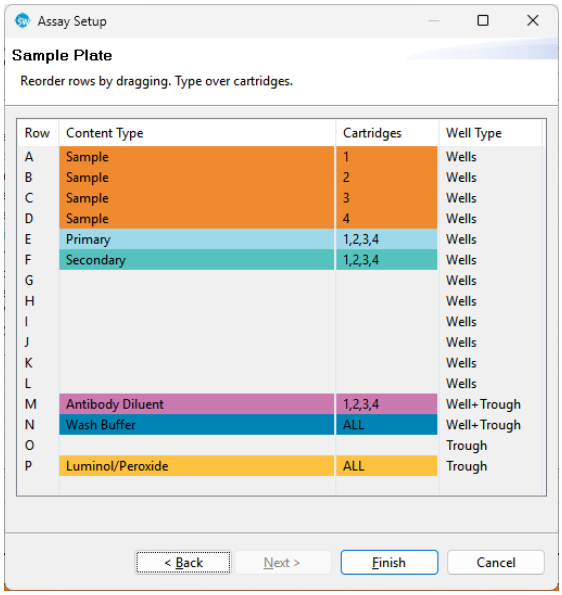
b. Select the number of sample, primary antibody, and secondary antibody rows pipetted on the Sample Plate using the drop-down list.




**NOTE:** An alert will appear if you try to specify a number of reagent rows that exceeds the number of rows available on the Sample Plate.

3. Click **Next**.

4. An 'Assay Setup - Sample Plate' prompt will appear.



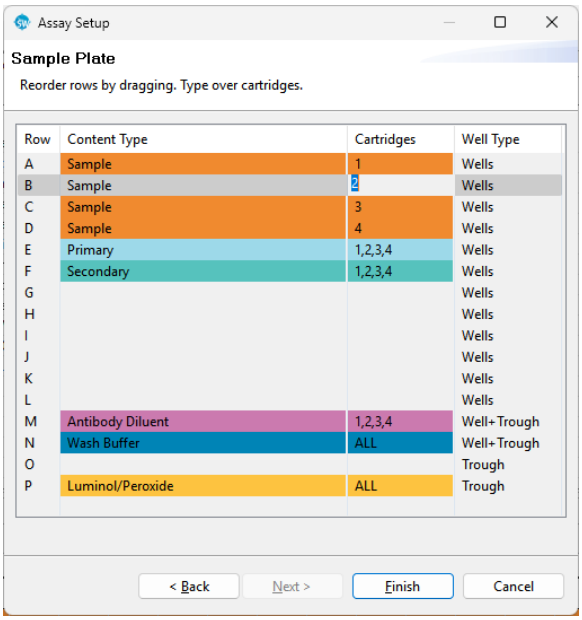

- **Row A–D** – Biotinylated ladder (A1, B1, C1, D1) and Samples (A2–25, B2–25, C2–25, D2–25)
- **Row E** – Antibody Diluent (E1) and Primary antibody (E2–E25)
- **Row F** – Streptavidin-HRP (F1) and Secondary conjugate (F2–F25)
- **Row M** – Antibody Diluent
- **Row N** – Wash Buffer
- **Row P** – Luminol/Peroxide mix

5. The default protocol automatically assigns all reagent locations for the assay. Reagents assignments to rows M–P should not be modified.

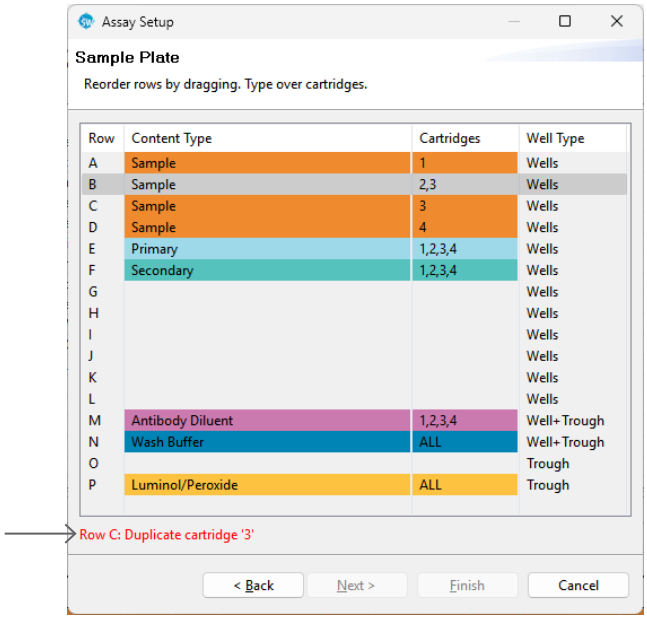
   **To modify the reagents assigned to rows A–L:**

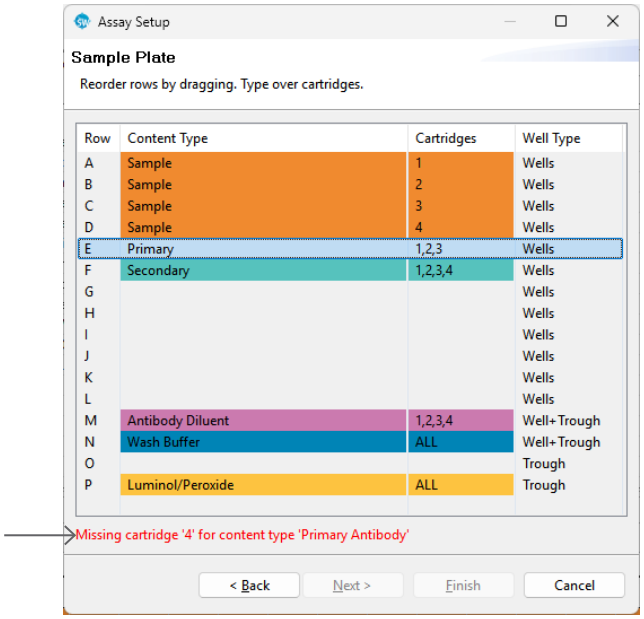
   a. Reorder row locations by clicking on the row and dragging it to the correct location.

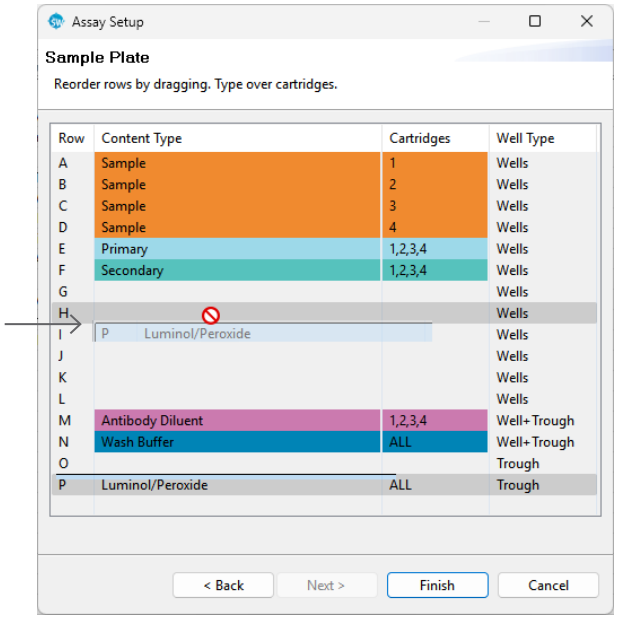
   b. Change the row a cartridge will load a reagent from by clicking on the cell in the Cartridges column and typing in the cartridge number.




   c. An alert will appear at the bottom of the prompt if too many rows are assigned to one cartridge, if a cartridge is missing a reagent assignment, or if you try to move a reagent to a row that is not allowed.



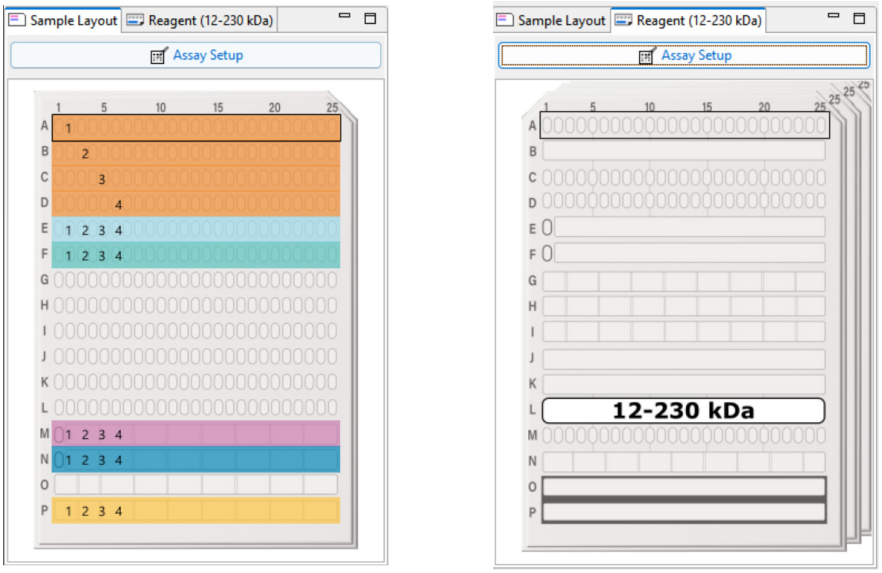



d. To add or remove a row, click the **Back** button and adjust the number of rows using the drop-down list.

6. Click **Finish**. You can also adjust the row a cartridge will load from later in the Protocol pane.

7. A Sample Plate with updated reagent locations will display in the Sample Layout tab of the Assay screen. Numbers on the Sample Plate indicate the row each cartridge will load reagents from. The Reagent tab will display the size range selected and the number of Pre-filled Reagent Plates required to perform your run.

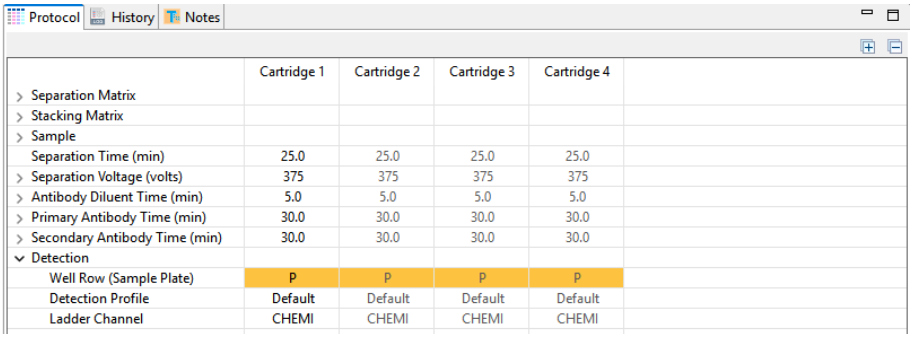



## Step 3: Modifying the Assay Protocol (Optional)

The capillary cartridges are all run in parallel, so voltage- and time-based parameters for all the cartridges must be the same. Changing the parameter for cartridge 1 will change the parameter for all other cartridges in the protocol. Grayed-out parameters or reagent locations cannot be changed.

To modify the protocol:

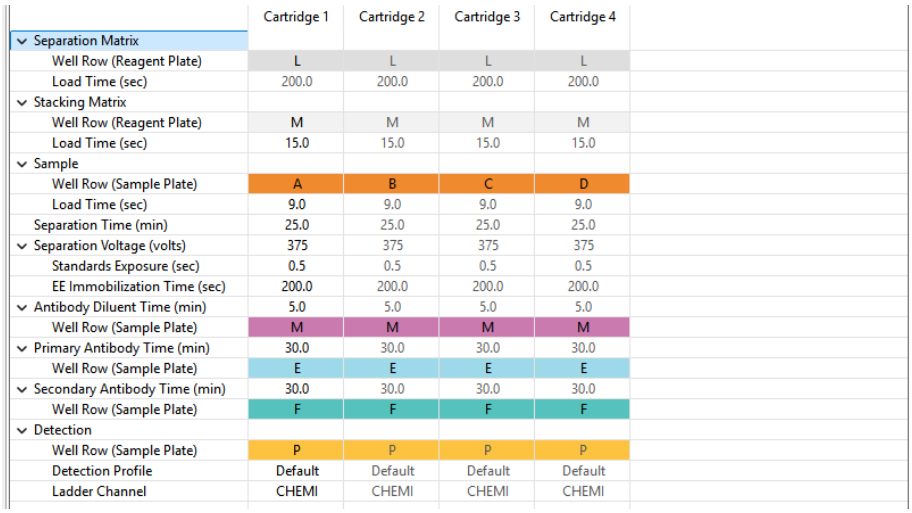
1. Click on the **Protocol** tab. This pane shows the individual steps of the assay protocol and allows you to change some assay parameters. The example below is for a chemiluminescence Immunoassay.




| | Cartridge 1 | Cartridge 2 | Cartridge 3 | Cartridge 4 |
|---|---|---|---|---|
| Separation Matrix | | | | |
| Stacking Matrix | | | | |
| Sample | | | | |
| Separation Time (min) | 25.0 | 25.0 | 25.0 | 25.0 |
| Separation Voltage (volts) | 375 | 375 | 375 | 375 |
| Antibody Diluent Time (min) | 5.0 | 5.0 | 5.0 | 5.0 |
| Primary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 |
| Secondary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 |
| Detection | | | | |
| Well Row (Sample Plate) | P | P | P | P |
| Detection Profile | Default | Default | Default | Default |
| Ladder Channel | CHEMI | CHEMI | CHEMI | CHEMI |

Each row contains an assay protocol step. Each step contains a Pre-filled Reagent Plate row assignment (gray) or Sample Plate row assignment (color-coded), and one or more parameter settings. To view the details for a step, click on

the gray arrow next to the step name. We recommend using default protocol settings for the assay. An expanded list of the default protocol step parameters is shown below:

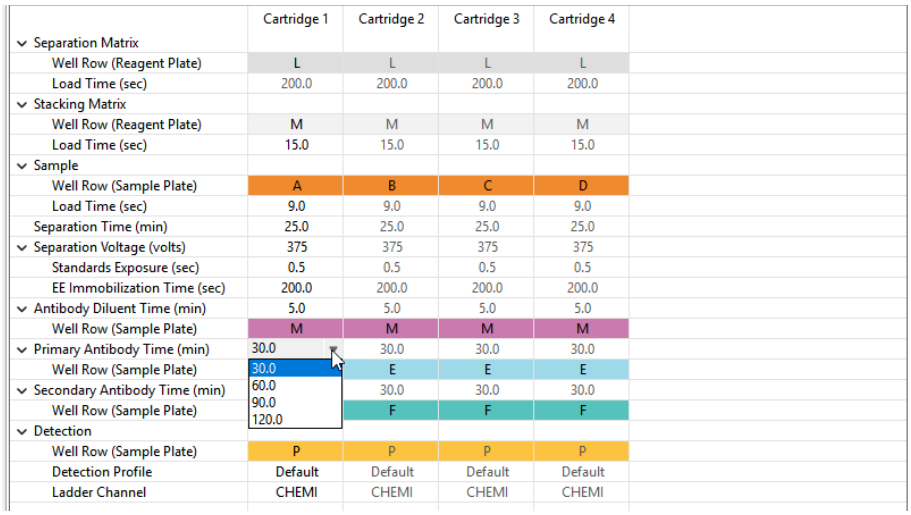


| | Cartridge 1 | Cartridge 2 | Cartridge 3 | Cartridge 4 |
|---|---|---|---|---|
| Separation Matrix | | | | |
| Well Row (Reagent Plate) | L | L | L | L |
| Load Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 |
| Stacking Matrix | | | | |
| Well Row (Reagent Plate) | M | M | M | M |
| Load Time (sec) | 15.0 | 15.0 | 15.0 | 15.0 |
| Sample | | | | |
| Well Row (Sample Plate) | A | B | C | D |
| Load Time (sec) | 9.0 | 9.0 | 9.0 | 9.0 |
| Separation Time (min) | 25.0 | 25.0 | 25.0 | 25.0 |
| Separation Voltage (volts) | 375 | 375 | 375 | 375 |
| Standards Exposure (sec) | 0.5 | 0.5 | 0.5 | 0.5 |
| EE Immobilization Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 |
| Antibody Diluent Time (min) | 5.0 | 5.0 | 5.0 | 5.0 |
| Well Row (Sample Plate) | M | M | M | M |
| Primary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | E | E | E | E |
| Secondary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | F | F | F | F |
| Detection | | | | |
| Well Row (Sample Plate) | P | P | P | P |
| Detection Profile | Default | Default | Default | Default |
| Ladder Channel | CHEMI | CHEMI | CHEMI | CHEMI |

**NOTE:** To expand all parameters, click the ⊞ button at the upper right of the pane. To collapse all parameters, click the ⊟ button at the upper right of the pane.

2. You can change the primary or secondary antibody incubation time.

   **To change the primary antibody incubation time:** Click the arrow in the cell next to Primary Antibody Time (min) in the 'Cartridge 1' column and select a new incubation time from the drop-down list. The values for the other cartridges will automatically update.



| | Cartridge 1 | Cartridge 2 | Cartridge 3 | Cartridge 4 |
|---|---|---|---|---|
| Separation Matrix | | | | |
| Well Row (Reagent Plate) | L | L | L | L |
| Load Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 |
| Stacking Matrix | | | | |
| Well Row (Reagent Plate) | M | M | M | M |
| Load Time (sec) | 15.0 | 15.0 | 15.0 | 15.0 |
| Sample | | | | |
| Well Row (Sample Plate) | A | B | C | D |
| Load Time (sec) | 9.0 | 9.0 | 9.0 | 9.0 |
| Separation Time (min) | 25.0 | 25.0 | 25.0 | 25.0 |
| Separation Voltage (volts) | 375 | 375 | 375 | 375 |
| Standards Exposure (sec) | 0.5 | 0.5 | 0.5 | 0.5 |
| EE Immobilization Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 |
| Antibody Diluent Time (min) | 5.0 | 5.0 | 5.0 | 5.0 |
| Well Row (Sample Plate) | M | M | M | M |
| Primary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | 30.0 / 60.0 / 90.0 / 120.0 | E | E | E |
| Secondary Antibody Time (min) | | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | | F | F | F |
| Detection | | | | |
| Well Row (Sample Plate) | P | P | P | P |
| Detection Profile | Default | Default | Default | Default |
| Ladder Channel | CHEMI | CHEMI | CHEMI | CHEMI |

**To change the secondary antibody incubation time:** Click the cell next to Secondary Antibody Time (min) in the 'Cartridge 1' column and enter a new value in minutes. The values for the other cartridges will automatically update.

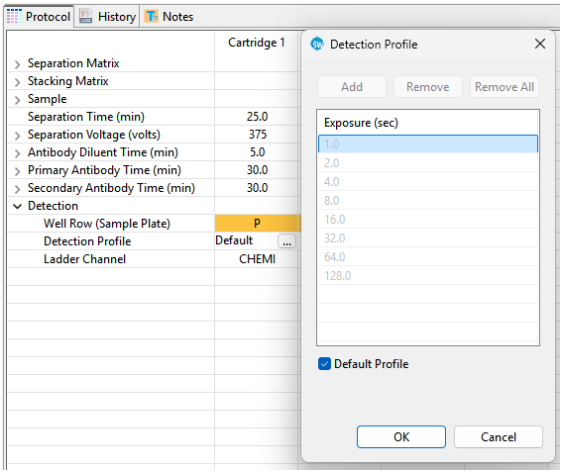
| | Cartridge 1 | Cartridge 2 | Cartridge 3 | Cartridge 4 |
|---|---|---|---|---|
| Separation Matrix | | | | |
| Well Row (Reagent Plate) | L | L | L | L |
| Load Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 |
| Stacking Matrix | | | | |
| Well Row (Reagent Plate) | M | M | M | M |
| Load Time (sec) | 15.0 | 15.0 | 15.0 | 15.0 |
| Sample | | | | |
| Well Row (Sample Plate) | A | B | C | D |
| Load Time (sec) | 9.0 | 9.0 | 9.0 | 9.0 |
| Separation Time (min) | 25.0 | 25.0 | 25.0 | 25.0 |
| Separation Voltage (volts) | 375 | 375 | 375 | 375 |
| Standards Exposure (sec) | 0.5 | 0.5 | 0.5 | 0.5 |
| EE Immobilization Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 |
| Antibody Diluent Time (min) | 5.0 | 5.0 | 5.0 | 5.0 |
| Well Row (Sample Plate) | M | M | M | M |
| Primary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | E | E | E | E |
| Secondary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | F | F | F | F |
| Detection | | | | |
| Well Row (Sample Plate) | P | P | P | P |
| Detection Profile | Default | Default | Default | Default |
| Ladder Channel | CHEMI | CHEMI | CHEMI | CHEMI |

3. You can change the location of Sample Plate reagents in rows A–L. Click the cell in the column next to the Well Row and select a different row on the Sample Plate:

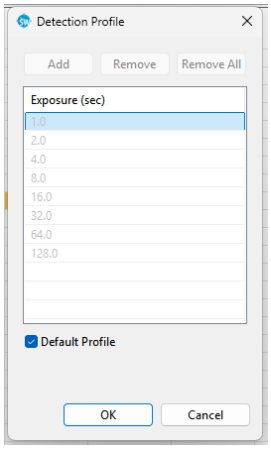
| | Cartridge 1 | Cartridge 2 | Cartridge 3 | Cartridge 4 |
|---|---|---|---|---|
| Separation Matrix | | | | |
| Well Row (Reagent Plate) | L | L | L | L |
| Load Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 |
| Stacking Matrix | | | | |
| Well Row (Reagent Plate) | M | M | M | M |
| Load Time (sec) | 15.0 | 15.0 | 15.0 | 15.0 |
| Sample | | | | |
| Well Row (Sample Plate) | A | B | C | D |
| Load Time (sec) | A | 9.0 | 9.0 | 9.0 |
| Separation Time (min) | B | 25.0 | 25.0 | 25.0 |
| Separation Voltage (volts) | C | 375 | 375 | 375 |
| Standards Exposure (sec) | D | 0.5 | 0.5 | 0.5 |
| EE Immobilization Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 |
| Antibody Diluent Time (min) | 5.0 | 5.0 | 5.0 | 5.0 |
| Well Row (Sample Plate) | M | M | M | M |
| Primary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | E | E | E | E |
| Secondary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | F | F | F | F |
| Detection | | | | |
| Well Row (Sample Plate) | P | P | P | P |
| Detection Profile | Default | Default | Default | Default |
| Ladder Channel | CHEMI | CHEMI | CHEMI | CHEMI |

**NOTE:** Only rows you've designated in the Assay Setup as the appropriate content type will appear in the drop-down list.

4. The default Leo protocol uses a default detection profile. Exposures in the assay can be adjusted if desired. To do this, click the gray arrow next to Detection to expand the row. Click the Default cell in the column next to Detection Profile and click the **...** button that appears to open the Detection Profile window.




Deselect the Default Profile checkbox. To remove times, click the **Remove** button. To add additional times to the protocol, click the **Add** button, enter the values, and select **OK**.




**NOTES:**
No more than two exposures can be longer than 32 seconds.

The Default Detection Profile for chemiluminescence in Immunoassays for Leo is similar to the High Dynamic Range (HDR) exposure series used for Jess and Abby assays, but removes the 512 second exposure. See “High Dynamic Range Detection Profile (Jess and Abby) and Default Detection Profile (Leo)” on page 100 for more information.

For more information on changing protocol step parameters other than incubation times, contact ProteinSimple Technical Support **support@bio-techne.com** (US/Canada) or **Instrument.Support.EMEA@bio-techne.com** (Europe). You can also contact your local Field Application Scientist for help.

## Steps 4–8

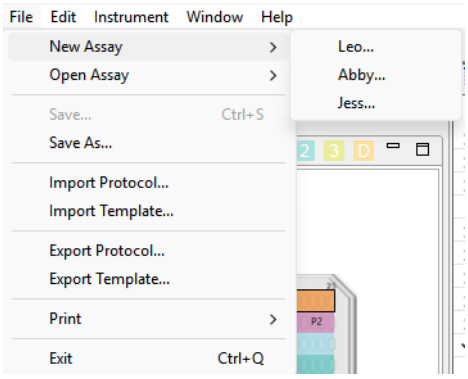
Steps 4 through 8 for creating an Immunoassay on Leo are the same as when you're creating an Immunoassay on Jess or Abby. Please go to "Step 4 - Add Assay Notes (Optional)" on page 51 to continue.

# RePlex Assays on Leo: Creating a New Assay

**NOTE:** To create a RePlex Assay on Jess or Abby, see "RePlex Assays on Jess and Abby: Creating a New Assay" on page 67.

## Step 1 – Open a Template Assay

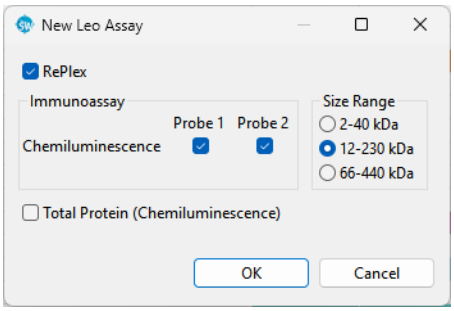
1. Select the **Assay** screen.
2. If Leo is not connected to Compass for Simple Western, select **File** in the main menu, click **New Assay**, and select **Leo**.




If Leo is connected to Compass for Simple Western, select **File** in the main menu and click **New Assay**.

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

3. Select **RePlex**.



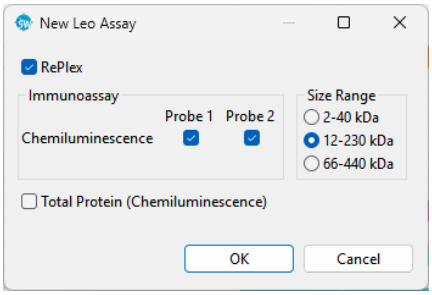
These RePlex Assay combinations are currently validated on Leo:

- **Chemiluminescence Immunoassay + Chemiluminescence Immunoassay using RePlex:** Performs a chemiluminescence immunoassay followed by another chemiluminescence immunoassay.
- **Chemiluminescence Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection**: Performs a chemiluminescence immunoassay followed by total protein detection.

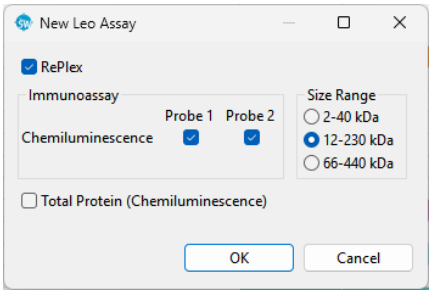
The assay selected for Probe 1 is run first. When Probe 1 is complete, Leo performs a wash, RePlex purge, and wash step combination prior to beginning the assay selected for Probe 2.

4. Select the type of assays.

   **To run a Chemiluminescence Immunoassay + Chemiluminescence Immunoassay using RePlex** – check the boxes for Probe 1 and Probe 2.




   **To run a Chemiluminescence Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection Assay** – check the boxes for Probe 1 and Total Protein (Chemiluminescence). The assay for Probe 1 will be performed first.



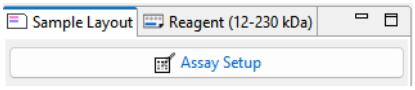

5. Select the **Size Range**.
6. Click **OK**.

A template assay with default reagent locations will display in the Sample Layout tab of the Assay screen. The numbers on the Sample Plate indicate the cartridge that will load reagents from that row. The Reagent tab will display the size range selected.

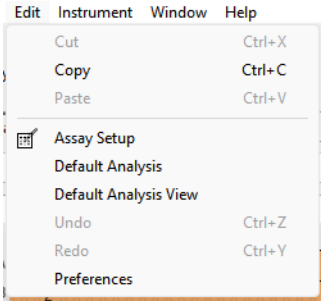
**NOTE:** If you make changes to the assay, such as defining the wells in the plate, be sure to select **File** and **Save** before proceeding.

## Step 2 - Assign Assay Plate Reagents

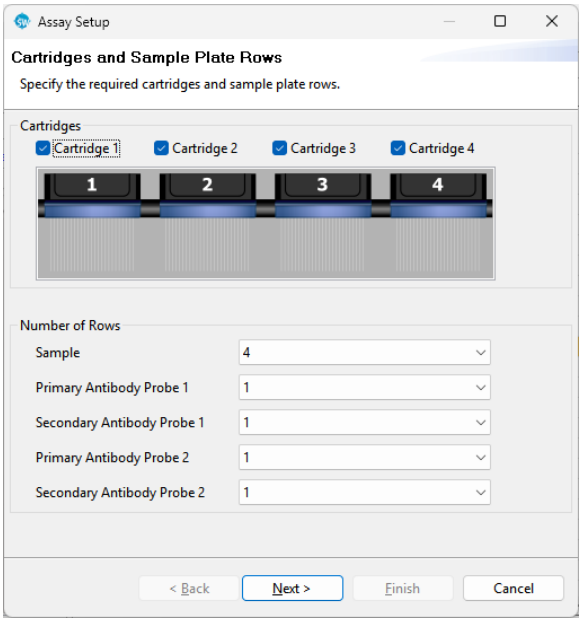
1. Click on the **Sample Layout** tab and click **Assay Setup**.




You can also select **Edit** in the main menu when the Assay pane is active and click **Assay Setup**.



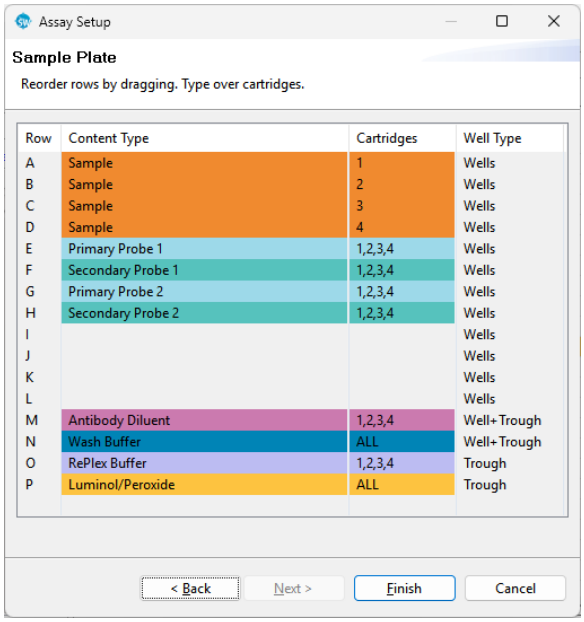

2. Define the assay parameters.
   a. Click the boxes next to the cartridge holder to indicate which holders will be loaded with a cartridge.
   b. Select the number of samples, primary antibody, and secondary antibody rows pipetted on the Sample Plate using the drop-down list. The example below is for a Chemiluminescence Immunoassay + Chemiluminescence Immunoassay using RePlex assay.



**NOTE:** An alert will appear if you try to specify a number of reagent rows that exceeds the number of rows available on the Sample Plate.

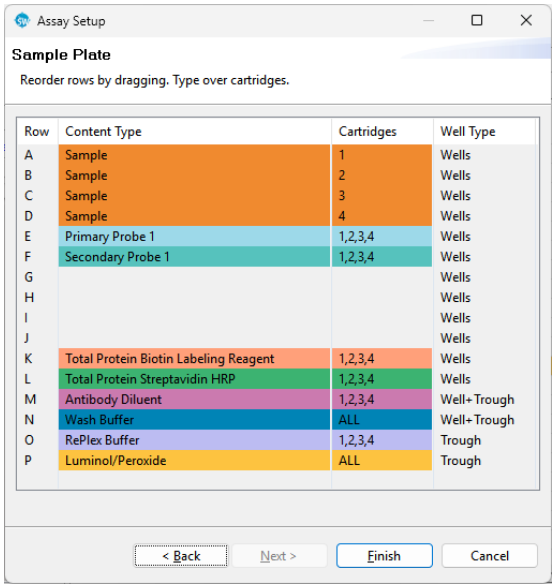
3. Click **Next**.
4. An 'Assay Setup – Sample Plate' prompt will appear.

### CHEMILUMINESCENCE IMMUNOASSAY + CHEMILUMINESCENCE IMMUNOASSAY USING REPLEX




- **Row A–D** – Biotinylated ladder (A1, B1, C1, D1) and Samples (A2–25, B2–25, C2–25, D2–25)
- **Row E** – Primary Probe 1: Antibody Diluent (E1) and Primary antibody for Probe 1 (E2–E25)
- **Row F** –Secondary Probe 1: Streptavidin-HRP (F1) and Secondary conjugate for Probe 1 (F2–F25)
- **Row G** – Primary Probe 2: Antibody Diluent (G1) and Primary antibody for Probe 2 (G2–G25)
- **Row H** –Secondary Probe 2: Antibody Diluent (H1) and Secondary conjugate for Probe 2 (H2–H25)
- **Row M** – Antibody Diluent
- **Row N** – Wash Buffer
- **Row O** – RePlex reagent mix
- **Row P** – Luminol/Peroxide mix

### CHEMILUMINESCENCE IMMUNOASSAY + TOTAL PROTEIN NORMALIZATION USING REPLEX AND CHEMILUMINESCENCE DETECTION



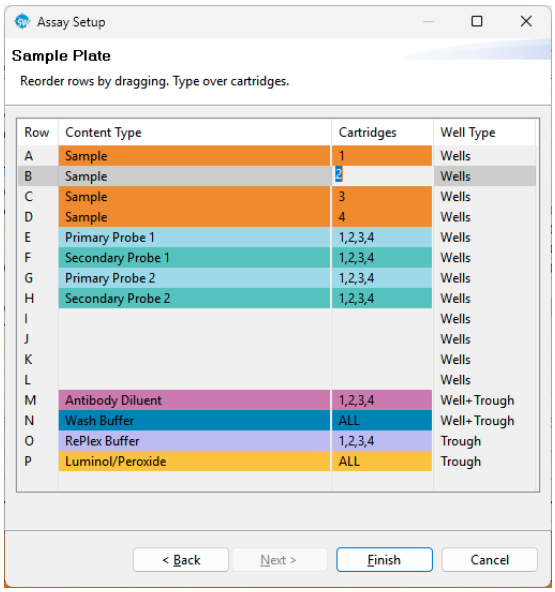

- **Row A–D** – Biotinylated ladder (A1, B1, C1, D1) and Samples (A2–25, B2–25, C2–25, D2–25)
- **Row E** – Primary Probe 1: Antibody Diluent (E1) and Primary antibody for Probe 1 (E2–E25)
- **Row F** – Secondary Probe 1: Streptavidin-HRP (F1) and Secondary conjugate for Probe 1 (F2–F25)
- **Row K** – Antibody Diluent (K1) and Total Protein Biotin Labeling Reagent (K2–K25)
- **Row L** – Total Protein Streptavidin–HRP for Probe 2 (L1–L25)
- **Row M** – Antibody Diluent
- **Row N** – Wash Buffer
- **Row O** – RePlex reagent mix
- **Row P** – Luminol/Peroxide mix

5. The default protocol automatically assigns all reagent locations for the assay. Regent assignments to rows M–P cannot be modified.

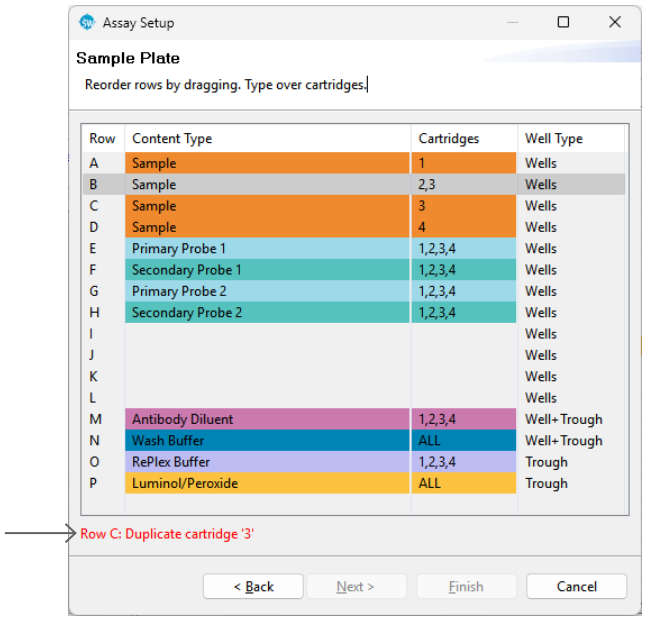
   **To modify the reagents assigned to rows A-L:**

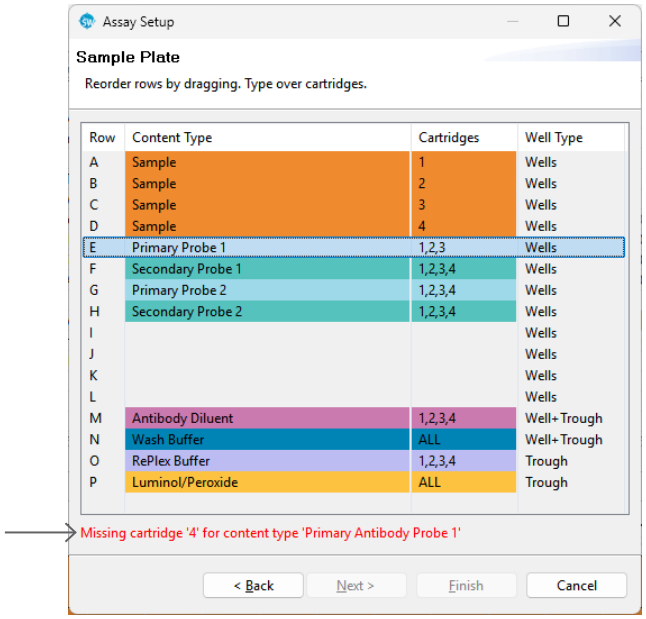
   a. Reorder row locations by clicking on the row and dragging it to the correct location.

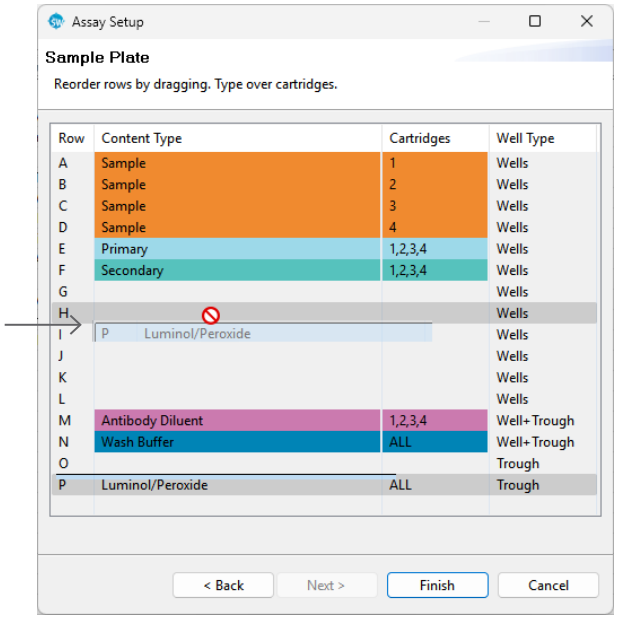
   b. Change the row a cartridge will load a reagent from by clicking on the cell in the Cartridges column and typing the cartridge number.



c. An alert will appear at the bottom of the prompt if too many rows are assigned to one cartridge or if a cartridge is missing a reagent assignment.



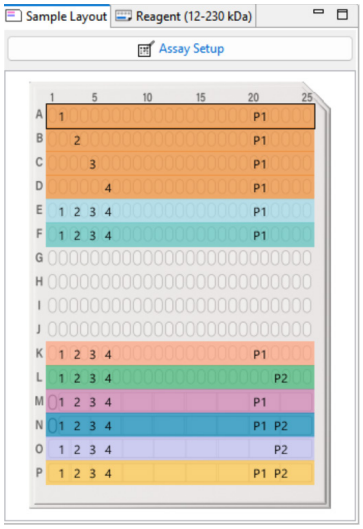





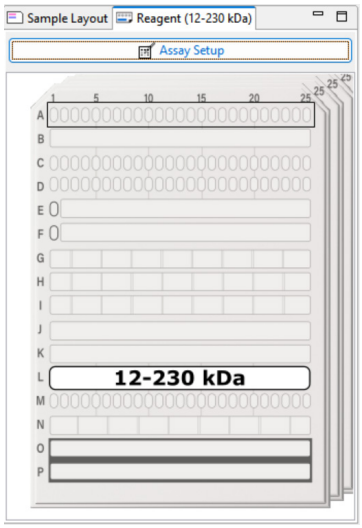

d. To add or remove a row, click the **Back** button and adjust the number of rows using the drop-down lists.

6. Click **Finish**. You can also adjust the row a cartridge will load from later in the Protocol pane.

7. A Sample Plate with updated reagent locations will display in the Sample Layout tab of the Assay screen. Numbers on the Sample Plate indicate the row each cartridge will load reagents from. Reagents used in Probe 1 are labeled ‘P1’ on the plate, those used in Probe 2 are labeled ‘P2’.



The Reagent tab will display the size range selected and the number of Pre-filled Reagent Plates required to perform your run.

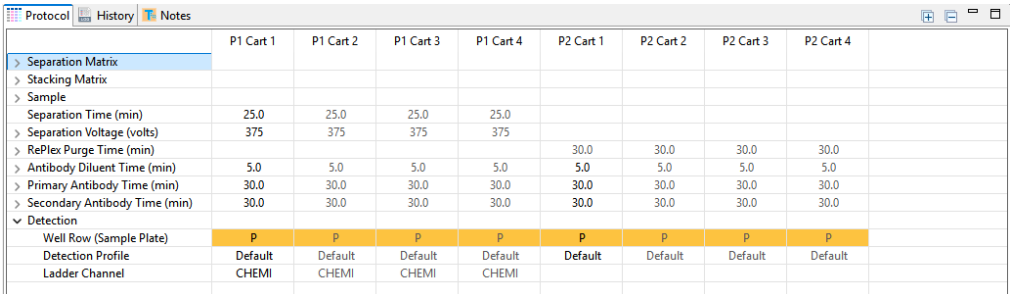



## Step 3: Modifying the Assay Protocol (Optional)

The capillary cartridges are all run in parallel, so voltage- and time-based parameters for all the cartridges must be the same. Changing the parameter for cartridge 1 will change the parameter for all other cartridges in the protocol. Grayed-out parameters or reagent locations cannot be changed.

**To modify the protocol:**

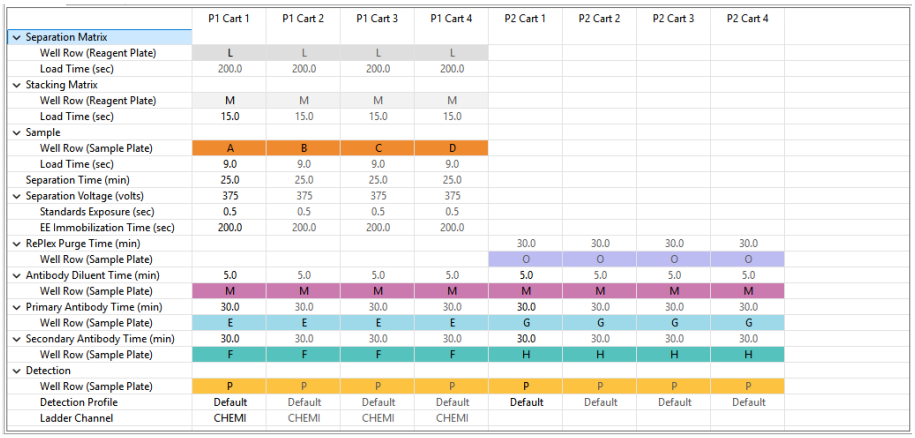
1. Click on the **Protocol** tab. This pane shows the individual steps of the assay protocol and allows you to change some assay parameters. The example below is for a 2 Chemiluminescence Immunoassay with RePlex assay.




| | P1 Cart 1 | P1 Cart 2 | P1 Cart 3 | P1 Cart 4 | P2 Cart 1 | P2 Cart 2 | P2 Cart 3 | P2 Cart 4 |
|---|---|---|---|---|---|---|---|---|
| Separation Matrix | | | | | | | | |
| Stacking Matrix | | | | | | | | |
| Sample | | | | | | | | |
| Separation Time (min) | 25.0 | 25.0 | 25.0 | 25.0 | | | | |
| Separation Voltage (volts) | 375 | 375 | 375 | 375 | | | | |
| RePlex Purge Time (min) | | | | | 30.0 | 30.0 | 30.0 | 30.0 |
| Antibody Diluent Time (min) | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| 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 (Sample Plate) | P | P | P | P | P | P | P | P |
| Detection Profile | Default | Default | Default | Default | Default | Default | Default | Default |
| Ladder Channel | CHEMI | CHEMI | CHEMI | CHEMI | | | | |

Each row contains an assay protocol step. Each step contains a Pre-filled Reagent Plate row assignment (gray) or Sample Plate row assignment (color-coded) and one or more parameter settings. To view the details for a step, click on the gray arrow next to the step name. We recommend using default protocol settings for the assay.

An expanded list of the default protocol step parameters is shown below:



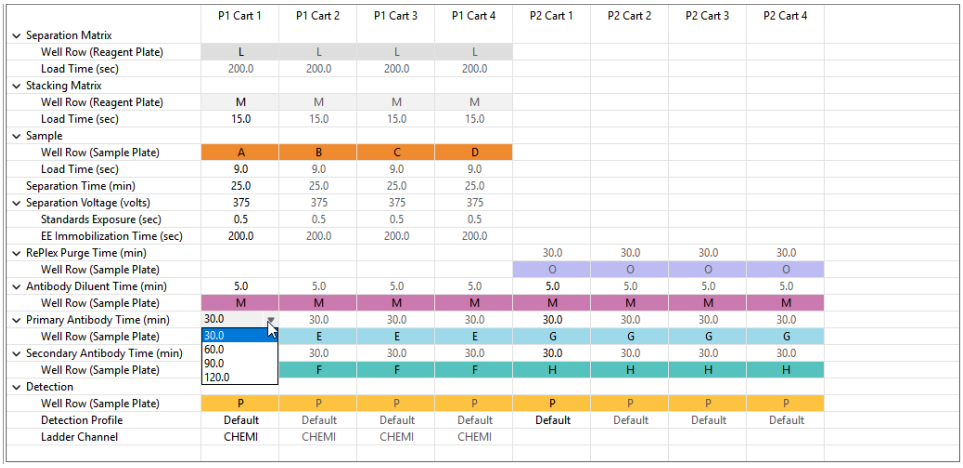
| | P1 Cart 1 | P1 Cart 2 | P1 Cart 3 | P1 Cart 4 | P2 Cart 1 | P2 Cart 2 | P2 Cart 3 | P2 Cart 4 |
|---|---|---|---|---|---|---|---|---|
| Separation Matrix | | | | | | | | |
| Well Row (Reagent Plate) | L | L | L | L | | | | |
| Load Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 | | | | |
| Stacking Matrix | | | | | | | | |
| Well Row (Reagent Plate) | M | M | M | M | | | | |
| Load Time (sec) | 15.0 | 15.0 | 15.0 | 15.0 | | | | |
| Sample | | | | | | | | |
| Well Row (Sample Plate) | A | B | C | D | | | | |
| Load Time (sec) | 9.0 | 9.0 | 9.0 | 9.0 | | | | |
| Separation Time (min) | 25.0 | 25.0 | 25.0 | 25.0 | | | | |
| Separation Voltage (volts) | 375 | 375 | 375 | 375 | | | | |
| Standards Exposure (sec) | 0.5 | 0.5 | 0.5 | 0.5 | | | | |
| EE Immobilization Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 | | | | |
| RePlex Purge Time (min) | | | | | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | | | | | O | O | O | O |
| Antibody Diluent Time (min) | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Well Row (Sample Plate) | M | M | M | M | M | M | M | M |
| Primary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | E | E | E | E | G | G | G | G |
| Secondary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | F | F | F | F | H | H | H | H |
| Detection | | | | | | | | |
| Well Row (Sample Plate) | P | P | P | P | P | P | P | P |
| Detection Profile | Default | Default | Default | Default | Default | Default | Default | Default |
| Ladder Channel | CHEMI | CHEMI | CHEMI | CHEMI | | | | |

**NOTE:** To expand all parameters, click the ⊞ button at the upper right of the pane. To collapse all parameters, click the ⊟ button at the upper right of the pane.

2. You can change the primary or secondary antibody incubation time. The examples shown below are for a 2 Chemiluminescence Immunoassay with RePlex assay.

   **To change the primary antibody incubation time:** Click the arrow in the cell next to Primary Antibody Time (min) in the 'P1 Cart 1' column for Probe 1 and 'P2 Cart 1' column for Probe 2. Select a new incubation time from the drop-down list. The values for other cartridges in that Probe will automatically update.

**NOTE:** The column header will change depending on the position of the first cartridge loaded in Leo. For example, if two cartridges are loaded in position 2 and 3, the first column header will be 'P1 Cart 2'.




| | P1 Cart 1 | P1 Cart 2 | P1 Cart 3 | P1 Cart 4 | P2 Cart 1 | P2 Cart 2 | P2 Cart 3 | P2 Cart 4 |
|---|---|---|---|---|---|---|---|---|
| Separation Matrix | | | | | | | | |
| Well Row (Reagent Plate) | L | L | L | L | | | | |
| Load Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 | | | | |
| Stacking Matrix | | | | | | | | |
| Well Row (Reagent Plate) | M | M | M | M | | | | |
| Load Time (sec) | 15.0 | 15.0 | 15.0 | 15.0 | | | | |
| Sample | | | | | | | | |
| Well Row (Sample Plate) | A | B | C | D | | | | |
| Load Time (sec) | 9.0 | 9.0 | 9.0 | 9.0 | | | | |
| Separation Time (min) | 25.0 | 25.0 | 25.0 | 25.0 | | | | |
| Separation Voltage (volts) | 375 | 375 | 375 | 375 | | | | |
| Standards Exposure (sec) | 0.5 | 0.5 | 0.5 | 0.5 | | | | |
| EE Immobilization Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 | | | | |
| RePlex Purge Time (min) | | | | | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | | | | | O | O | O | O |
| Antibody Diluent Time (min) | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Well Row (Sample Plate) | M | M | M | M | M | M | M | M |
| Primary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | 30.0 | E | E | E | G | G | G | G |
| Secondary Antibody Time (min) | 60.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | 90.0 | F | F | F | H | H | H | H |
| Detection | 120.0 | | | | | | | |
| Well Row (Sample Plate) | P | P | P | P | P | P | P | P |
| Detection Profile | Default | Default | Default | Default | Default | Default | Default | Default |
| Ladder Channel | CHEMI | CHEMI | CHEMI | CHEMI | | | | |

**To change the secondary antibody incubation time:** Click the cell next to Secondary Antibody Time (min) in the 'P1 Cart 1' column for Probe 1 or the 'P2 Cart 1' column for Probe 2 and enter a new value in minutes. The values for the other cartridges in the Probe will automatically update.

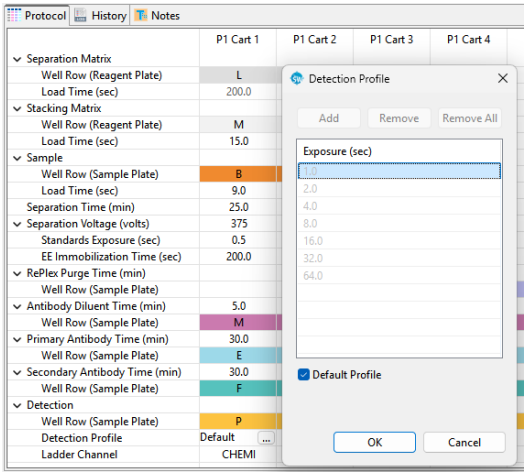
| | P1 Cart 1 | P1 Cart 2 | P1 Cart 3 | P1 Cart 4 | P2 Cart 1 | P2 Cart 2 | P2 Cart 3 | P2 Cart 4 |
|---|---|---|---|---|---|---|---|---|
| Separation Matrix | | | | | | | | |
| Well Row (Reagent Plate) | L | L | L | L | | | | |
| Load Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 | | | | |
| Stacking Matrix | | | | | | | | |
| Well Row (Reagent Plate) | M | M | M | M | | | | |
| Load Time (sec) | 15.0 | 15.0 | 15.0 | 15.0 | | | | |
| Sample | | | | | | | | |
| Well Row (Sample Plate) | A | B | C | D | | | | |
| Load Time (sec) | 9.0 | 9.0 | 9.0 | 9.0 | | | | |
| Separation Time (min) | 25.0 | 25.0 | 25.0 | 25.0 | | | | |
| Separation Voltage (volts) | 375 | 375 | 375 | 375 | | | | |
| Standards Exposure (sec) | 0.5 | 0.5 | 0.5 | 0.5 | | | | |
| EE Immobilization Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 | | | | |
| RePlex Purge Time (min) | | | | | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | | | | | O | O | O | O |
| Antibody Diluent Time (min) | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Well Row (Sample Plate) | M | M | M | M | M | M | M | M |
| Primary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | E | E | E | E | G | G | G | G |
| Secondary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | F | F | F | F | H | H | H | H |
| Detection | | | | | | | | |
| Well Row (Sample Plate) | P | P | P | P | P | P | P | P |
| Detection Profile | Default | Default | Default | Default | Default | Default | Default | Default |
| Ladder Channel | CHEMI | CHEMI | CHEMI | CHEMI | | | | |

3. You can change the location of Sample Plate reagents in rows A–L. Click the cell in the column next to the Well Row and select a different row on the Sample Plate:

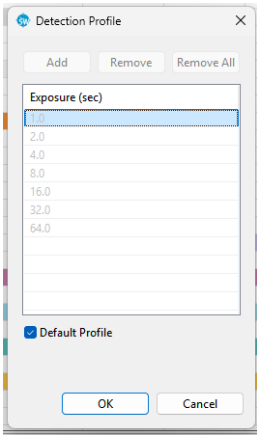
| | P1 Cart 1 | P1 Cart 2 | P1 Cart 3 | P1 Cart 4 | P2 Cart 1 | P2 Cart 2 | P2 Cart 3 | P2 Cart 4 |
|---|---|---|---|---|---|---|---|---|
| Separation Matrix | | | | | | | | |
| Well Row (Reagent Plate) | L | L | L | L | | | | |
| Load Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 | | | | |
| Stacking Matrix | | | | | | | | |
| Well Row (Reagent Plate) | M | M | M | M | | | | |
| Load Time (sec) | 15.0 | 15.0 | 15.0 | 15.0 | | | | |
| Sample | | | | | | | | |
| Well Row (Sample Plate) | A | B | C | D | | | | |
| Load Time (sec) | A | 9.0 | 9.0 | 9.0 | | | | |
| Separation Time (min) | B | 25.0 | 25.0 | 25.0 | | | | |
| Separation Voltage (volts) | C | 375 | 375 | 375 | | | | |
| Standards Exposure (sec) | D | 0.5 | 0.5 | 0.5 | | | | |
| EE Immobilization Time (sec) | 200.0 | 200.0 | 200.0 | 200.0 | | | | |
| RePlex Purge Time (min) | | | | | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | | | | | O | O | O | O |
| Antibody Diluent Time (min) | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Well Row (Sample Plate) | M | M | M | M | M | M | M | M |
| Primary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | E | E | E | E | G | G | G | G |
| Secondary Antibody Time (min) | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| Well Row (Sample Plate) | F | F | F | F | H | H | H | H |
| Detection | | | | | | | | |
| Well Row (Sample Plate) | P | P | P | P | P | P | P | P |
| Detection Profile | Default | Default | Default | Default | Default | Default | Default | Default |
| Ladder Channel | CHEMI | CHEMI | CHEMI | CHEMI | | | | |

**NOTE:** Only rows you've designated in the Assay Setup as the appropriate content type will appear in the drop-down list.

4. The default Leo protocol uses a default detection profile. Exposures in the assay can be adjusted if desired. To do this, click the gray arrow next to Detection to expand the row. Click the Default cell in the column next to Detection Profile and click the **...** button that appears to open the Detection Profile window.




Deselect the **Default Profile** checkbox. To remove exposure times, click the **Remove** button. To add additional exposure times to the protocol, click the **Add** button and enter the values. Select **OK** when you are done.




**NOTES:**
A total of seven exposures are allowed, but no more than one exposure can be longer than 32 seconds.

The Default Detection Profile in RePlex assays for Leo is the same as the RePlex Dynamic Range (RDR) detection profile used for Jess and Abby assays. See “RePlex Dynamic Range Detection Profile (Jess and Abby) and Default Detection Profile for RePlex assays (Leo)” on page 102 for more information.

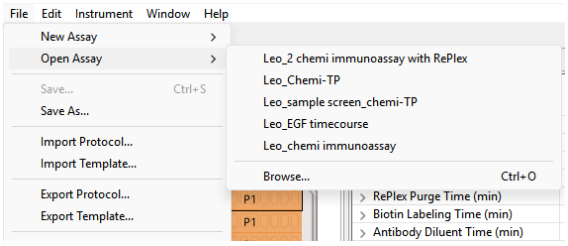
For more information on changing protocol step parameters other than incubation times contact ProteinSimple Technical Support at **support@bio-techne.com** (US/Canada) or **Instrument.Support.EMEA@bio-techne.com** (Europe). You can also contact your local Field Application Scientist for help.

## Steps 4–8

Steps 4 through 8 for creating an RePlex assay on Leo are the same as when you're creating an Immunoassay on Jess or Abby. Please go to "Step 4 - Add Assay Notes (Optional)" on page 51 to continue.

# Making Changes to an Existing Assay

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



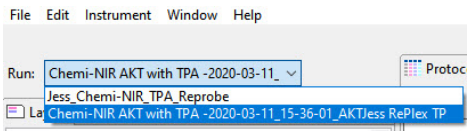

3. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the assay folder and select a different assay.
4. Follow the steps for the appropriate assay to make changes and save the assay:

- "Immunoassays on Jess and Abby: Creating a New Assay" on page 41.
- "Stellar Assays on Jess: Creating a New Assay" on page 59.
- "Protein Normalization on Jess: Creating a New Assay" on page 65.
- "RePlex Assays on Jess and Abby: Creating a New Assay" on page 67.
- "Immunoassays on Leo: Creating a New Assay" on page 78.
- "RePlex Assays on Leo: Creating a New Assay" on page 87.

# 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.

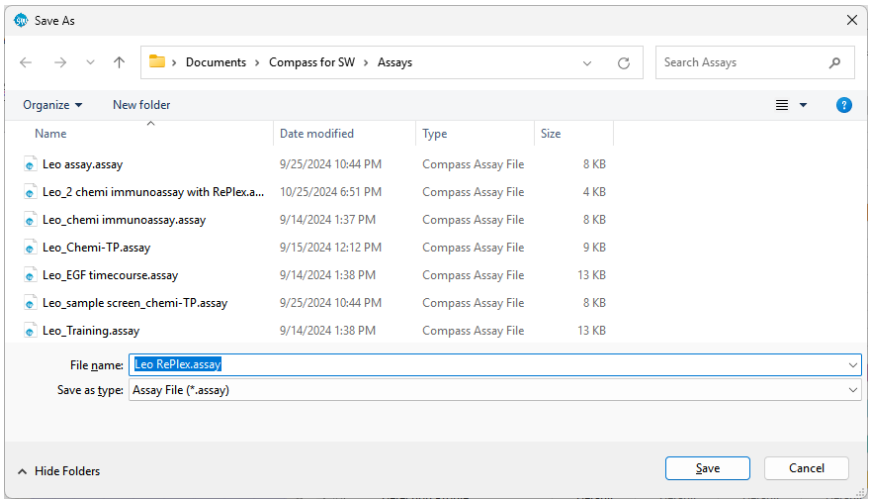
# Creating a Template Assay

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

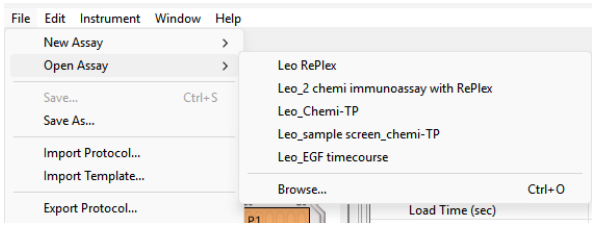
1. Select the **Assay** screen.
2. Select **File** in the main menu. Click **Open Assay** to open an existing assay or click **New Assay** to open an existing template assay.
3. Follow the steps for the appropriate assay to make changes to the assay:

- “Immunoassays on Jess and Abby: Creating a New Assay” on page 41.
- “Stellar Assays on Jess: Creating a New Assay” on page 59.
- “Protein Normalization on Jess: Creating a New Assay” on page 65.
- “RePlex Assays on Jess and Abby: Creating a New Assay” on page 67.
- “Immunoassays on Leo: Creating a New Assay” on page 78.
- “RePlex Assays on Leo: Creating a New Assay” on page 87.

4. When changes are complete, select **File** in the main menu and click **Save As**. Select the **New Assays** folder:

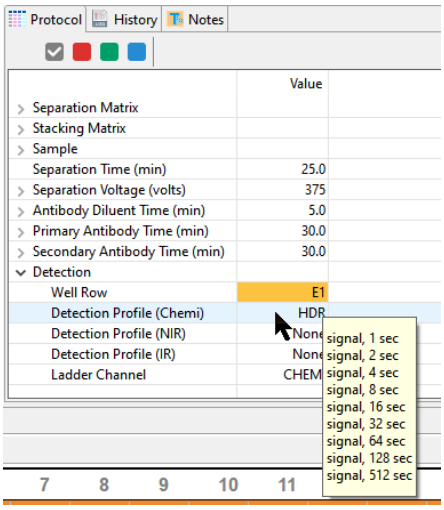



5. Type the name for the new template assay and click **Save**.
6. Select **File** in the main menu and click **Open Assay**. The new template assay will now be available in the drop-down list:



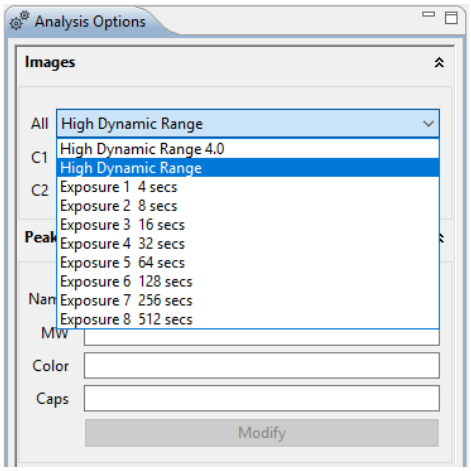
# Viewing and Changing the Detection Exposures

To view the current detection profile, roll the cursor over the exposures cell in Protocol pane. In this example, a Jess chemiluminescence + fluorescence immunoassay is shown:




## High Dynamic Range Detection Profile (Jess and Abby) and Default Detection Profile (Leo)

Starting with Compass for Simple Western 4.0 and the Jess instrument, HDR (high dynamic range) is the default detection profile for chemiluminescent detection, and with Compass for Simple Western 6.1 and higher, HDR is the default detection profile for Stellar fluorescence detection (Jess only). In Compass for Simple Western 7.0, the Default detection profile for the Leo system is similar to the chemiluminescence HDR exposure series used for Jess and Abby assays, but removes the 512 second exposure. Exposure times for HDR have been optimized for best performance and can't be edited. These exposures were selected for maximum performance for each instrument when HDR mode is selected. Below is an example of different exposure settings:

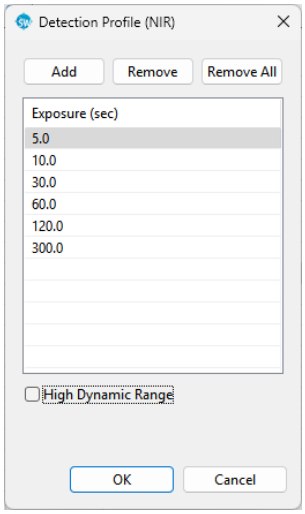


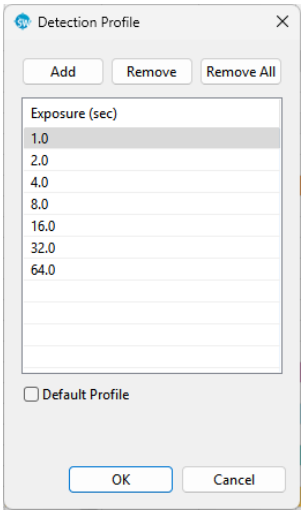
To achieve a broader detection dynamic range, you need Compass for Simple Western version 4.0 or higher. To download the latest version of the software, go to **Help** > **Check for Updates**.

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 (chemiluminescence only) and minimizing the time between substrate aspiration and imaging (chemiluminescence only).

**NOTE:** See “Images Analysis Settings” on page 287 to find out more about how data sets are analyzed under HDR and standard detection profiles.

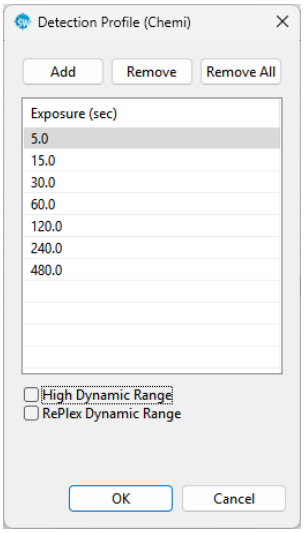
To use a detection profile used in a previous run or if you prefer not to use HDR (Jess and Abby) or Default (Leo) detection profiles, uncheck the box next to **High Dynamic Range** or **Default Profile**, respectively, in the Detection Profile window. Edit the exposure times as desired.



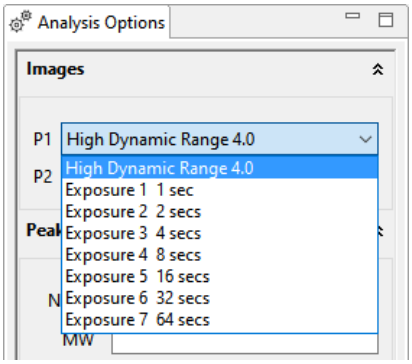

**NOTE:** Only two exposures greater than 32 seconds can be added to a Leo chemiluminescence assay Detection Profile.

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



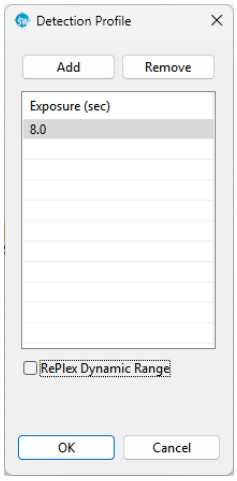

## RePlex Dynamic Range Detection Profile (Jess and Abby) and Default Detection Profile for RePlex assays (Leo)

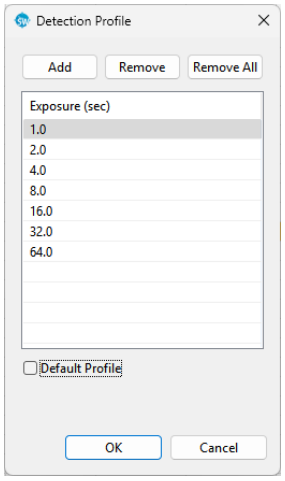
For RePlex assays that use chemiluminescent detection, RDR (RePlex dynamic range) is the default detection for Jess and Abby. The Default profile on Leo is the same as the RDR exposure series used for Jess and Abby RePlex assays. Exposure times for these detection profiles use the short exposure times from HDR, eliminating the 128 and 512 second exposures. Below is an example of the RDR exposure settings:




**NOTE:** See “Images Analysis Settings” on page 287 to find out more about how data sets are analyzed under HDR and standard detection profiles.

If you prefer not use the RDR (Jess/Abby) or Default (Leo) detection profile, uncheck the box next to **RePlex Dynamic Range** or **Default Profile**, respectively, in the Detection Profile window and edit the exposure times as desired. Up to 7 exposures may be added to the RePlex assay Detection Profile. For more information on RDR, please refer to Simple Western assay optimization guidelines.



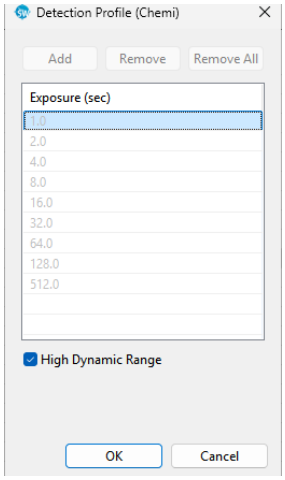



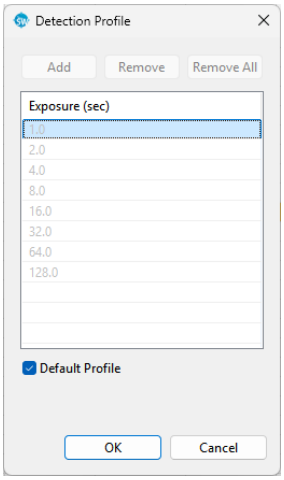

**NOTE:** Only one exposure greater than 32 seconds can be added to a Leo RePlex assay Detection Profile for chemiluminescence.

## Changing the Detection Profile

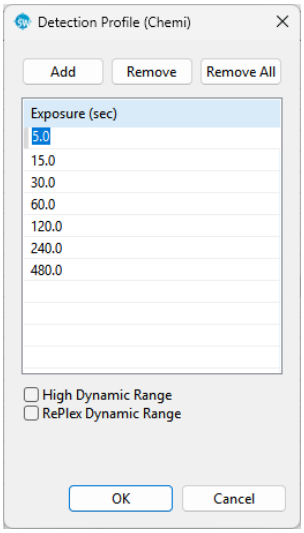
While we recommend using the default detection profile, you can change the profile, exposure times and exposure sequences if needed. To do this:

1. To open a Detection Profile, select the exposures cell in the Protocol pane and click the **...** button. Examples of the Detection Profile screen for chemiluminescence are shown below:

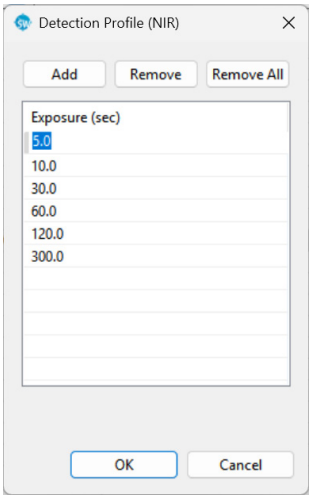


a. **To change an existing chemiluminescence exposure time** - Uncheck High Dynamic Range or RePlex Dynamic Range (Jess and Abby) or Default Profile (Leo) if checked. Then click in the exposure cell and enter a new time in seconds. An example from Jess is shown below:




b. **To change an existing fluorescence exposure time** - Click in the exposure cell in the Detection Profile window and enter a new time in seconds. An example from Jess is shown below:




c. **To delete an existing exposure** - Select an exposure cell and click **Remove**. For runs performed on Jess with fluorescence detection, you can delete all exposures if the ladder was not run in that channel or if it is not the only channel used in the probe by selecting **Remove All**.

d. **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 names and attributes 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

You can copy cells or an entire row from your assay template to paste into another document.

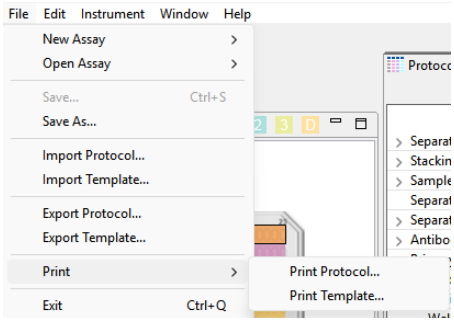
1. Click on the **Template** tab.
2. Select the cell(s) or row.
3. Select **Edit** in the main menu and click **Copy**.
4. Open a document (Microsoft® Word®, Excel®, etc.). Right-click in the document and select **Paste**. The assay template will be copied into the document as an image, exactly as it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

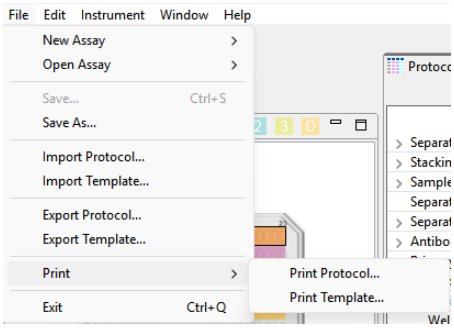
1. Select **File** in the main menu, click **Print**, and then click **Print Protocol**.




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. 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 name and attribute information. This allows the same assay protocol and template information to be imported into another assay later, 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.

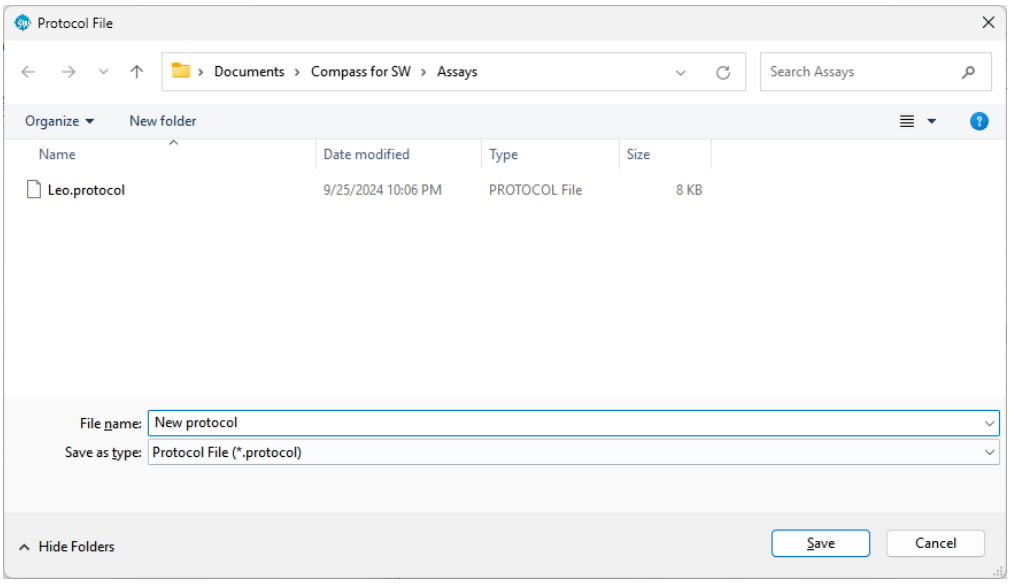
1. Open the assay you want to import the assay protocol in to.
2. Select **File** in the main menu and click **Import Protocol**.
3. Select a protocol file (*.protocol) and click **OK**. The imported information will display in the Protocol pane.

## 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.

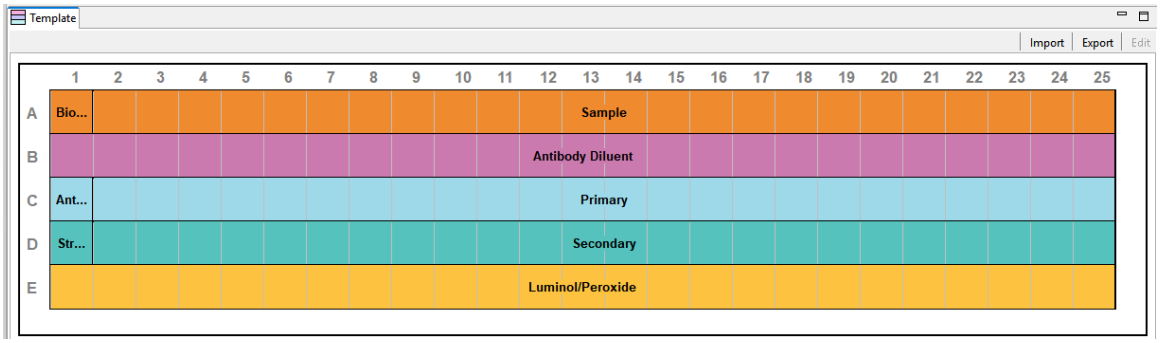
2. Select **File** in the main menu and click **Export Protocol**. The following window displays:




3. The default directory is Compass for SW/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.



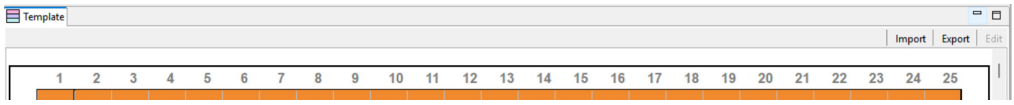

There are two file format options:

- A comma separated CSV file that is best opened in a spreadsheet.
- A tab delimited TXT file that can be opened in a spreadsheet even when the decimal separator is set to a comma.

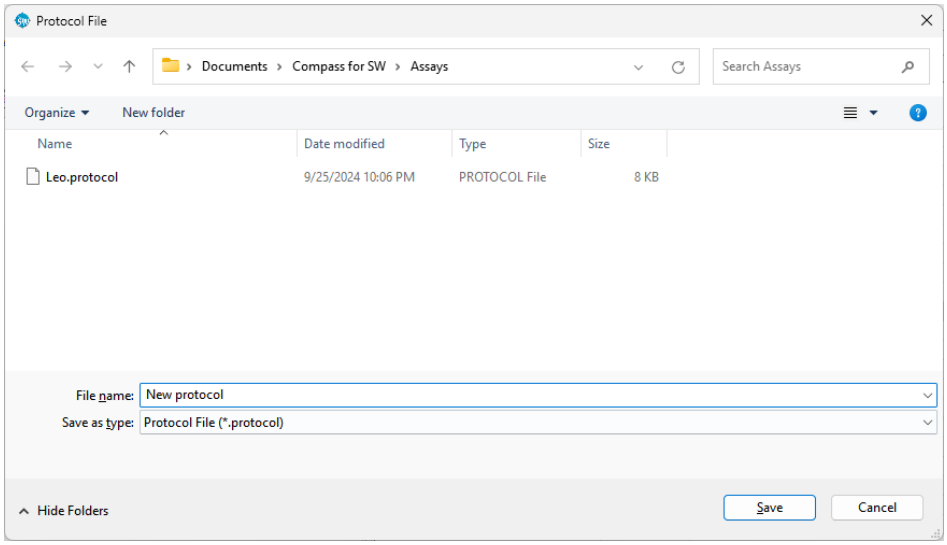
## Exporting then Importing the Template to a CSV file

Follow the steps below to easily export a template from Compass for Simple Western, populating it with reagent names (sample, antibody, etc.) and their corresponding attributes (concentration, dilution factor, etc.).

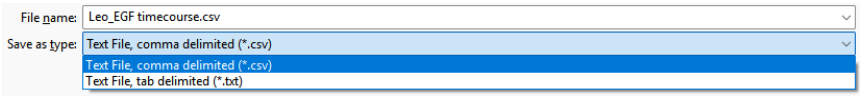
1. Select **File** in the main menu and click **Export Template**. You can also select the **Export** button in the top right corner of the Template (Jess and Abby) or Sample Template (Leo) pane.




2. The following window displays:




It is recommended to set the 'Save as type' to CSV.




3. Enter a template name and click **Save**. The template will be saved as a *.csv file.

4. 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 |

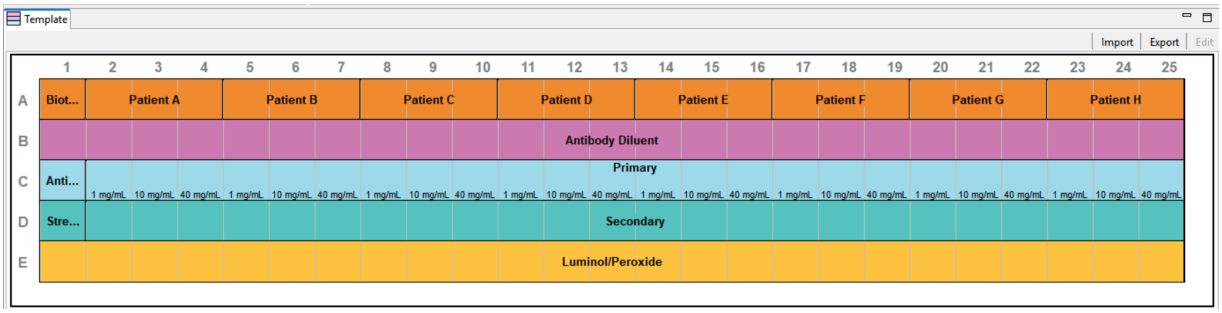
The names in the spreadsheet are arranged in the same order as the Compass Template, and the rows alternate between names and attributes.

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

5. 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.

6. To import the edited CSV file into Compass for Simple Western, select **File** in the main menu and click **Import Template**. You can also select the **Import** button in top right corner of the Template (Jess and Abby) or Sample Template (Leo) pane. Browse to the .csv file you just saved. Once imported, the edited CSV file displays the edited Sample names and Primary Antibody with attributes in the Template.



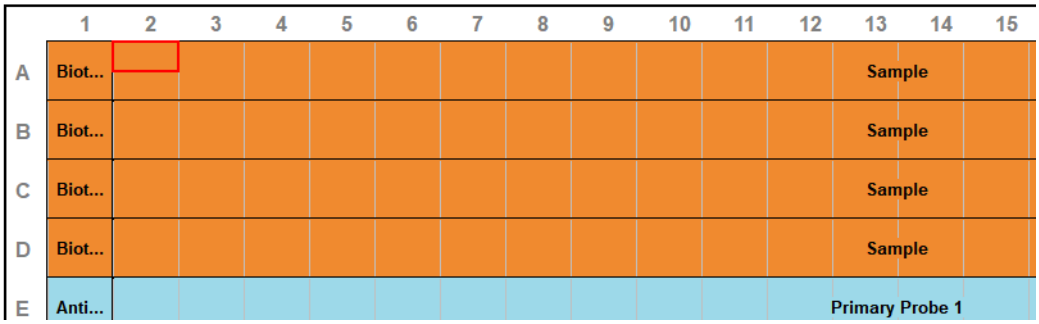

## Template Cut and Paste

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®.

Enter names in a spreadsheet row, then copy to the clipboard. Next, select the top of a well in the Template and paste a name from the clipboard or select the bottom of a well and past an attribute 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:

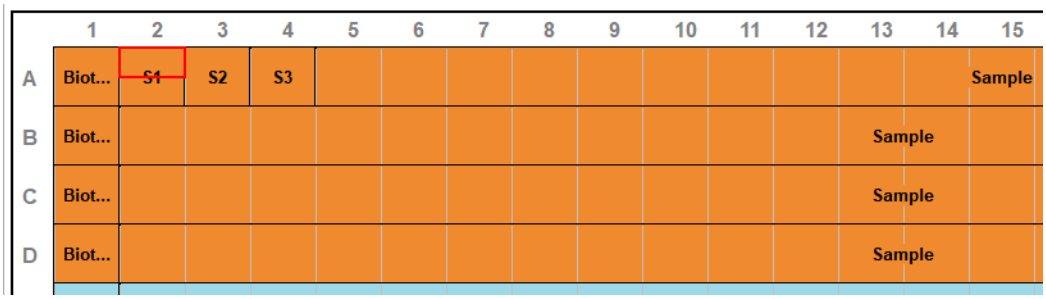
1. Copy these three rows from a spreadsheet:

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

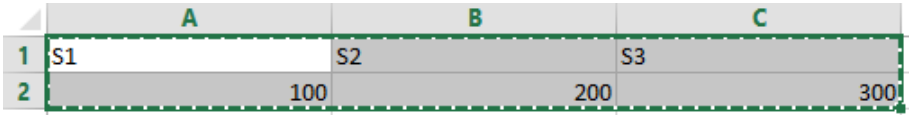



3. Paste the names from the clipboard into the first row.

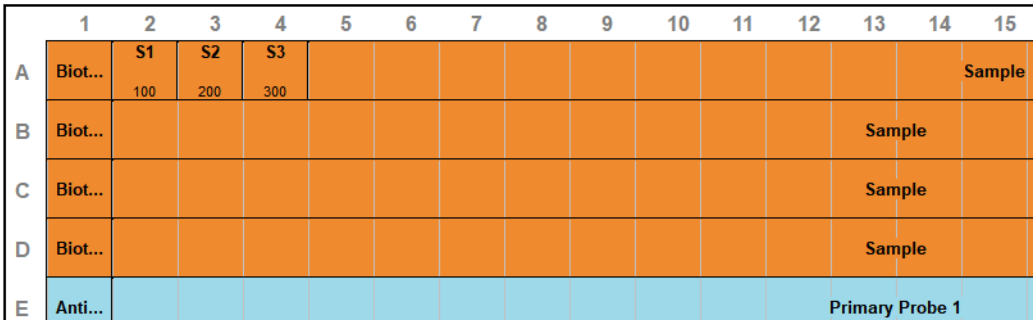
**NOTE:** Make sure not to select any of the ladder wells (A1, B1, C1, etc.)




4. Paste the attributes from the clipboard into the second row:



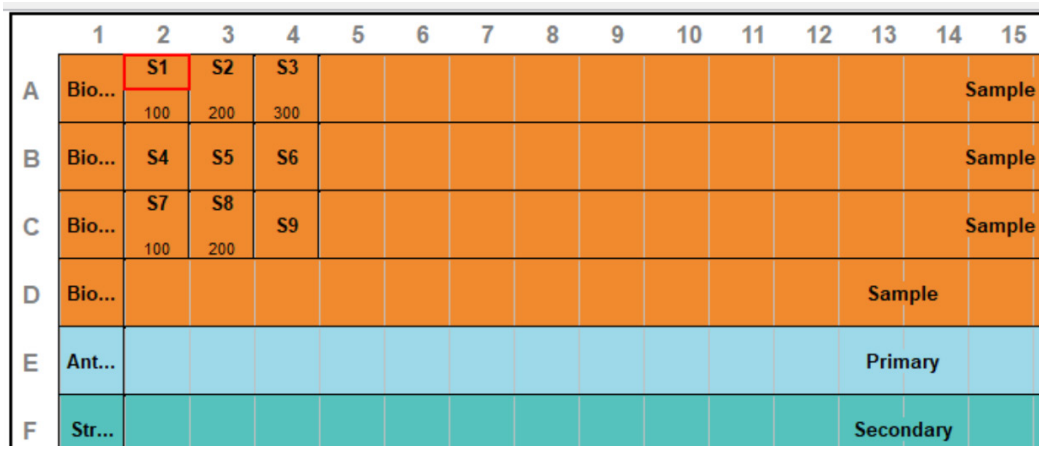

5. Pasting into the Template will update the names and attributes.



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 | S4 | S5 | S6 |
| 4 | | | |
| 5 | S7 | S8 | S9 |
| 6 | 100 | 200 | |

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

**NOTE:** If you are using a Wes, Peggy Sue, Sally Sue, or NanoPro 1000, Compass for Simple Western version 7.0 and higher does not support these instruments. You can still use software versions 5.x and lower for Peggy Sue, Sally Sue, or NanoPro 1000 and software versions 6.x and lower for Wes to create, load, and run assays on these systems, and to analyze data. Lower versions of Compass can be downloaded at the I**nstrument Software Download Center** on the Bio-Techne website.

## Step 1 - Get Ready

1. Turn your system on and allow it to remain on for at least 1.5 hours before starting a run to ensure the instrument reaches its temperature setpoint.
2. Open Compass for Simple Western Software.
3. Create or open an assay file in Compass for Simple Western.
4. Prepare the assay plate for Jess and Abby using the information provided in the Simple Western kit product insert. Prepare the Sample Plate for Leo using the information provided in the Leo Quick Start Guide.

**IMPORTANT**
If a row on the assay plate contains reagents in some wells but not in others, pipette DI water or the diluent used for other reagents in the row into the empty wells of that row. This prevents air aspiration and bubble formation in the capillary cartridge. To prevent well evaporation and ensure best results, keep a lid on the assay plate until ready to use.

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

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

6. For Leo only – place the Pre-filled Reagent Plate(s) into the Pre-Filled Reagent Plate holder(s) in Leo.
7. Remove the capillary cartridge(s) from the packaging and insert the into the cartridge holder(s).
8. Place Assay Plate/Sample Plate into the sample tray/sample plate holder of the instrument.

## Step 2 - Start the Run

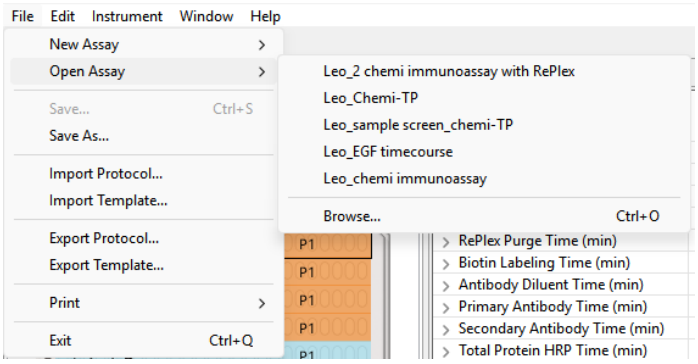
You can start a run in one of two ways depending on whether you want to run an assay using existing parameters or set up a new assay.

**NOTES:**
Start runs from the Assay screen only.

Your Leo, Jess, or Abby system must be connected to Compass for Simple Western to start a run.

1. New run of an existing assay:

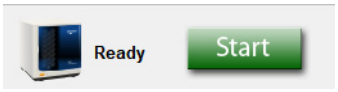
   a. In the Assay screen, select **File** in the main menu and click **Open Assay**.




   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 Leo, Jess, or Abby.

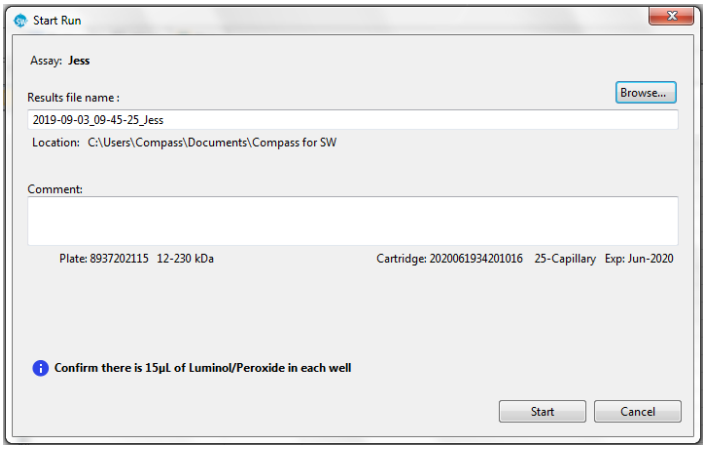
   a. The Start button will display. This indicates that an assay has been loaded.

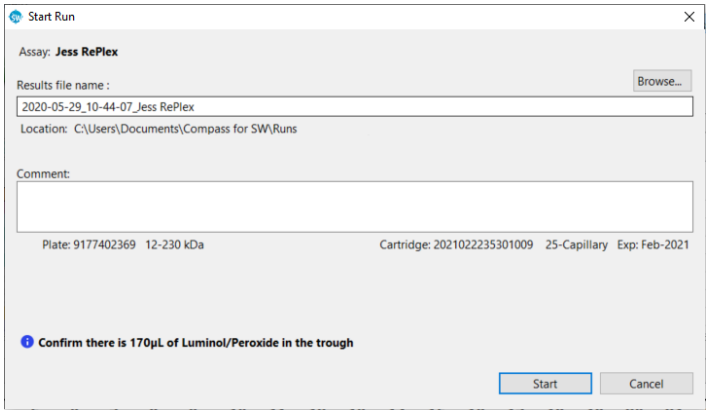


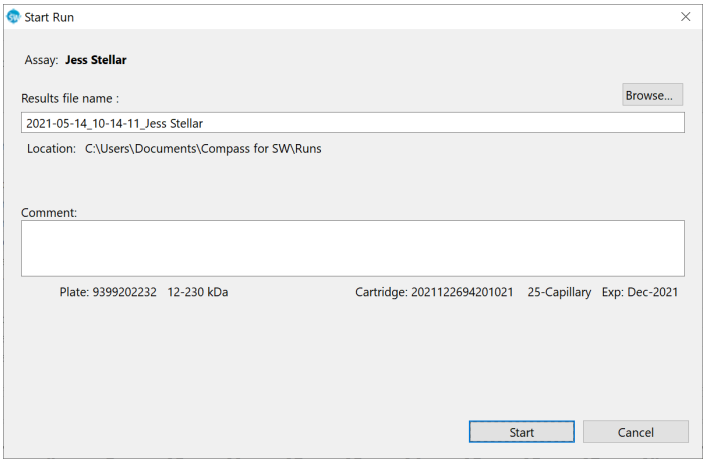
3. Click **Start** to begin the run.

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

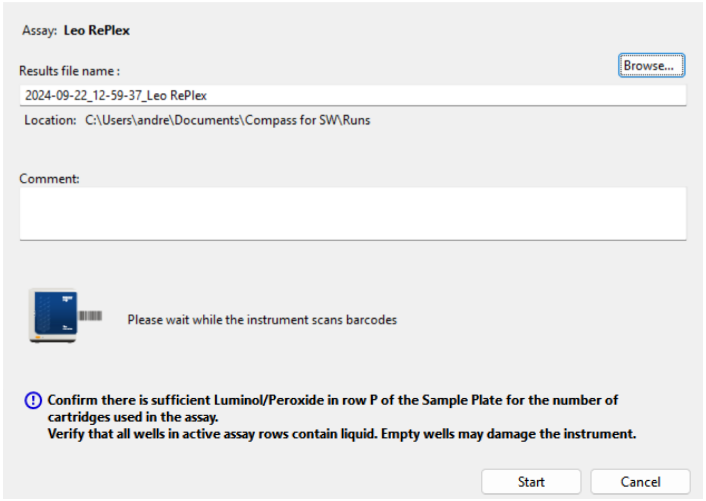
**NOTE:** Run file names should not exceed 255 characters. Only space, -, _, A-Z, a-z, 0-9 are allowed when naming run files.






4. The instrument will scan cartridge and plate barcodes after the door is closed. The instrument status indicator will blink **blue** until the instrument has initialized and completed the barcode scan:



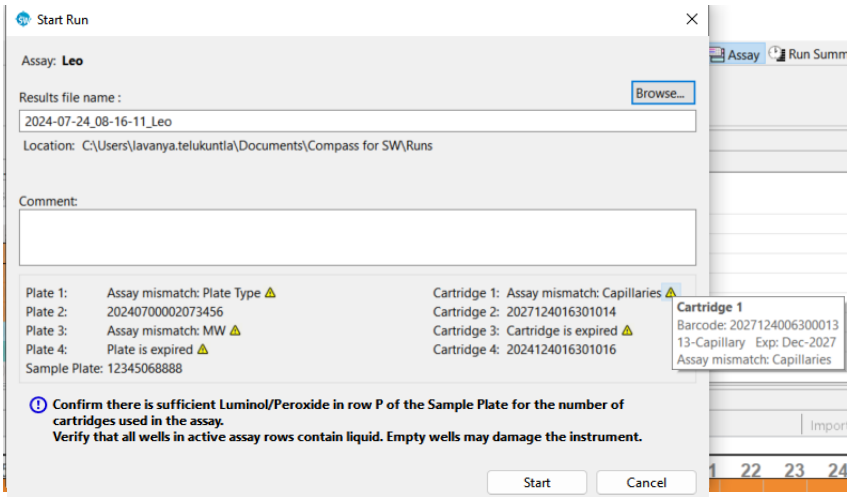

   a. For Jess and Abby: confirms that the cartridge type and plate installed matches the assay selected for the run.

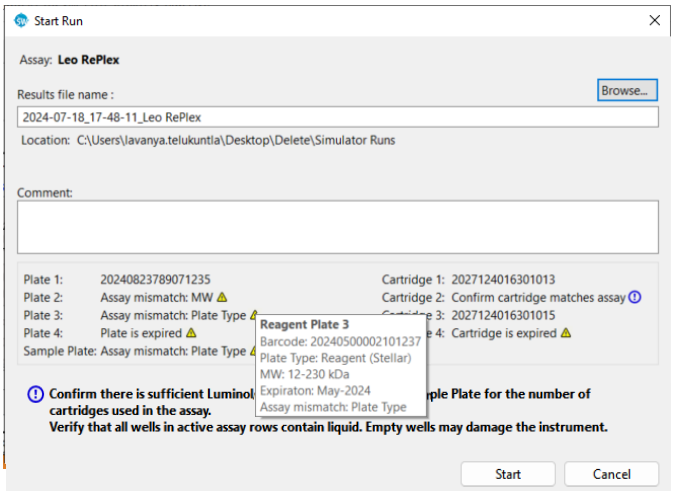
   b. For Leo: confirms that the number of cartridges and Pre-filled Reagent Plates matches the assay setup, that the Pre-filled Reagent Plates match the assay selected for the run, and that a Sample Plate has been loaded in the instrument.

   c. Check the cartridge and plate expiration dates. You can still start the run if these are expired, but performance is not guaranteed.

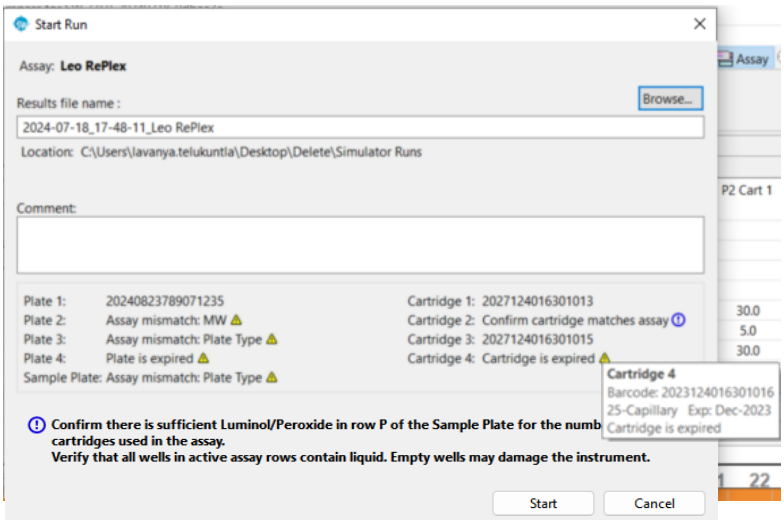
5. Scanning barcodes will initiate Start Run check.

    a. If the cartridge type or plate installed in the instrument doesn't match the assay selected for the run or the number of cartridges and Pre-filled Reagent Plates for a Leo run doesn't match the assay setup, 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.



b. If a cartridge or plate is expired or a barcode is not readable, a warning will appear. You will still be able to start the run and the warning information will be saved in the run History.




6. 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:


## Step 3 - Post-Run Procedures

1. Remove the capillary cartridge(s).
2. Remove the Jess and Abby assay plate or remove the Leo Sample Plate and Pre-filled Reagent Plate(s).
3. Dispose of the plate(s) and capillary cartridge(s). Disposal will depend on the samples that have been assayed. If sample origins are unknown, we recommend that used capillary cartridges and plates be disposed of as biohazard waste.

**WARNING!** SHARPS HAZARD
The capillaries may present a potential sharps hazard. Dispose of used capillary cartridges in biomedical waste sharps containers.

**!WARNING!** BIOHAZARD
Samples and plates 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 **https://www.cdc.gov/labs/bmbl/index.html**.

Depending on the samples used, residual plate contents may constitute a biohazard. Dispose of plates 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 plates before you store, handle or dispose of chemical waste.

# Stopping a Run

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

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(s) and plate(s) and discard them. The stopped status, date and time will display in the Run Summary screen.

Chapter 4:

# Imaging a Blot on Jess

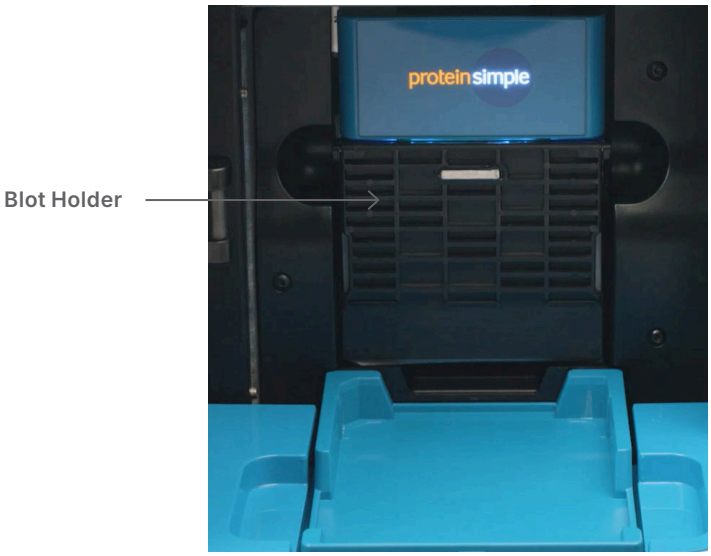
## Chapter Overview

- Starting a Run
- Stopping 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.

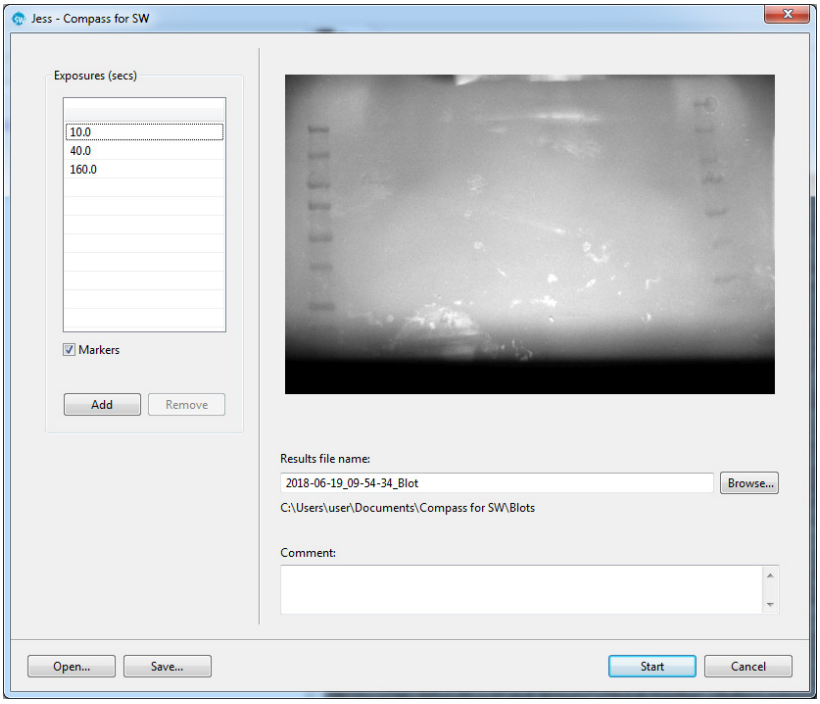



The software will automatically change to the Blot screen:


## Step 2 - Start the Imaging Run

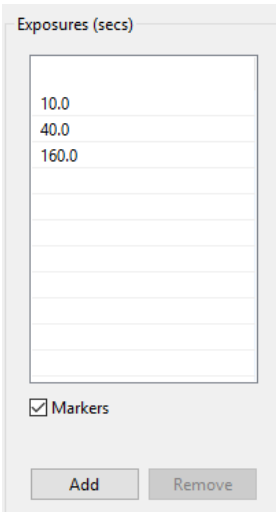
**NOTE:** The Jess system door must be closed before starting the imaging run.

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

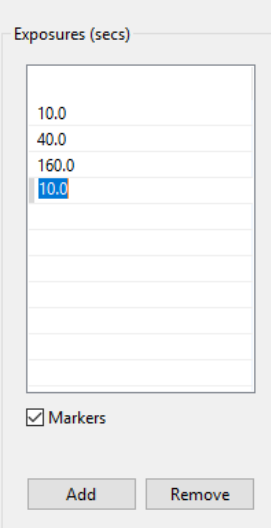



1. Edit the exposure settings in the Exposures table.

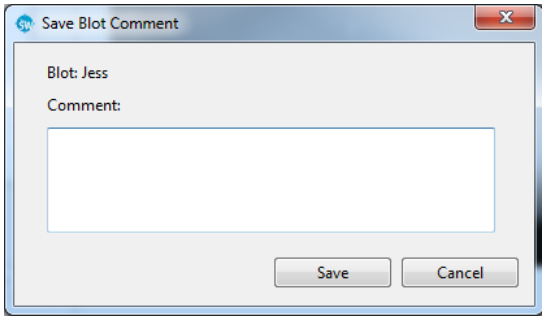
- **To add an exposure:** Click **Add** then select the exposure time to change it.
- **To remove an exposure:** Select the exposure and click **Remove.**
- **To edit exposure time:** Click on an exposure and enter a new time.



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**.

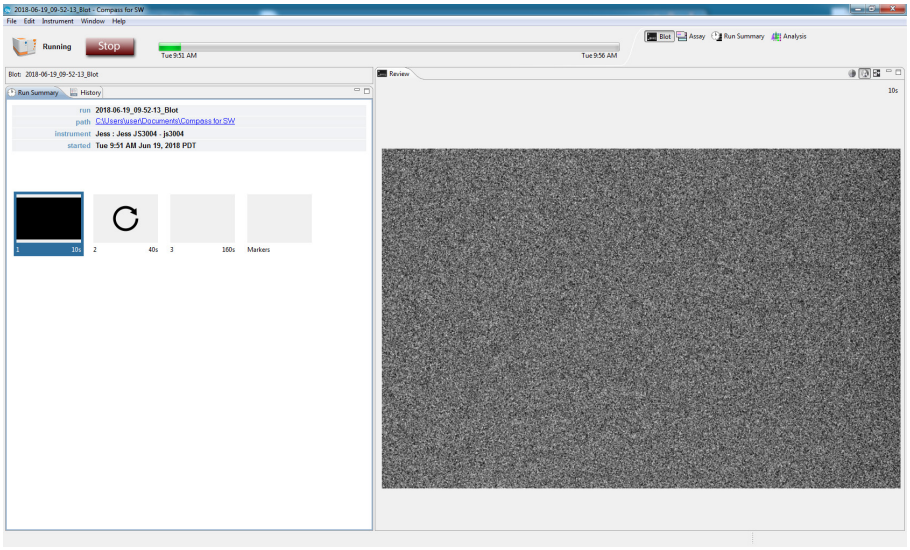



6. Optional: To save the settings associated with this blot imaging protocol for future use, click **Save**.
7. Click **Start** to begin imaging.

# Step 3 - Imaging Run Summary and Analysis

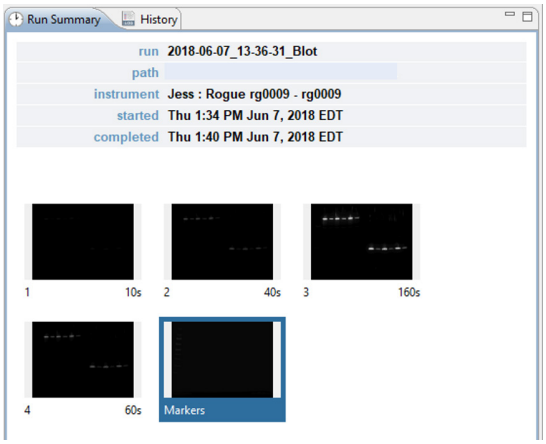
## Reviewing Imaging Data

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:

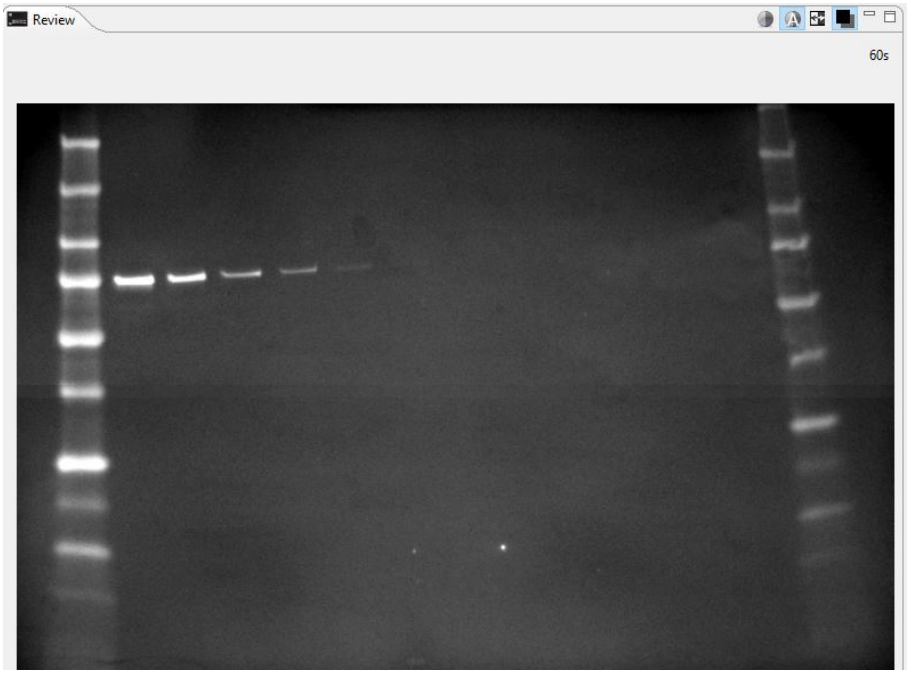
 Contrast Adjustment

 Auto Contrast

 Invert

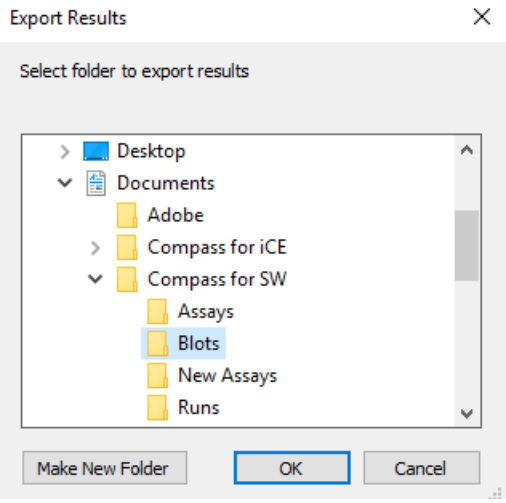
 Overlay Markers

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:

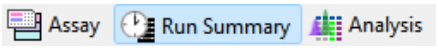
# Run Status

## Chapter Overview

- Run Summary Screen Overview
- 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:




The run in progress will automatically load when the instrument is connected to Compass for Simple Western Software.

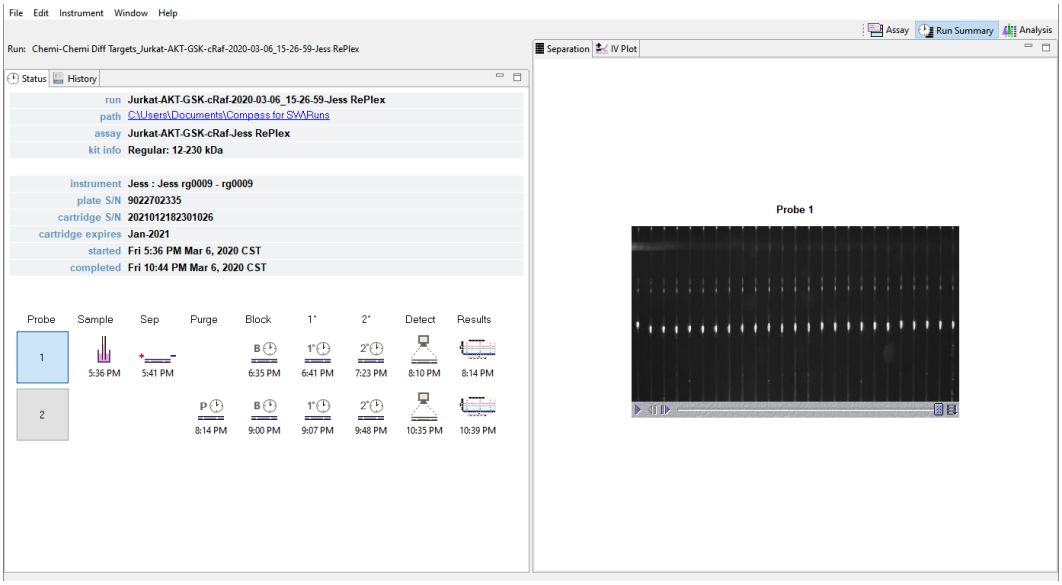
## Run Summary Screen Panes

The Run Summary screen has the following panes:

- **Status** – Displays run file information and current status of a run in progress.

> **NOTE:** If status doesn't update during a run, there may be a disconnect between the instrument and computer. If this happens and the status light on the instrument is still blinking, let the run complete first and then reconnect to the instrument.

- **Separation** – Lets you view a movie of the fluorescent standards separation for each cartridge in the experimental run.
- **IV Plots** – Lets you view plots of the total current and voltage measured during separation for all capillaries for each cartridge in the experimental run.
- **Barcodes (Leo only)** – Displays the barcodes and expiration dates for the cartridge(s), and the barcodes for the Pre-filled Reagent Plate(s) and Sample Plate used in a run. This information can be found in the Status pane on Jess and Abby systems.



# Software Menus Active in the Run Summary Screen

The following software menus are available:

- File
- Edit
- Instrument

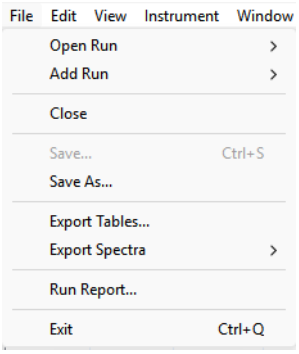
> **NOTE:** The Instrument submenu options will change depending on whether Compass is connected to an instrument.

- Window
- Help

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

## File Menu

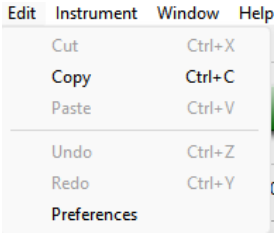
The following options can be found in the File menu:




- **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. See “Running Reports” on page 206 for more information
- **Exit** – Closes Compass for Simple Western.

### Edit Menu

The following options can be found in the Edit menu:

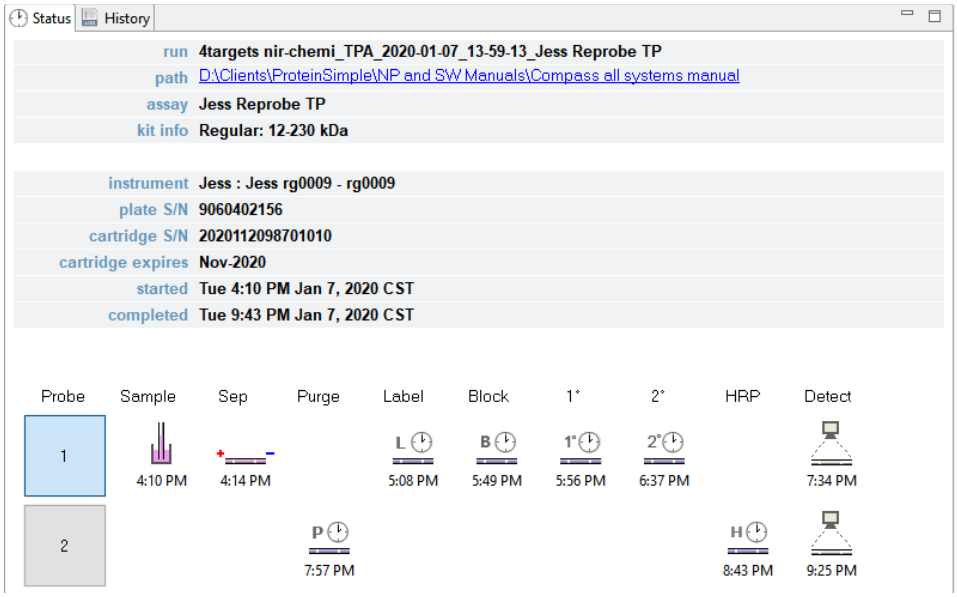



- **Preferences** – Set and save custom preferences for data export, and plot colors in the graph. See Chapter 8: "Setting Your Preferences" for more information.

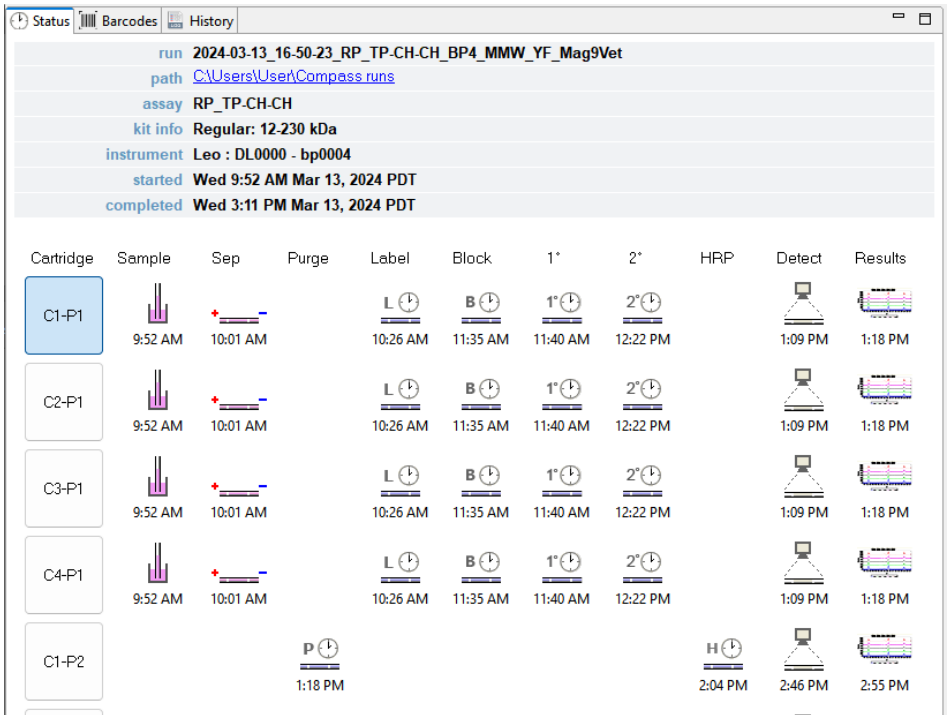
# Viewing File and Run Status Information

Information specific to each run file is shown in the Status pane for Jess and Abby, and the Status pane and Barcode pane for Leo.

#### JESS STATUS PANE



### LEO STATUS PANE

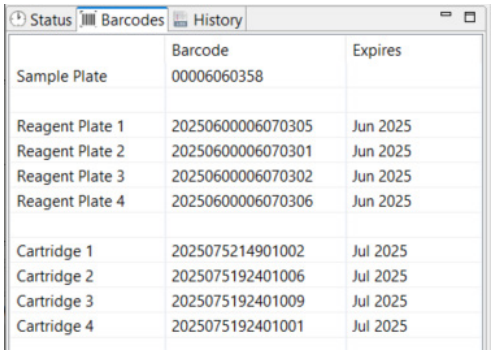



The run file name, path (directory location) and assay used is displayed along with instrument serial number and the run start/complete date and time.

- **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 Path**. 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 or total protein) and the molecular range.
- **Plate S/N (Jess and Abby)** – The plate serial number (S/N) information is captured and displayed for all assays.
- **Cartridge S/N (Jess and Abby)** – the cartridge serial number (S/N) information is captured and displayed for all assays.
- **Cartridge expires (Jess and Abby)** – the cartridge expiration date is captured and displayed.

The Leo Barcode pane records the scanned barcodes and expiration dates for the Sample Plate, Pre-filled Reagent Plate(s), and Cartridge used in the run.

#### LEO BARCODE PANE




| | Barcode | Expires |
|---|---|---|
| Sample Plate | 00006060358 | |
| | | |
| Reagent Plate 1 | 20250600006070305 | Jun 2025 |
| Reagent Plate 2 | 20250600006070301 | Jun 2025 |
| Reagent Plate 3 | 20250600006070302 | Jun 2025 |
| Reagent Plate 4 | 20250600006070306 | Jun 2025 |
| | | |
| Cartridge 1 | 2025075214901002 | Jul 2025 |
| Cartridge 2 | 2025075192401006 | Jul 2025 |
| Cartridge 3 | 2025075192401009 | Jul 2025 |
| Cartridge 4 | 2025075192401001 | Jul 2025 |

## Assay Steps: Size-based Assays

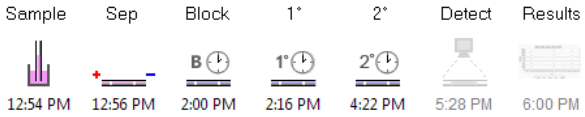
Each assay step that is executed during the run is represented by an icon. The time the individual step is scheduled to start is shown under each icon. Details on what happens during each assay step is as follows:

#### STANDARD SIZE ASSAYS

| Step | Description |
|---|---|
| Sample  12:54 PM | **Sample Loading Step** - Separation Matrix and Stacking Matrix are aspirated first into the capillaries followed by biotinylated ladder and samples. Capillaries are then transferred to the running buffer trough. |
| Sep  12:56 PM | **Separation Step** - Samples and fluorescent standards are separated in the capillaries. 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. Capillaries are then exposed to UV light, which immobilizes the separated proteins to the walls of each capillary. After separation, Matrix Removal Buffer is aspirated. |
| PN  4:38 PM | **Protein Normalization Step (Jess only) -** Protein normalization reagent is aspirated and incubated in the capillary. When the incubation is complete, Wash Buffer is aspirated. |
| Block  2:00 PM | **Blocking Step** - Blocking reagent (Antibody Diluent) is aspirated and incubated in the capillary. When the incubation is complete, Wash Buffer is aspirated. |
| 1°  2:16 PM | **Primary Antibody (1°) Step -** Primary antibody is aspirated and incubated in the capillary. When incubation is complete, Wash Buffer is aspirated. |

| Step | Description |
|---|---|
| 2°<br>4:22 PM | **Secondary Antibody (2°) Step -** Secondary HRP/NIR/IR antibody conjugate and Streptavidin-HRP/NIR is aspirated and incubated in the capillary. When incubation is complete, Wash Buffer is aspirated. |
| Detect<br>5:28 PM | **Detect Step** - Luminol-Peroxide solution is aspirated into the capillary (chemiluminescence only). Emitted chemiluminescent light is detected with the CCD camera. For fluorescence detection on Jess, emitted fluorescent signal is detected. |
| Results<br>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 gray (inactive). In the following example, the Secondary Antibody (2°) step is executing and the Detect and Results steps have not started:

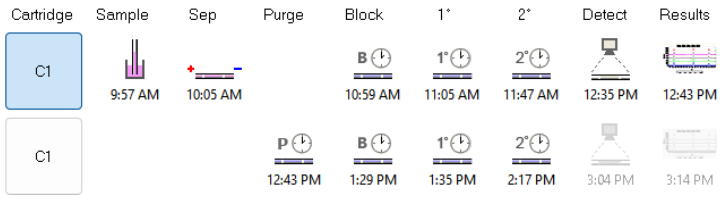



### REPLEX ASSAYS

| Step | Description |
|---|---|
| Sample<br>12:54 PM | **Sample Loading Step** - Separation Matrix and Stacking Matrix are aspirated first into the capillaries followed by biotinylated ladder and samples. Capillaries are then transferred to the running buffer trough. |
| Sep<br>12:56 PM | **Separation Step** - Samples and fluorescent standards are separated in the capillaries. 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. Capillaries are then exposed to UV light, which immobilizes the separated proteins to the walls of each capillary. After separation, Matrix Removal Buffer is aspirated. |
| Label<br>5:08 PM | **Label Step (Total Protein only)** - Total Protein Biotin Labeling Reagent is aspirated and incubated in the capillary. When incubation is complete, Wash Buffer is aspirated. |
| Block<br>2:00 PM | **Blocking Step** - Blocking reagent (Antibody Diluent) is aspirated and incubated in the capillary. When incubation is complete, Wash Buffer is aspirated. |
| 1°<br>2:16 PM | **Primary Antibody (1°) Step** - Primary antibody is aspirated and incubated in the capillary. When incubation is complete, Wash Buffer is aspirated. |

| Step | Description |
|---|---|
| 2°<br>4:22 PM | **Secondary Antibody (2°) Step** - Secondary HRP/NIR/IR antibody conjugate and Streptavidin-HRP/NIR is aspirated and incubated in the capillary. When incubation is complete, Wash Buffer is aspirated. |
| HRP<br>8:43 PM | **HRP Step (Total Protein only)** - Total Protein Streptavidin-HRP is aspirated and incubated in the capillary. When incubation is complete, Wash Buffer is aspirated. |
| Detect<br>5:28 PM | **Detect Step** - Luminol-Peroxide solution is aspirated into the capillary (chemiluminescence only). Emitted chemiluminescent light is detected with the CCD camera. For fluorescence detection on Jess, emitted fluorescent signal is detected. |
| Purge<br>7:57 PM | **Purge Step** - Wash Buffer and then RePlex reagent mix is aspirated to remove the Probe 1 primary and secondary antibodies from the immobilized sample proteins. This is followed by another round of Wash Buffer. |
| Results<br>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 gray (inactive). In the following example, the Secondary Antibody (2°) step is executing and the Detect and Results steps have not started:

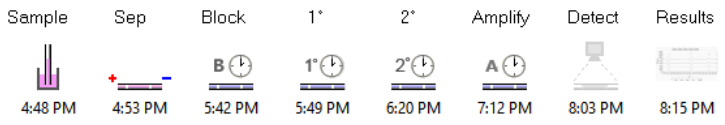



### STELLAR ASSAYS (JESS ONLY)

| Step | Description |
|---|---|
| Sample<br>12:54 PM | **Sample Loading Step** - Separation Matrix and Stacking Matrix are aspirated first into the capillary followed by biotinylated ladder and samples. Capillaries are then transferred to the running buffer trough. |
| Sep<br>12:56 PM | **Separation Step** - Samples and fluorescent standards are separated in the capillaries. 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. Capillaries are then exposed to UV light, which immobilizes the separated proteins to the walls of each capillary. After separation, Matrix Removal Buffer is aspirated. |

| Step | Description |
|---|---|
| Label   | **Label Step (Total Protein only)** - Total Protein Biotin Labeling Reagent is aspirated and incubated in the capillary. When incubation is complete, Wash Buffer is aspirated. |
| Block   | **Blocking Step** - Blocking reagent (Antibody Diluent) is aspirated and incubated in the capillary. When incubation is complete, Wash Buffer is aspirated. |
| 1°   | **Primary Antibody (1°) Step** - Primary antibody is aspirated and incubated in the capillary. When incubation is complete, Wash Buffer is aspirated. |
| 2°   | **Secondary Antibody (2°) Step** - Stellar secondary antibody is aspirated and incubated in the capillary. When incubation is complete, Wash Buffer is aspirated. |
| Amplify   | **Amplify Step** - Stellar IR or NIR Anneal is aspirated into the capillary followed by Stellar IR or NIR Label. These steps are repeated 10 times. When amplification is complete, Wash Buffer is aspirated. |
| Detect   | **Detect Step** - Luminol-Peroxide solution is aspirated into the capillary (chemiluminescence only). Emitted chemiluminescent light is detected with the CCD camera. For Stellar fluorescence detection, emitted fluorescent signal is detected. |
| Results   | **Results Step** - Results are available in the Analysis screen. |

When a run is in progress, icons for steps that have not executed yet will be gray (inactive). In the following example, the Amplify step is executing and the Detect and Results steps have not started:

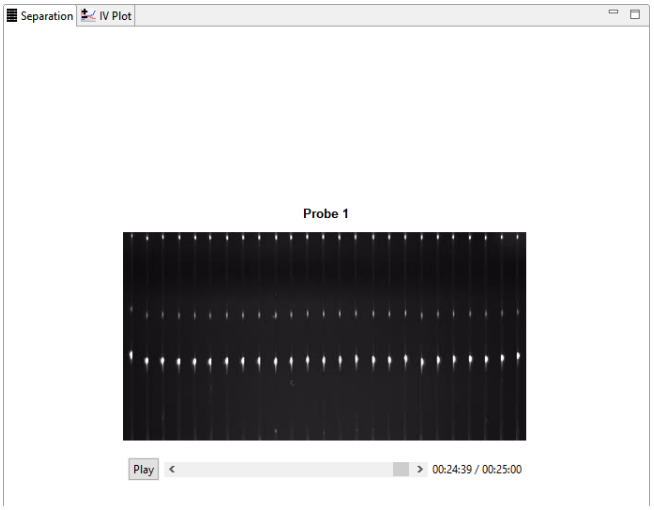


# Watching Standards Separation Movies

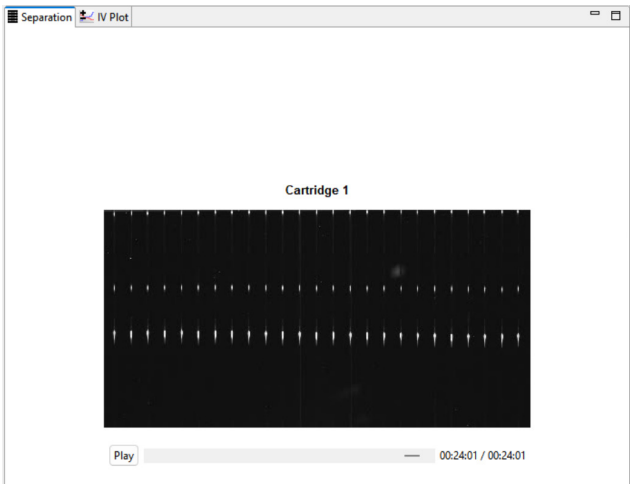
You can view a movie of the fluorescent standards separation in the capillaries. To do this:

1. Click the **Separation** tab.

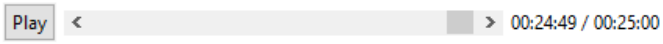
**JESS/ABBY REPLEX**



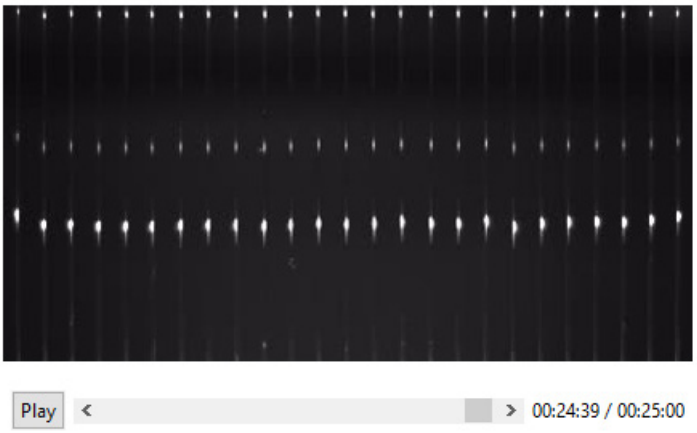

**LEO**




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:



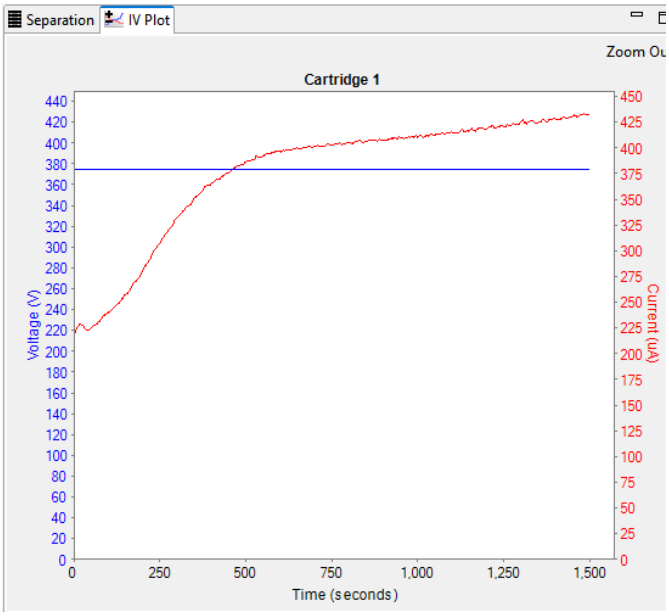
Click **Play** (button on far left) to view the movie. In the example below, standards for a Jess size assay are shown:




**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 still 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. To do this, click the **IV Plot** tab. The example below is from a Leo Chemiluminescence Immunoassay.



The blue Y-axis and plot show the separation voltage in volts (V), and the red Y-axis and plot shows the separation current in microamps (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.

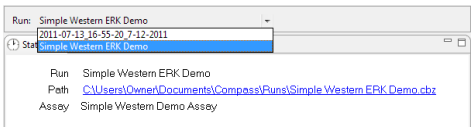
**NOTE:** The IV plot for a run in progress will not be available until the separation step starts executing. The plot is then displayed in real-time.

# Switching Between Open Run Files

**NOTE:** See “Opening Run Files” on page 153 for more information.

If more than one run file is open, you can switch between viewing the run information in each while in the Run Summary screen. 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.



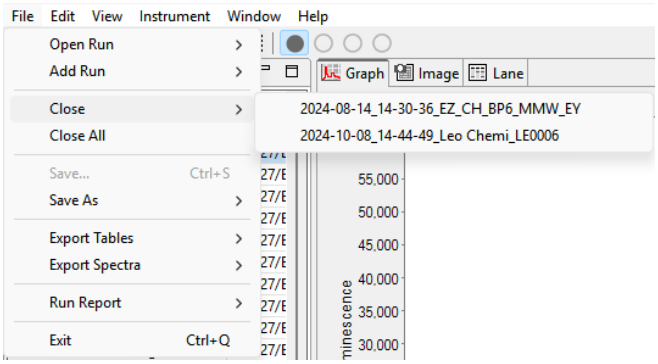

# Closing Run Files

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 one run file**

  a. Select **File** from the main menu and then click **Close** to open the submenu.

b. Select the run you want to close.




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

Chapter 6:

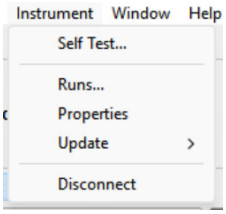
# Controlling Jess, Abby, and Leo

## Chapter Overview

- Instrument Control
- Self Test
- Viewing and Changing System Properties
- Viewing Log Files
- Software Updates
- Instrument Software (Embedded) Updates
- Status Modes

# Instrument Control

The instrument menu allows you to control Jess, Abby, and Leo.



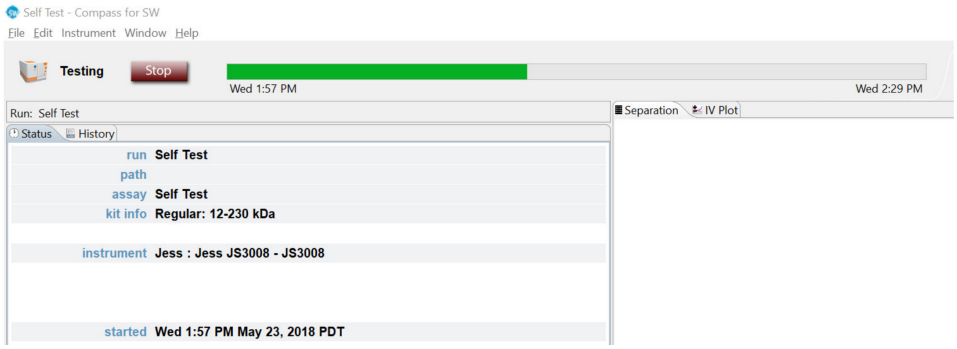

**NOTES:**
Instrument menu options are active only when a computer with Compass for Simple Western is connected directly to Jess, Abby, or Leo. Available functions will vary depending on the instrument.

When your instrument is not connected to Compass, the Instrument menu allows you to connect Jess, Abby, or Leo to the software.

If the instrument is in an Error state, a Reset option will appear in the menu and the Self Test option will be disabled.

# Self Test

Jess, Abby, and Leo can perform a series of 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 for Jess and Abby and approximately 20 minutes for Leo.



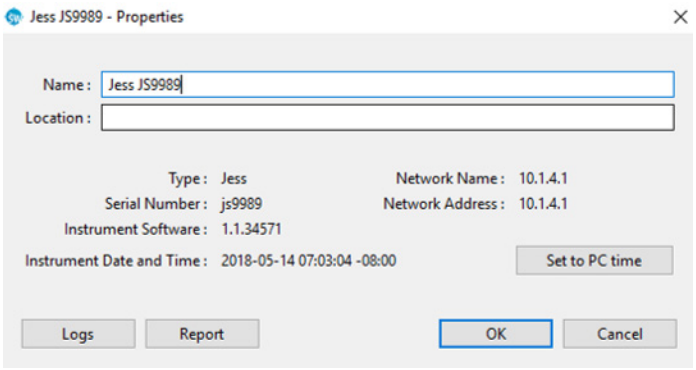

**NOTE:** We recommend performing the self test monthly or when encountering issues with the system. When performing a self test, start the test before preparing your sample, reagents, and pipetting plates for your instrument.

To view the test log at completion of the test, select **Instrument**, click **Properties** and click **View Logs**. See "Self Test Report" on page 145 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 (embedded)
- 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**.

> **NOTE:** Reboot the computer and instrument and wait for about one minute.

To confirm the time was set properly after syncing:

1. Open Compass for SW.
2. Go to **Instrument** > **Properties**.
3. Check if Instrument Date and Time matches your local time.
4. If it doesn't match, click **Set to PC** time again.

If the time zone is not set correctly:

1. In Windows, go to **Date and Time Settings** and turn on **Set time zone automatically**.

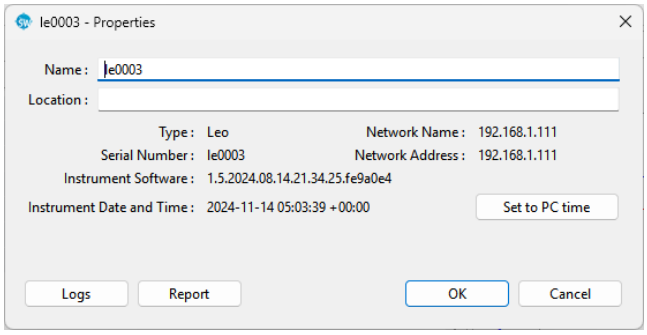
> **NOTE:** Windows may handle Daylight Savings Time differently.

2. Under Synchronize your clock, click **Sync now**.
3. Confirm that the computer now has the correct time and time zone.
4. Start Compass for Simple Western.
5. Connect the software to your instrument.
6. Select **Instrument** and click **Properties**.
7. Click **Set to PC Time**.
8. Close Compass for Simple Western.
9. Turn the instrument off.
10. Turn the instrument on.
11. Open Compass for Simple Western.
12. Connect the software to your instrument.
13. Select **Instrument** and click **Properties**.
14. Confirm the instrument time.

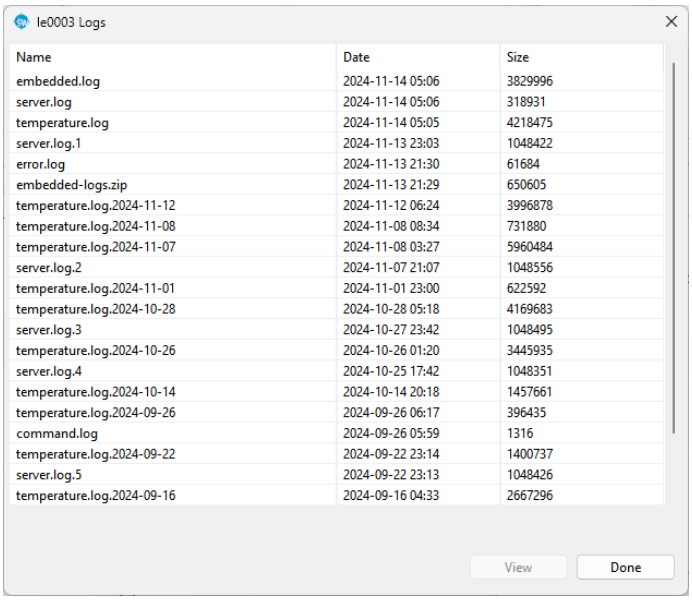
# Viewing Log Files

## System Logs

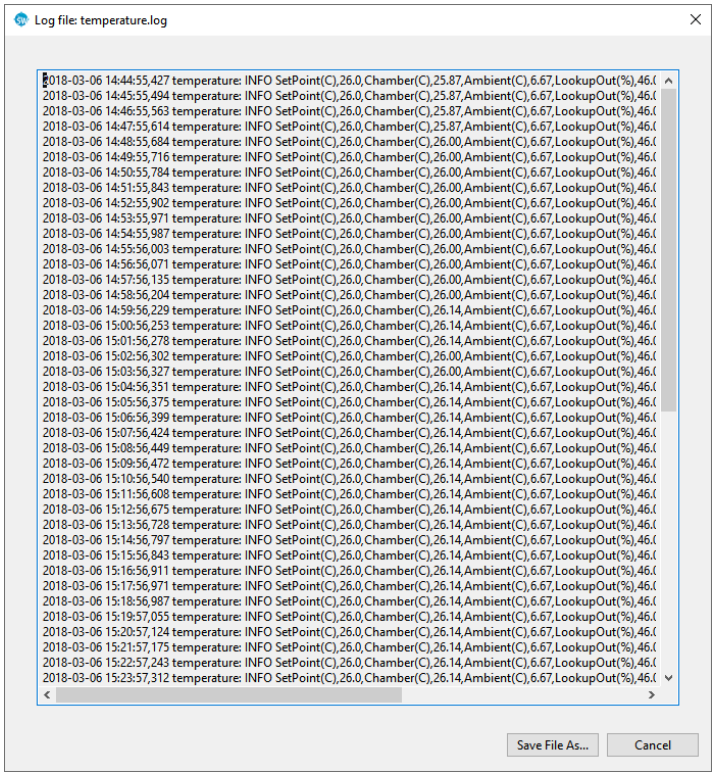
1. Select **Instrument** in the main menu and click **Properties** to display system properties.



2. Select **Logs** and then click **All Logs**. A list of system logs will display:




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

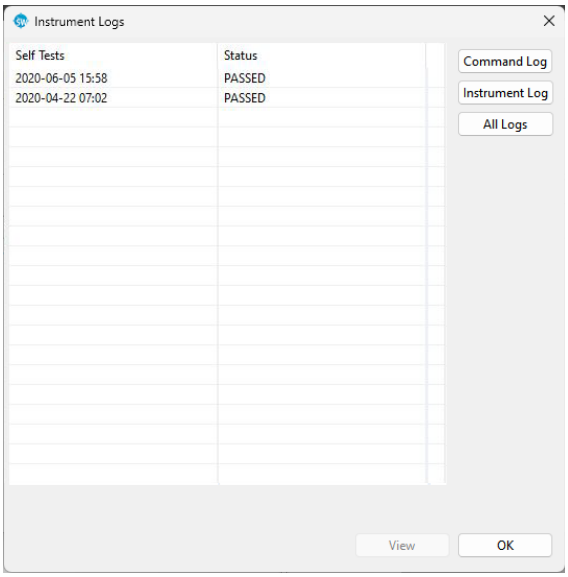



4. Click **Save File As** to save a copy of the log file.

# Self Test Report

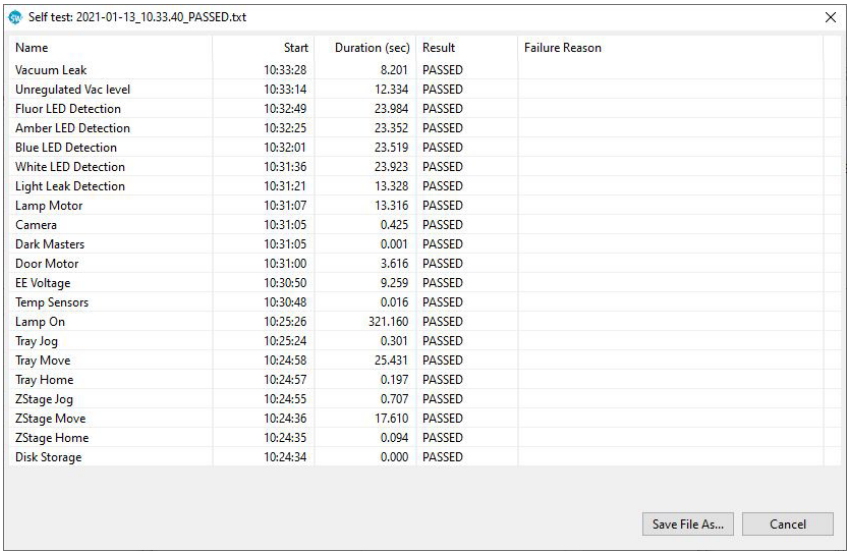
**To view a self test report from the Instrument Log.**

1. Select **Instrument** in the main menu and click **Properties** to display system properties.
2. Click **Logs**. A list of self tests that have been performed will display:




**NOTE:** The “All Logs” button will not appear when Compass for SW is connected to a Jess instrument.

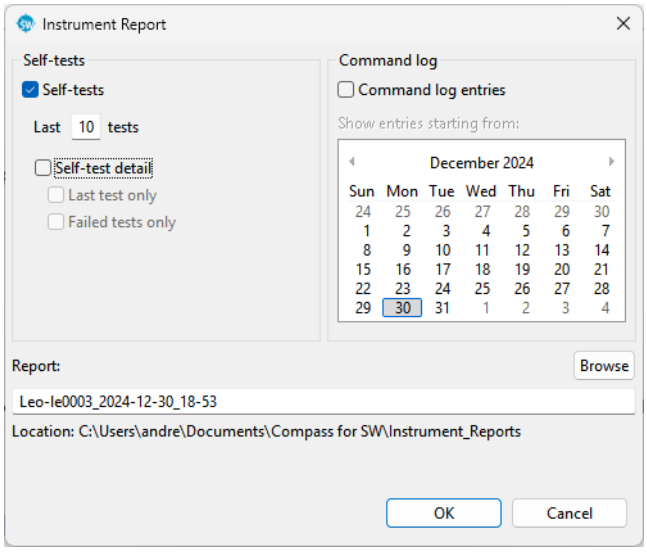
3. Select a self test and click **View**. The individual test details will display:



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

**To download self test reports.**

1. Select **Instrument** in the main menu and click **Properties** to display system properties.
2. Click **Reports**.
3. Select the number of self test reports to download and the self test details to include. You can also select a date to download reports from that day onward.
4. Enter a file name for the report.




5. Click **OK** to save the self test report(s) to the designated file directory.

# Software Updates

To check for software updates, go to Compass for Simple Western, select **Help** in the main menu, and click **Check for Updates**.

# Instrument Software (Embedded) Updates

To view the version installed, open Compass for Simple Western, select **Instrument** in the main menu, then click **Properties**. The current version of system software will be displayed.

To check for embedded updates, go to Compass for Simple Western, select **Instrument** in the main menu, then **Update**, and select **Network**. If you are not on the network, contact ProteinSimple Technical Support for assistance on how to obtain the latest update.

# Status Modes

The instrument status bar displays status, buttons, and progress bars depending on what Jess, Abby, or Leo is doing.

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

Chapter 7:

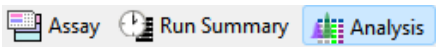
# Size Assay Data Analysis

## 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
- Undo/Redo
- 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
- Creating Annotated Lane View Figures
- Changing the Electropherogram View
- Closing Run Files
- Analysis Settings Overview
- Standards Settings
- Ladders Settings
- Images Analysis Settings
- Normalization
- Peak Names Settings
- Standard Curve Settings
- Cartridge Corrections (Leo only)
- Loading Controls Settings
- Peak Fit Analysis Settings
- Signal to Noise Settings
- Advanced Analysis 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:

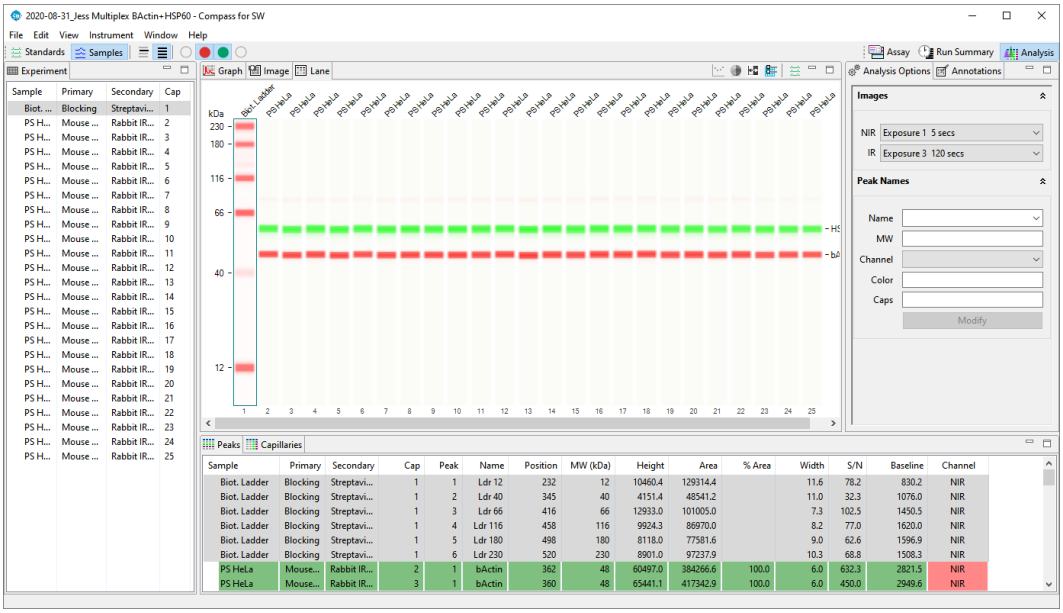



## Analysis Screen Panes

The Analysis screen has seven panes, each displays the following data for capillaries in the experimental run:

- **Experiment** – Lists sample, reagent, cartridge, and capillary information for the data generated. Sample and reagent information is defined the assay template and is automatically updated in the Experiment pane.
- **Graph** – Displays electropherogram data for sample proteins or fluorescent standards.
- **Image** – Displays an image of the separated sample proteins or fluorescent standards in the capillaries
- **Lane** – Displays data for sample proteins or fluorescent standards 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.
- **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.
- **Annotations** – Creates annotated lane view figures.

**NOTE:** If you don't see data in the Analysis screen after a run, there may have been a disconnection between the instrument and computer during the run. If this happens, the instrument's embedded computer will have a copy of the run file. Go to **Instrument** > **Runs...** to find and save a copy of the run file.




**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.[1,2]

## Software Menus Active in the Analysis Screen

The following software menus are available:

- File
- Edit
- View
- Instrument

**NOTE:** The Instrument submenu options will change depending on whether Compass is connected to an instrument.

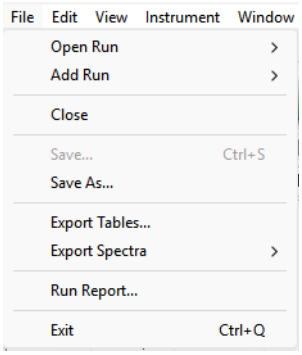
- Window
- Help

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

[1] *Electrophoresis. 2021 Aug;42(14-15):1521-1531. doi: 10.1002/elps.202100068.*
[2] *Electrophoresis. 2021 Feb;42(3):206-218. doi: 10.1002/elps.202000199.*

## File Menu

The following options can be found in the File menu:




- **Open Run** – Opens a run file.
- **Add Run** – Opens and views other run files in addition to those that are already open in the active Compass window.

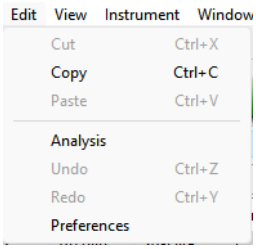
**NOTES:**
The run files must be the same assay type and use the same detection channels.

When multiple runs are open in the active Compass window, a menu expand arrow will appear next to the close, save as, export tables, export spectra, and run report menu options. Choose the run you want to close, save, or export information from.

- **Close** – Closes the run file currently being viewed.
- **Close All** – Closes all open run files in the active Compass window.
- **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

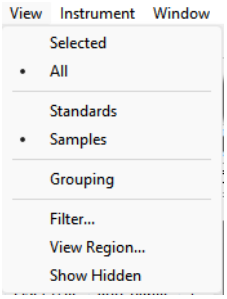
The following options can be found in the Edit menu:




- **Copy** – Lets you copy data shown in the graph, image, lane, peaks, or capillaries panes. See "Copying Data Views and Results Tables" on page 203 for more information.
- **Analysis** – Displays the analysis settings used to analyze the run data when a run file is open and lets you change them as needed. See "Analysis Settings Overview" on page 277" for more information.
- **Preferences** – Lets you set and save custom preferences for data export and plot colors in the graph. See Chapter 8: "Setting Your Preferences" for more information.

### View Menu

The following option can be found in the View menu:




- **Selected View** – Displays data in a per capillary (single) view format.
- **All View** – Displays data in a multiple capillary view format.
- **Standards** – Lets you change the data view to show only the fluorescent standards.
- **Samples** – Lets you change the data view to show sample proteins.
- **Filter** – Lets you display data only for specific capillaries, cartridges (Leo only), or named proteins.
- **View Region** – Lets you change the molecular weight (x-axis) range of the data displayed.
- **Show Hidden** – Shows capillaries that have been hidden from the data view.

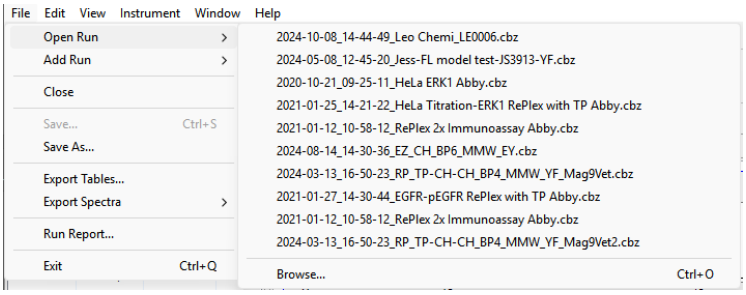
# Opening Run Files

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 and disconnect the instrument by clicking **Instrument** and selecting **Disconnect**. Then open the run file.

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

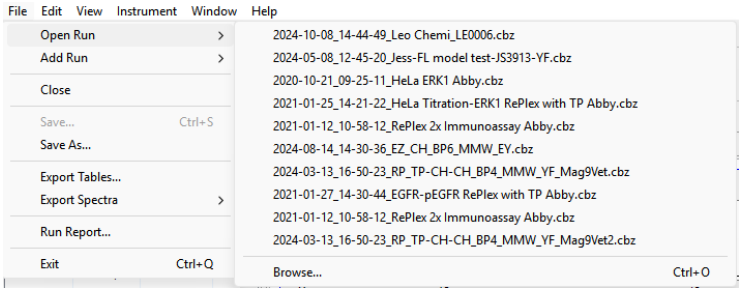



2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file.

## Opening Multiple Run Files

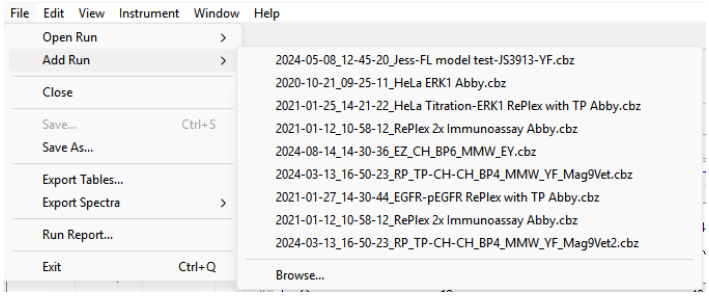
**NOTE:** If you need to open a run file when another run is executing, launch another instance of Compass for Simple Western and disconnect the instrument by clicking **Instrument** and selecting **Disconnect**. Then open the run file.

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

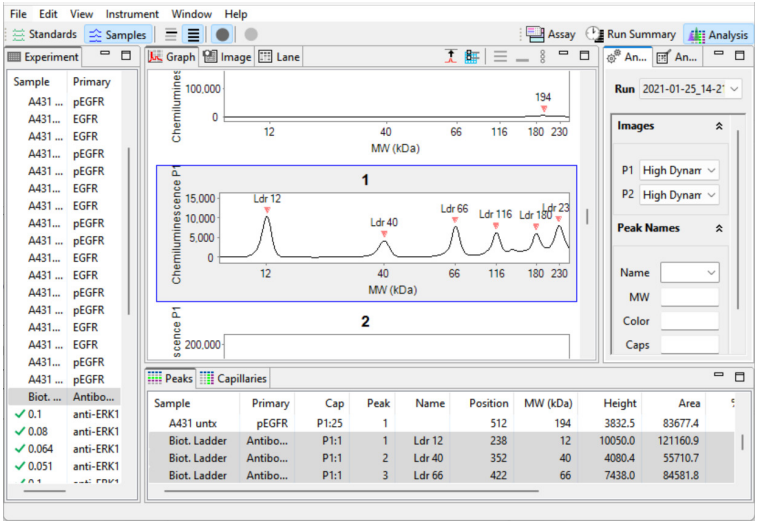


2. A list of the last 10 runs opened will display. Select one of these runs or click **Browse** to open the Runs folder and select a different file. When connected to an instrument, you can also select **Instrument** in the main menu and click **Runs** to select a different run file.

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 capillaries (Jess/Abby/Leo) 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:**
A Leo run cannot be added to a Jess/Abby run even if the channels match.

When adding multiple data files for analysis, the assay type and detection channel (for example chemi, IR/NIR fluorescence, or IR/NIR fluorescence with Normalization) between the runs must be the same.

When adding multiple RePlex data files, both probes must contain matching probe types and channels. For example, a chemiluminescence with Total Protein data file can't be added to a 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, cartridge (Leo only), and capillary information for each of the capillaries in the run. A view of the Experiment panes for some assay types are shown below.

**CHEMILUMINESCENCE IMMUNOASSAY ON ABBY**

Experiment

| Sample | Primary | Cap |
|---|---|---|
| EZ (66-440k... | SA-HRP | 1 |
| ✓ EZ (66-440k... | SA-HRP | 2 |
| ✓ EZ (66-440k... | SA-HRP | 3 |
| ✓ EZ (66-440k... | SA-HRP | 4 |
| ✓ EZ (66-440k... | SA-HRP | 5 |
| ✓ EZ (66-440k... | SA-HRP | 6 |
| ✓ EZ (66-440k... | SA-HRP | 7 |
| ✓ EZ (66-440k... | SA-HRP | 8 |
| ✓ EZ (66-440k... | SA-HRP | 9 |
| ✓ EZ (66-440k... | SA-HRP | 10 |
| ✓ EZ (66-440k... | SA-HRP | 11 |
| ✓ EZ (66-440k... | SA-HRP | 12 |

**REPLEX ASSAY ON JESS**

Experiment

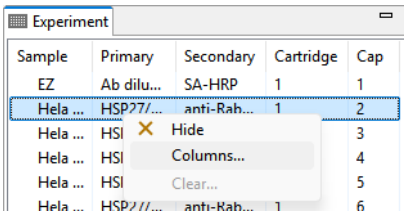
| Sample | Primary 1 | Primary 2 | Cap |
|---|---|---|---|
| Biot. ... | Antibod... | Primary II | 1 |
| 0.2 m... | MFAD | bActin | 2 |
| 0.2 m... | bActin | bActin | 3 |
| 0.2 m... | Park7 | bActin | 4 |
| 0.2 m... | MFAD | bActin | 5 |
| 0.2 m... | bActin | bActin | 6 |
| 0.2 m... | Park7 | bActin | 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 |

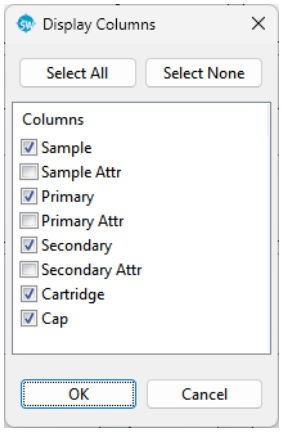
**CHEMILUMINESCENCE IMMUNOASSAY ON LEO**

Experiment

| Sample | Primary | Secondary | Cartridge | Cap |
|---|---|---|---|---|
| EZ | Ab dilu... | SA-HRP | 1 | 1 |
| Hela ... | HSP27/... | anti-Rab... | 1 | 2 |
| Hela ... | HSP27/... | anti-Rab... | 1 | 3 |
| Hela ... | HSP27/... | anti-Rab... | 1 | 4 |
| Hela ... | HSP27/... | anti-Rab... | 1 | 5 |
| Hela ... | HSP27/... | anti-Rab... | 1 | 6 |
| Hela ... | HSP27/... | anti-Rab... | 1 | 7 |
| Hela ... | HSP27/... | anti-Rab... | 1 | 8 |
| Hela ... | HSP27/... | anti-Rab... | 1 | 9 |
| Hela ... | HSP27/... | anti-Rab... | 1 | 10 |
| Hela ... | HSP27/... | anti-Rab... | 1 | 11 |
| Hela ... | HSP27/... | anti-Rab... | 1 | 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.
- **To select columns for display** – Right-click in the Experiment pane, then select **Columns...** and select the columns to display. Options that appear will depend on the assay run.



Experiment pane 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.
- **Sample Attr** – Sample attribute. If sample attributes were entered in the assay template (Assay screen), those names will display here.

**NOTE:** Data notification icons will display in the sample column if Compass for Simple Western detected a potential analysis issue or if data was manually modified by the user. For more information see “Run Data Notifications and Warnings” on page 189.

- **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 Attr** – Primary antibody attribute. If primary antibody attributes were entered in the assay template (Assay screen), those names will display here.
- **Primary 1 (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 Probe 1 (default name) will display.
- **Primary Attr 1 (RePlex Assays only)** – Primary antibody attribute for Probe 1. If primary antibody attributes were entered in the assay template (Assay screen), those names will display here.
- **Primary 2 (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 Probe 2 (default name) will display.
- **Primary Attr 2 (RePlex Assays only)** – Primary antibody attribute for Probe 2. If primary antibody attributes were entered in the assay template (Assay screen), those names will display here.
- **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.
- **Secondary Attr** – Secondary antibody attribute. If secondary antibody attributes were entered in the assay template (Assay screen), those names will display here.
- **Secondary 1 (RePlex Assays only)** – Secondary antibody name for Probe 1. If secondary antibody names were entered in the assay template (Assay screen), those names will display here.
- **Secondary Attr 1 (RePlex Assays only)** – Secondary antibody attribute for Probe 1. If secondary antibody attributes were entered in the assay template (Assay screen), those names will display here.
- **Secondary 2 (RePlex Assays only)** – Secondary antibody name for Probe 2. If secondary antibody names were entered in the assay template (Assay screen), those names will display here.
- **Secondary Attr 2 (RePlex Assays only)** – Secondary antibody attribute for Probe 2. If secondary antibody attributes were entered in the assay template (Assay screen), those names will display here.

**NOTE:** The Secondary column will not display for RePlex Assays with two immunoassays by default.

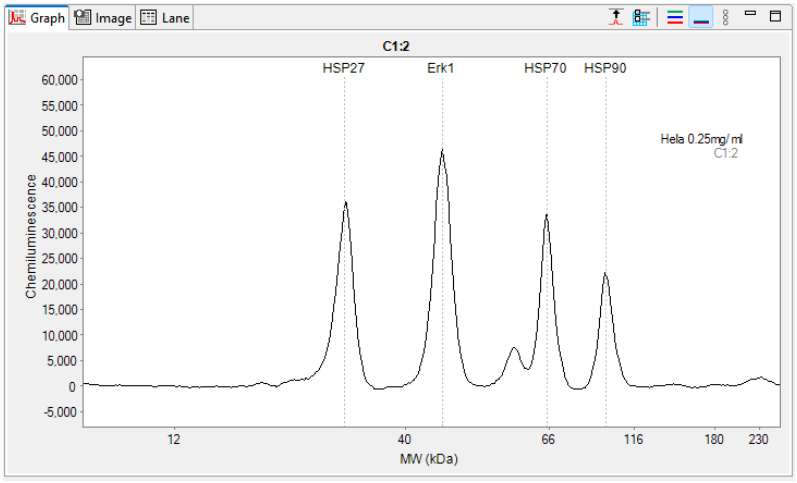
- **Cartridge (Leo only)** – Cartridge number.
- **Cap** – Capillary number.

**NOTES:**
Selection of columns applies to Compass views only. These column selection changes are not reflected in exports and reports.

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 189.

## Graph Pane: Electropherogram Data

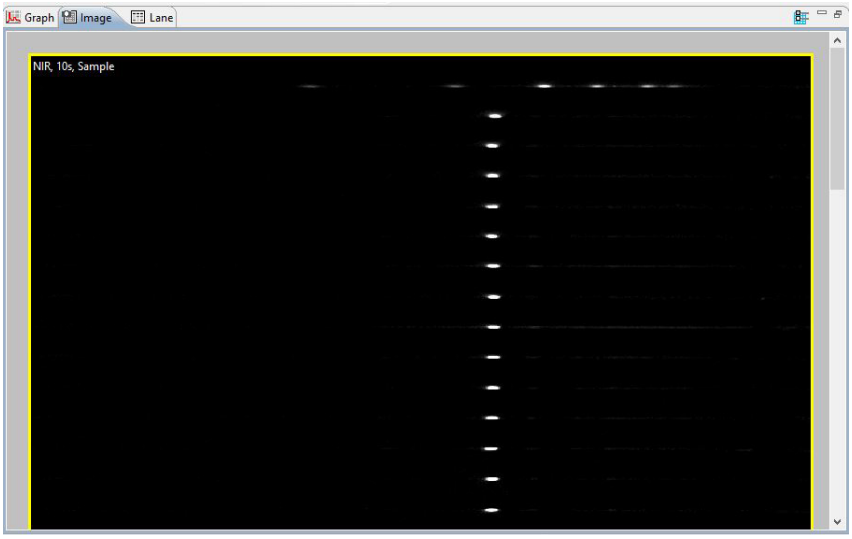
Click the **Graph** tab to view data for sample proteins or fluorescent standards. Data for samples is shown in the following example, and proteins are displayed as peaks:




More Graph view options will be described in more detail in “Graph Options: Customizing the Data Display” on page 259.

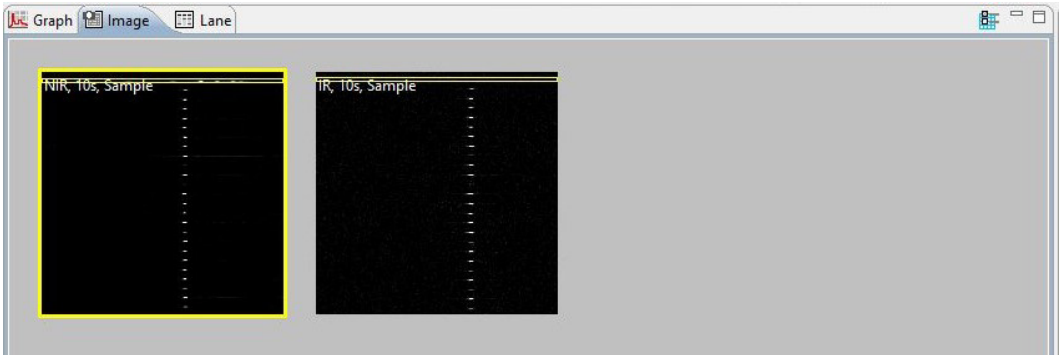
## Image Pane: Capillary Separation Image Data

Click the **Image** tab to view final images of sample proteins or fluorescent standards. Image data for samples is shown in the following example:




Click the blue **Show All Images** icon at the top right of the Image pane to display images for all exposures in the run for sample, raw (fluorescence only), and background (fluorescence only) images. Use the mouse to scroll down to view the images.

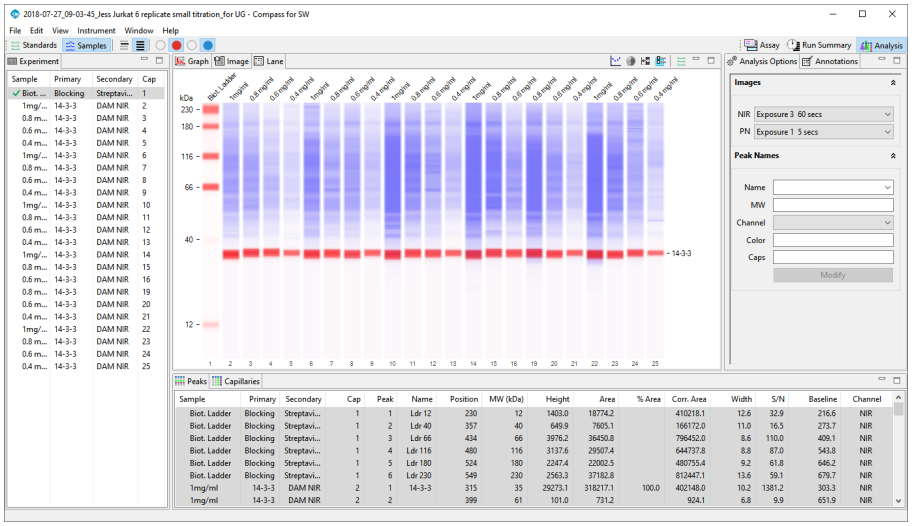

You can also view all the sample images for the selected exposure time in one pane by clicking **View** in the main menu and selecting **All**. For runs with multiple detection channels, select the desired channels to display. The image data for multiple detection channels (NIR and IR) is shown in the following example:

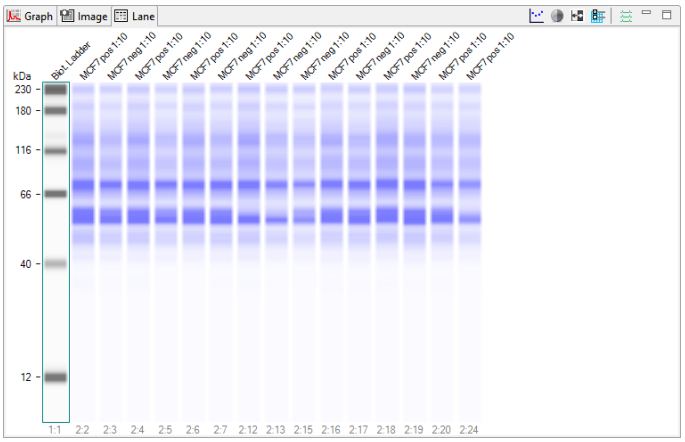


# Lane Pane: Virtual Blot-Like Image Data

**NOTE:** Lane view is a digitally created image to aid with data visualization and is not quantitative.

Click the **Lane** tab to view data for sample proteins or fluorescent standards 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 with Protein Normalization (for Jess only), and from an assay with Total Protein Normalization using RePlex are displayed as bands in the examples below.





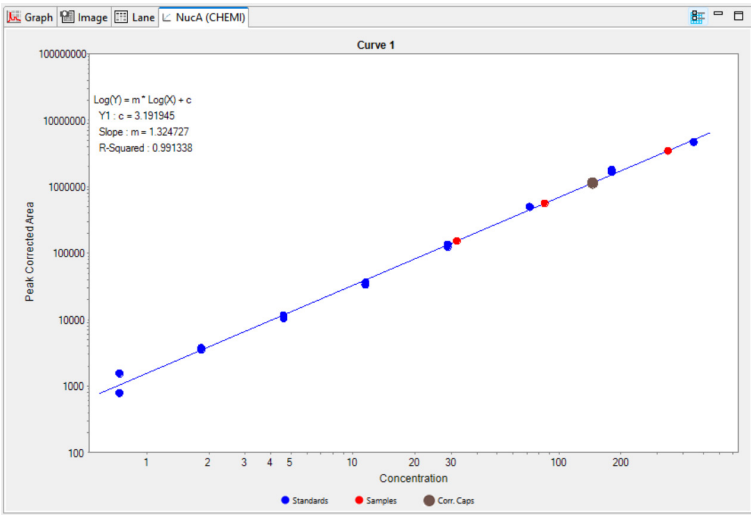

To view information for a band, roll the mouse over a band until the info box appears.

**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.[1,2]

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 220.

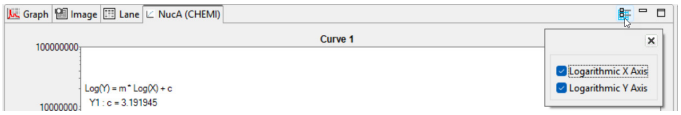
## Std Curve Pane: Standard Curve Fit Data

Click on the tab displaying the name of the standard curve sample and detection channel to view a linear, 4-parameter, or Log-Log curve fit of your standard curve proteins. Data for the samples in the standard curve view is shown in the following example for a standard curve performed on a Leo instrument where individual data points are displayed as dots.

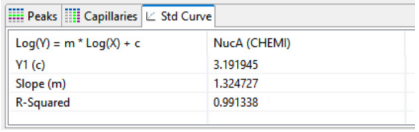



Concentration of the sample proteins is automatically determined by Compass based on defined values of the standard curve. Refer to “Standard Curve Settings” on page 300 for more information.

Click on the **Curve Options** button to adjust the x-axis and y-axis scale.




The Std Curve pane displays the equation and parameters used for the standard curve.



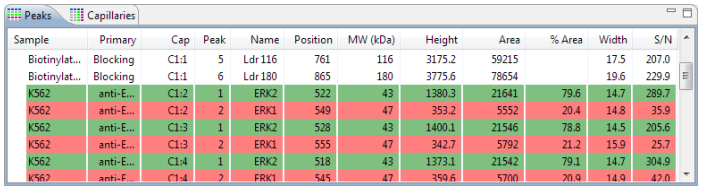

| Log(Y) = m * Log(X) + c | NucA (CHEMI) |
|---|---|
| Y1 (c) | 3.191945 |
| Slope (m) | 1.324727 |
| R-Squared | 0.991338 |

[1] *Electrophoresis. 2021 Aug;42(14-15):1521-1531. doi: 10.1002/elps.202100068.*
[2] *Electrophoresis. 2021 Feb;42(3):206-218. doi: 10.1002/elps.202000199.*

# Peaks Pane: Calculated Results

Click the **Peaks** tab to view tabulated results for sample proteins, normalized sample proteins shown as Corr. Area (when Normalization, Loading Control, or Cartridge Correction is enabled), or fluorescent standards. Each row in the table shows the individual results for each peak detected in each capillary. Some examples of data for samples in the Peaks table are shown below:

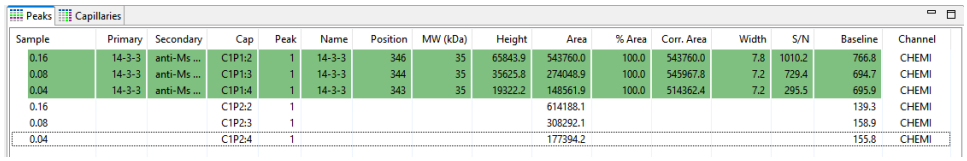
### IMMUNOASSAY



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 | 47.0 |

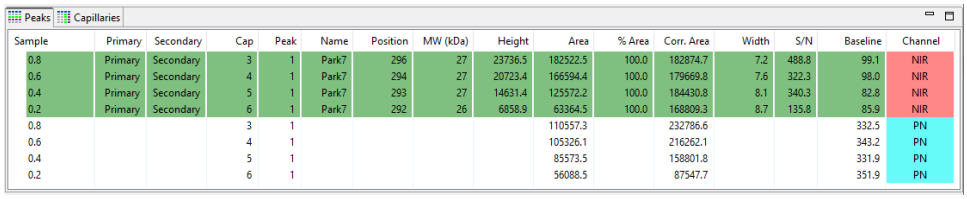
### TOTAL PROTEIN USING REPLEX: PROBE 1, CHEMILUMINESCENCE, PROBE 2, TOTAL PROTEIN NORMALIZATION



Peaks | Capillaries

| Sample | Primary | Secondary | Cap | Peak | Name | Position | MW (kDa) | Height | Area | % Area | Corr. Area | Width | S/N | Baseline | Channel |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.16 | 14-3-3 | anti-Ms ... | C1P1:2 | 1 | 14-3-3 | 346 | 35 | 65843.9 | 543760.0 | 100.0 | 543760.0 | 7.8 | 1010.2 | 766.8 | CHEMI |
| 0.08 | 14-3-3 | anti-Ms ... | C1P1:3 | 1 | 14-3-3 | 344 | 35 | 35625.8 | 274048.9 | 100.0 | 545967.8 | 7.2 | 729.4 | 694.7 | CHEMI |
| 0.04 | 14-3-3 | anti-Ms ... | C1P1:4 | 1 | 14-3-3 | 343 | 35 | 19322.2 | 148561.9 | 100.0 | 514362.4 | 7.2 | 295.5 | 695.9 | CHEMI |
| 0.16 | | | C1P2:2 | 1 | | | | | 614188.1 | | | | | 139.3 | CHEMI |
| 0.08 | | | C1P2:3 | 1 | | | | | 308292.1 | | | | | 158.9 | CHEMI |
| 0.04 | | | C1P2:4 | 1 | | | | | 177394.2 | | | | | 155.8 | CHEMI |

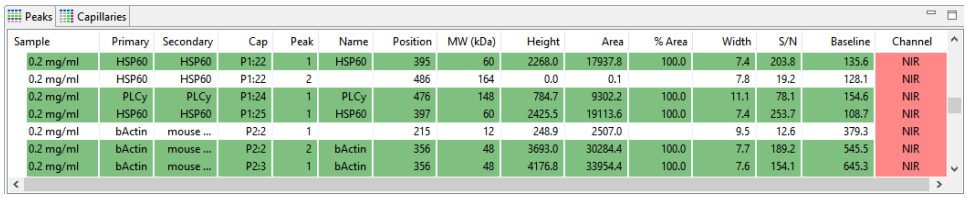
### PROTEIN NORMALIZATION (JESS ONLY)



Peaks | Capillaries

| Sample | Primary | Secondary | Cap | Peak | Name | Position | MW (kDa) | Height | Area | % Area | Corr. Area | Width | S/N | Baseline | Channel |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.8 | Primary | Secondary | 3 | 1 | Park7 | 296 | 27 | 23736.5 | 182522.5 | 100.0 | 182874.7 | 7.2 | 488.8 | 99.1 | NIR |
| 0.6 | Primary | Secondary | 4 | 1 | Park7 | 294 | 27 | 20723.4 | 166594.4 | 100.0 | 179669.8 | 7.6 | 322.3 | 98.0 | NIR |
| 0.4 | Primary | Secondary | 5 | 1 | Park7 | 293 | 27 | 14631.4 | 125572.2 | 100.0 | 184430.8 | 8.1 | 340.3 | 82.8 | NIR |
| 0.2 | Primary | Secondary | 6 | 1 | Park7 | 292 | 26 | 6858.9 | 63364.5 | 100.0 | 168809.3 | 8.7 | 135.8 | 85.9 | NIR |
| 0.8 | | | 3 | 1 | | | | | 110557.3 | | 232786.6 | | | 332.5 | PN |
| 0.6 | | | 4 | 1 | | | | | 105326.1 | | 216262.1 | | | 343.2 | PN |
| 0.4 | | | 5 | 1 | | | | | 85573.5 | | 158801.8 | | | 331.9 | PN |
| 0.2 | | | 6 | 1 | | | | | 56088.5 | | 87547.7 | | | 351.9 | PN |

### REPLEX ASSAY



Peaks | Capillaries

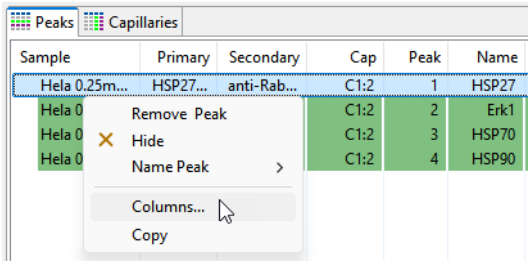
| Sample | Primary | Secondary | Cap | Peak | Name | Position | MW (kDa) | Height | Area | % Area | Width | S/N | Baseline | Channel |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.2 mg/ml | HSP60 | HSP60 | P1:22 | 1 | HSP60 | 395 | 60 | 2268.0 | 17937.8 | 100.0 | 7.4 | 203.8 | 135.6 | 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 |

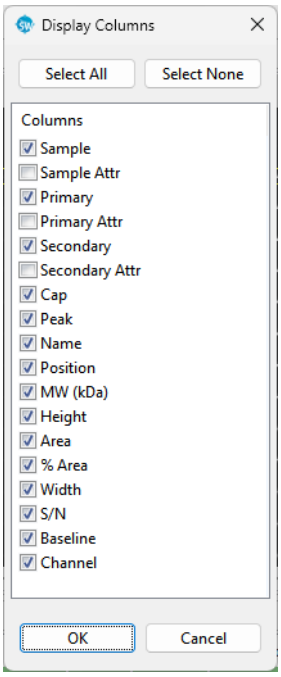
**NOTES:**
Peaks named automatically by Compass for Simple Western using the user-defined peak name analysis parameters are color-coded.

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.[1,2]

- **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.
- **To select columns for display** – Right-click in the Peaks pane, then select **Columns...** and select the Peak table columns to display.

**NOTE:** Selection of columns applies to Compass views only. These column selection changes are not reflected in exports and reports.




[1] *Electrophoresis. 2021 Aug;42(14-15):1521-1531. doi: 10.1002/elps.202100068.*
[2] *Electrophoresis. 2021 Feb;42(3):206-218. doi: 10.1002/elps.202000199.*

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.
- **Sample Attr** - Sample attribute. If sample attributes were entered in the assay template (Assay screen), those names will display here.
- **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 Attr** - Primary antibody attribute. If primary antibody attributes were entered in the assay template (Assay screen), those names will display here.
- **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.
- **Secondary Attr** - Secondary antibody attribute. If secondary antibody attributes were entered in the assay template (Assay screen), those names will display here.
- **Cap**
  - **Jess/Abby Immunoassays (non-RePlex):** Capillary number.
  - **Jess/Abby RePlex Assays:** Probe and capillary number. For example, P1:4 indicates Probe 1, capillary 4.
  - **Leo Immunoassays (non-RePlex):** Cartridge and capillary number. For example, C1:4 indicates cartridge 1, capillary 4
  - **Leo RePlex Assays:** Cartridge, Probe, and capillary number. For example, C1P1:4 indicates cartridge 1, Probe 1, capillary 4.
- **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** - Displays the calculated percent area for the named peak compared to all named peaks within the same Peak Names Analysis Group. 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 Lines methods ("Peak Find Settings" on page 317 for more information). Displays the calculated percent area for the peak compared to the total peak 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.
- **Known Conc (Concentration)** – Displays the known concentration of a protein for the named peak used to generate the standard curve. 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, Cartridge Corrections is enabled, 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 * Area2).
  - **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

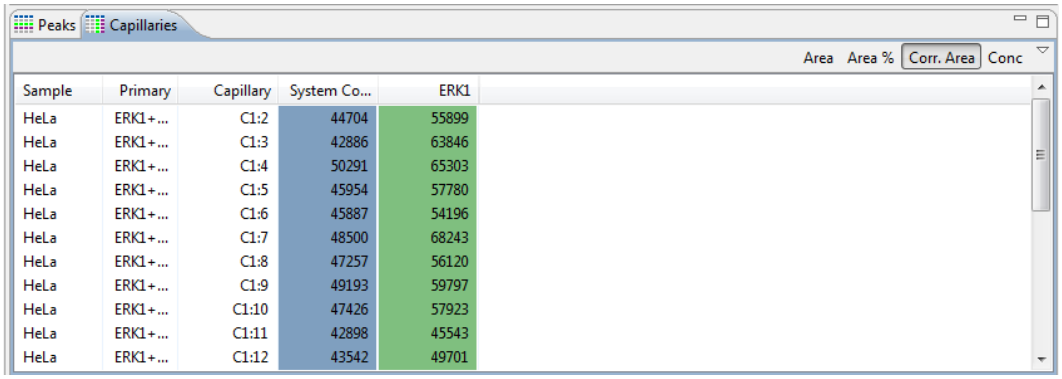
    Then,

    Corr. Area = Peak area/Capillary Normalization factor.
- **For Cartridge Corrections** - Corrected Area of the selected Peak is based on the Correction Factor per cartridge where Corr. Area = Peak Area * Corr. Factor. Refer to “Cartridge Corrections (Leo only)” on page 311 for more information.
- **Width** - Displays the calculated peak width, in pixels at half height (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 either based on a peak finding algorithm to provide a relative ratio comparing all peaks within the electropherogram or a signal to noise ratio that follows USP/NF regulations. These settings are selected in Advanced Analysis settings. For more information see “Signal to Noise Settings” on page 326.
- **Baseline** - Displays the raw baseline signal of each peak
- **Channel** - Detection channel (Chemi, NIR, IR, PN)

# Capillaries Pane: User-Specified Peak Names

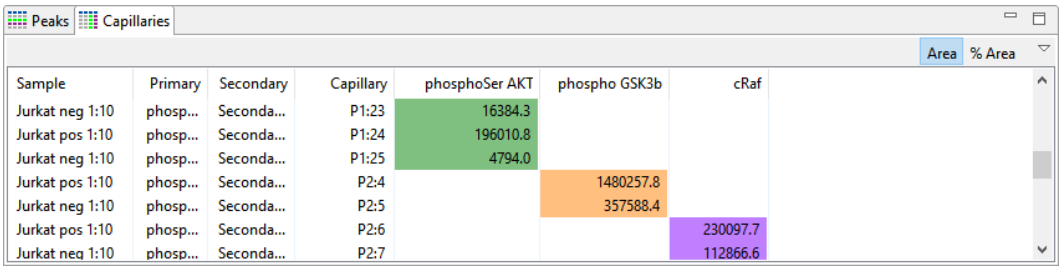
Click the **Capillaries** tab to view tabulated results for sample proteins associated with specific primary antibodies, Protein Normalization area, or Total Protein area in the run data. Compass for Simple Western labels these sample peaks automatically using user-defined peak name settings. Each row in the table shows the individual results for the named peaks detected in each capillary. Examples of data for samples in the Capillaries table are shown.

#### CHEMILUMINESCENCE IMMUNOASSAY




| 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 |

#### CHEMILUMINESCENCE IMMUNOASSAY + CHEMILUMINESCENCE IMMUNOASSAY USING REPLEX




| Sample | Primary | Secondary | Capillary | phosphoSer AKT | phospho GSK3b | cRaf |
|---|---|---|---|---|---|---|
| Jurkat neg 1:10 | phosp... | Seconda... | P1:23 | 16384.3 | | |
| Jurkat pos 1:10 | phosp... | Seconda... | P1:24 | 196010.8 | | |
| Jurkat neg 1:10 | phosp... | Seconda... | P1:25 | 4794.0 | | |
| Jurkat pos 1:10 | phosp... | Seconda... | P2:4 | | 1480257.8 | |
| Jurkat neg 1:10 | phosp... | Seconda... | P2:5 | | 357588.4 | |
| Jurkat pos 1:10 | phosp... | Seconda... | P2:6 | | | 230097.7 |
| Jurkat neg 1:10 | phosp... | Seconda... | P2:7 | | | 112866.6 |

Each row in the table shows the individual results for the named peaks detected in each capillary.

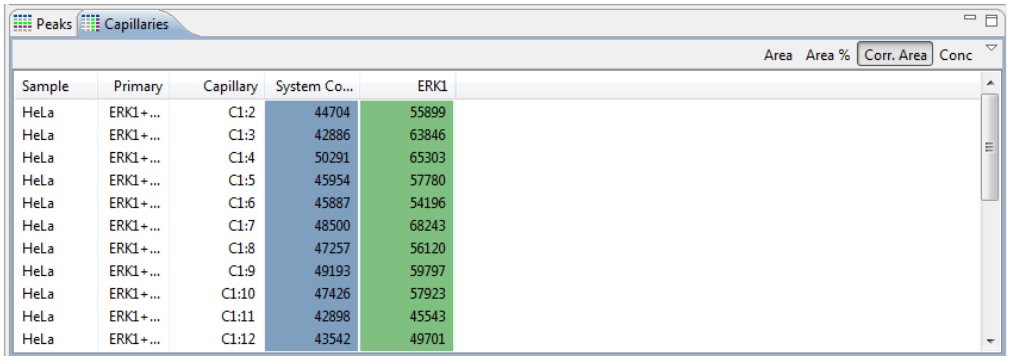
## Viewing the Capillaries Pane

- **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.
- **To select columns for display** - Right-click in the Capillaries pane, then select **Columns...** and select the Capillaries table columns to display.

**NOTE:** Selection of columns applies to Compass views only. These column selection changes are not reflected in exports and reports.

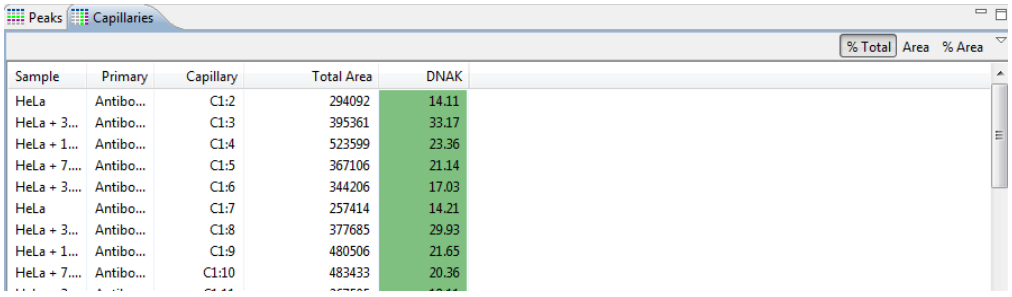
Capillaries table column descriptions are as follows:

- **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.
- **Sample Attr** - Sample attribute. If sample attributes were entered in the assay template (Assay screen), those names will display here.
- **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 Attr** - Primary antibody attribute. If primary antibody attributes were entered in the assay template (Assay screen), those names will display here.
- **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.
- **Secondary Attr** - Secondary antibody attribute. If secondary antibody attributes were entered in the assay template (Assay screen), those names will display here.
- **Capillary**
  - **Jess/Abby Immunoassays (non-RePlex):** Capillary number.
  - **Jess/Abby RePlex Assays:** Probe and capillary number. For example, P1:4 indicates Probe 1, capillary 4.
  - **Leo Immunoassays (non-RePlex):** Cartridge and capillary number. For example, C1:4 indicates cartridge 1, capillary 4
  - **Leo RePlex Assays:** Cartridge, Probe, and capillary number. For example, C1P1:4 indicates cartridge 1, Probe 1, capillary 4.
- **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 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.




| 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 |

- **Viewing % area in the peak name columns** - Select **% Area** 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 peaks within the same Peak Names Analysis Group for the capillary and multiplying by 100.
- **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 when a Loading Control is defined in the Analysis settings, or correction capillary and cartridge when Cartridge Corrections is enabled in the Analysis settings, or the reference capillary when 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 when a Standard Curve is defined in the Analysis settings.
- **To view % total in the peak name columns** - The Dropped Lines or Dropped Lines (manual) area calculation setting must first be selected. To do this, select **Analysis** from the **Edit** menu, then select **Peak Fit**. In the Peak Find box, select **Dropped Lines** or **Dropped Lines (manual)** for the Area Calculation setting. 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.

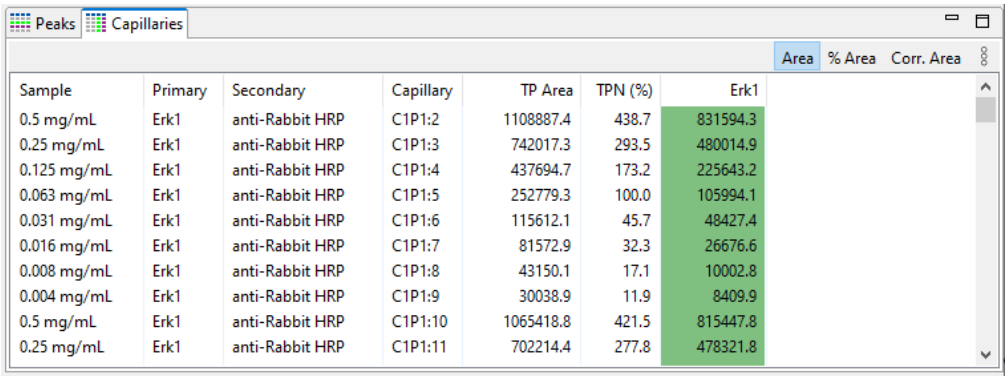



| Sample | Primary | Capillary | Total Area | DNAK |
|---|---|---|---|---|
| HeLa | Antibo... | C1:2 | 294092 | 14.11 |
| HeLa + 3... | Antibo... | C1:3 | 395361 | 33.17 |
| HeLa + 1... | Antibo... | C1:4 | 523599 | 23.36 |
| HeLa + 7.... | Antibo... | C1:5 | 367106 | 21.14 |
| HeLa + 3.... | Antibo... | 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 |

## RePlex and Stellar Assays with Total Protein

For RePlex and Stellar Assays with Total Protein enabled, each row in the table shows the individual results for the total protein and named peaks detected in each capillary, as shown below.

- **TP Area** - Total area measured in the capillary for the Total Protein signal.




| Sample | Primary | Secondary | Capillary | TP Area | TPN (%) | Erk1 |
|---|---|---|---|---|---|---|
| 0.5 mg/mL | Erk1 | anti-Rabbit HRP | C1P1:2 | 1108887.4 | 438.7 | 831594.3 |
| 0.25 mg/mL | Erk1 | anti-Rabbit HRP | C1P1:3 | 742017.3 | 293.5 | 480014.9 |
| 0.125 mg/mL | Erk1 | anti-Rabbit HRP | C1P1:4 | 437694.7 | 173.2 | 225643.2 |
| 0.063 mg/mL | Erk1 | anti-Rabbit HRP | C1P1:5 | 252779.3 | 100.0 | 105994.1 |
| 0.031 mg/mL | Erk1 | anti-Rabbit HRP | C1P1:6 | 115612.1 | 45.7 | 48427.4 |
| 0.016 mg/mL | Erk1 | anti-Rabbit HRP | C1P1:7 | 81572.9 | 32.3 | 26676.6 |
| 0.008 mg/mL | Erk1 | anti-Rabbit HRP | C1P1:8 | 43150.1 | 17.1 | 10002.8 |
| 0.004 mg/mL | Erk1 | anti-Rabbit HRP | C1P1:9 | 30038.9 | 11.9 | 8409.9 |
| 0.5 mg/mL | Erk1 | anti-Rabbit HRP | C1P1:10 | 1065418.8 | 421.5 | 815447.8 |
| 0.25 mg/mL | Erk1 | anti-Rabbit HRP | C1P1:11 | 702214.4 | 277.8 | 478321.8 |

TPN (%) is calculated as follows:

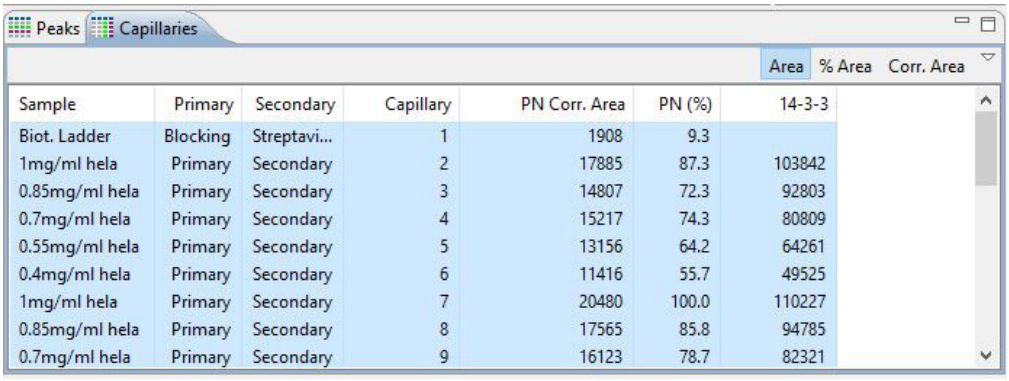
**Capillary Normalization Factor** = Total Protein signal for chosen capillary/Total Protein signal for reference capillary in Analysis settings

Then,

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

## Peak Normalization Jess

When using the Protein Normalization Module on Jess, where the PN Corr.Area column accounts for the Protein Normalization signal automatically, the Capillaries pane is shown below.




| 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 calculated as follows:

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

PN (%) is calculated as follows:

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

Then,

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

## Cartridge Corrections (Leo only)

To view the Corr.Area in the Capillaries pane when Cartridge Corrections is enabled, click the **Corr.Area** button at the upper, right corner of the Capillaries pane. Cartridge Corrections is only applied to the named peak chosen under Peak in Cartridge Corrections analysis settings.

Cartridge Correction Corr.Area is determined by first calculating the cartridge correction factor for each cartridge using the correction capillaries and either a single correction cartridge or the cartridge average, and then correcting peak area for each signal as follows:

- **Using a single correction cartridge:** Correction Factor for a cartridge = Average peak area of the named peak for the chosen Correction Capillaries on a cartridge/Average peak area of the named peak for the chosen Correction Capillaries on the Correction Cartridge
- **Using the cartridge average:** Correction Factor for a cartridge = Average peak area of the named peak for the chosen Correction Capillaries on a cartridge/Average peak area of the named peak for the chosen Correction Capillaries on all cartridges

Then,

**Corr.Area** = Peak Area * Correction Factor

**NOTE:** Peaks that Compass for Simple Western names automatically with user-defined peak name settings are color-coded. Information displayed for fluorescent standards data will be for identified standards.

# Viewing Run Data

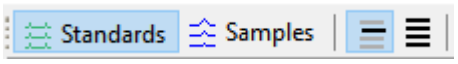
Each run file contains the following data for up to 25 capillaries for Jess and Abby and up to 100 capillaries for Leo:

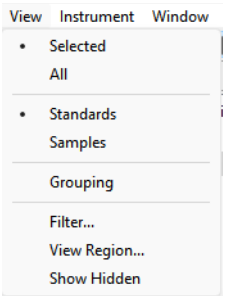
- **Sample data** - For the proteins in the sample.
- **Standards data** - For the fluorescent standards run with each sample.

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

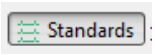
## Switching Between Sample and Standards Data Views

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

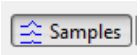


Data buttons in the View bar:

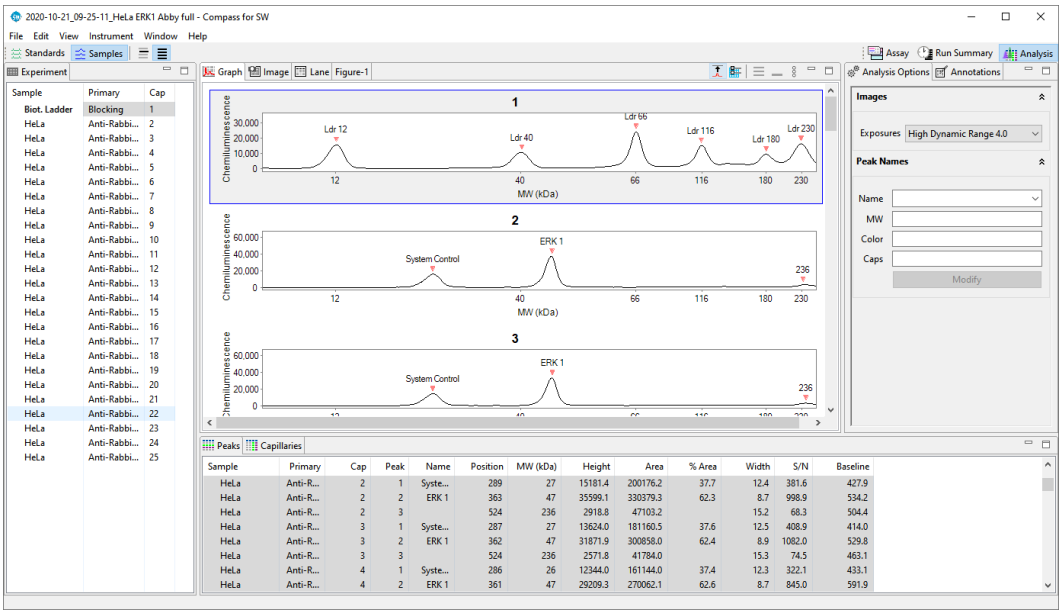



Show Standards




Show Samples

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



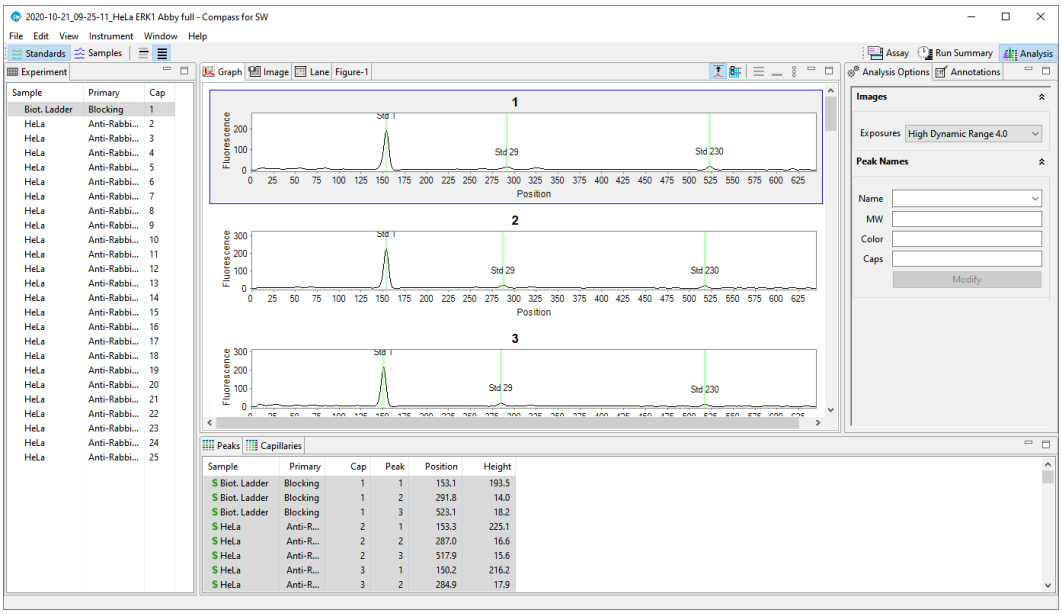

- Data in this view is for sample proteins only.
- Graph view data displays electropherograms in chemiluminescence, NIR or IR units (y-axis) and molecular weight in kDa (x-axis). If Protein Normalization was performed in the same immunoassay run, the overlay is also displayed in the Graph view.
- Lane 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.[1,2]

[1] *Electrophoresis. 2021 Aug;42(14-15):1521-1531. doi: 10.1002/elps.202100068.*
[2] *Electrophoresis. 2021 Feb;42(3):206-218. doi: 10.1002/elps.202000199.*

For information on checking and identifying sample peaks, see “Step 3 – Checking the Ladder” on page 195 or “Step 4 – Checking Samples” on page 197.

- **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 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 the Peaks tables and marked with an **S**.

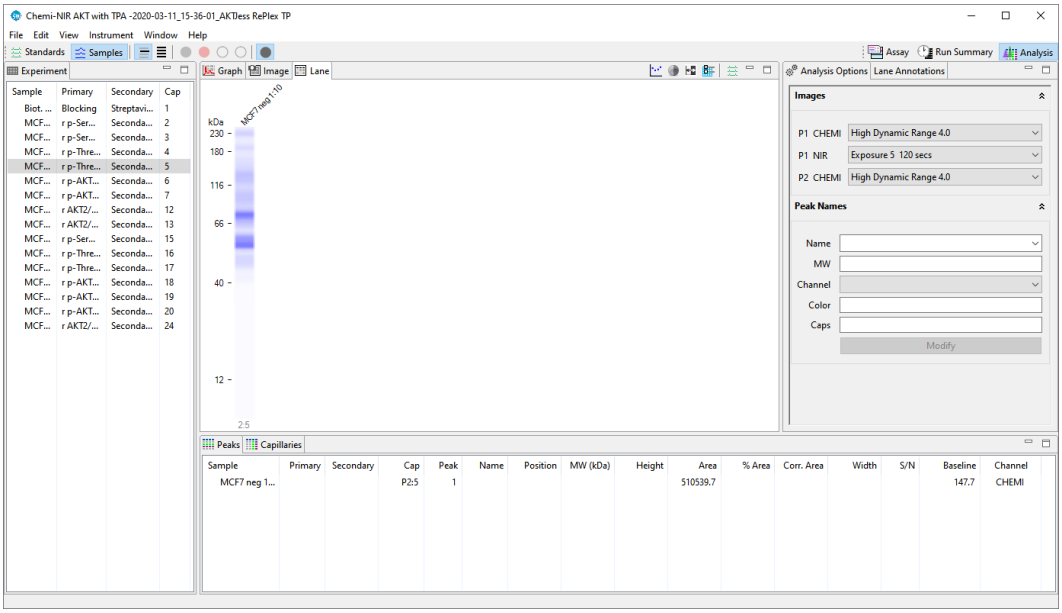
  For information on checking and identifying standards peaks, see “Step 2 – Checking Fluorescent Sizing Standards” on page 193.

## Selecting and Displaying Capillary Data

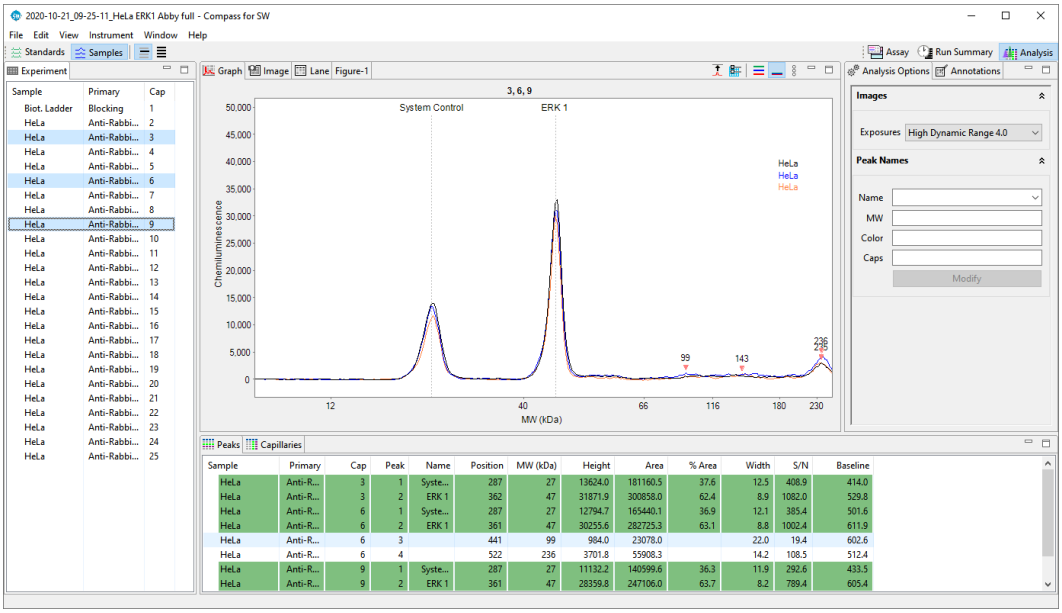
You can choose to view data from one, multiple, or all capillaries at once.

1. Select **View** in the main menu and click **View Selected**.

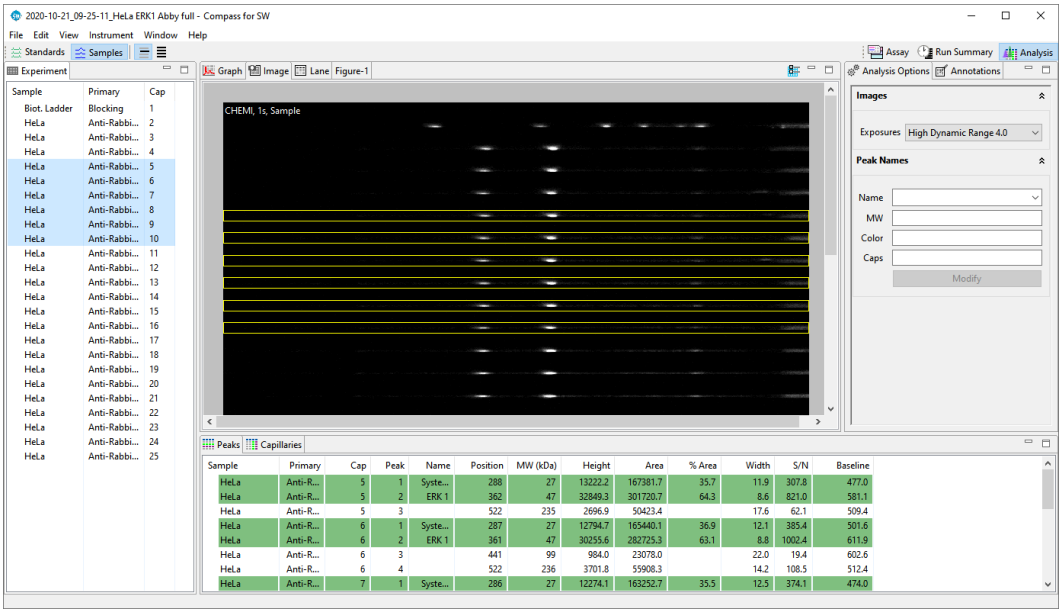
- **To look at data for one capillary** - Click a row in the Experiment pane. Data for just the row selected will display in all data views and tables. The following example shows sample data displayed in the Lane and Peaks panes when one sample is selected:



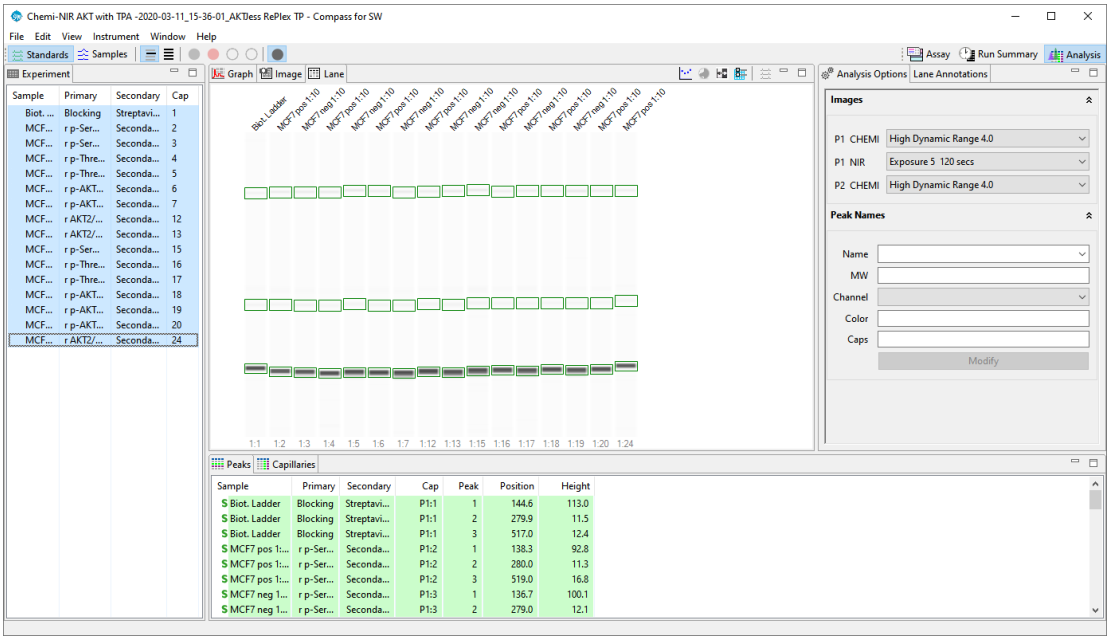

- **To look at data for multiple non-sequential capillaries** - Hold the **Ctrl** key and select the rows you want to view in the Experiment pane. Data for only the rows selected will display in all data views and results tables. The following example shows sample data displayed in the Graph (in Overlay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



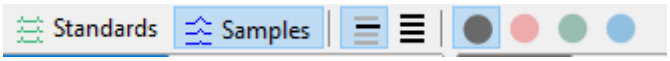

- **To look at data for all capillaries** - Hold the **Shift** key and select the first and last rows in the Experiment pane. Data for all rows will display in all data views and results tables. You can also select **All** in the **View** menu. The following example shows standards data displayed in the Lane and Peaks panes when all rows are selected.



## 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.

Detection channels in the View bar:

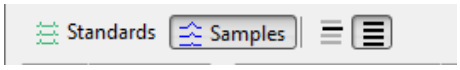


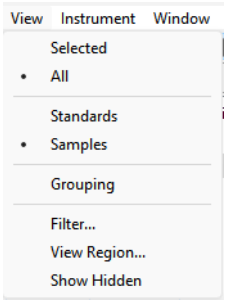

Detection channels in the View bar:

- Overlay chemiluminescent channel (gray)
- Overlay near infrared channel (red)
- Overlay infrared channel (green)
- Overlay protein normalization channel (blue)
- Unused channel (no color)
- Color is available but not selected

## 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 a multiple capillary format. This view is selected in the View bar or the View menu.

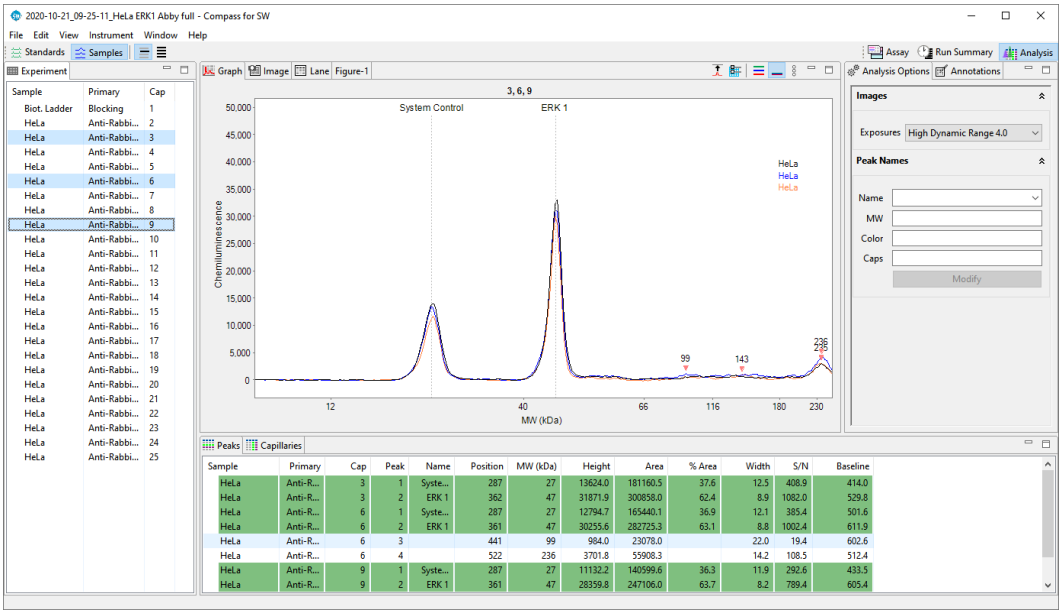



Capillary view buttons in the View bar:

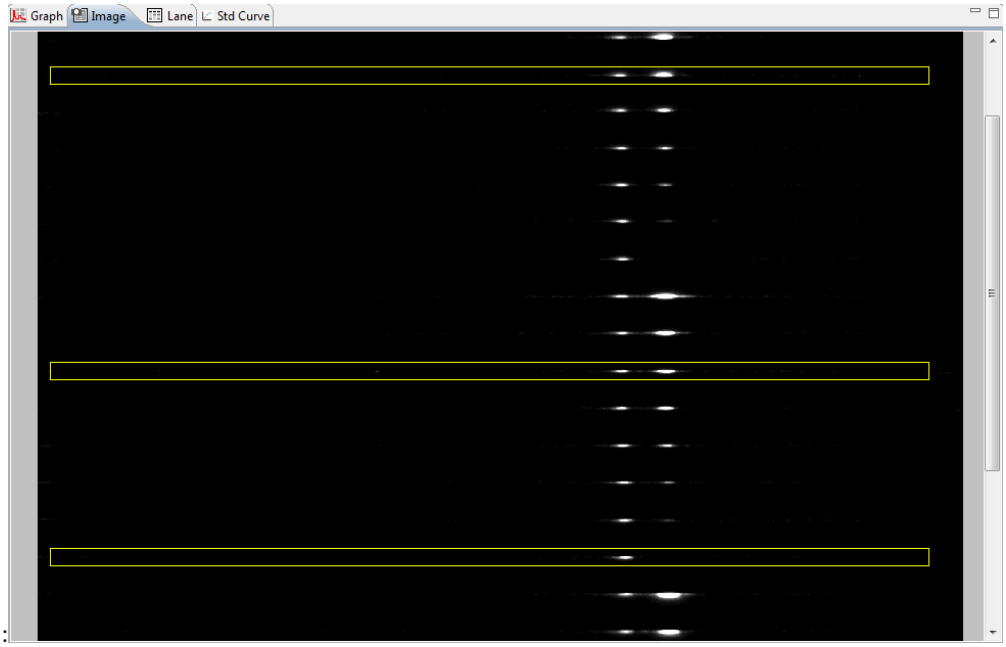
- View Selected
- View All

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

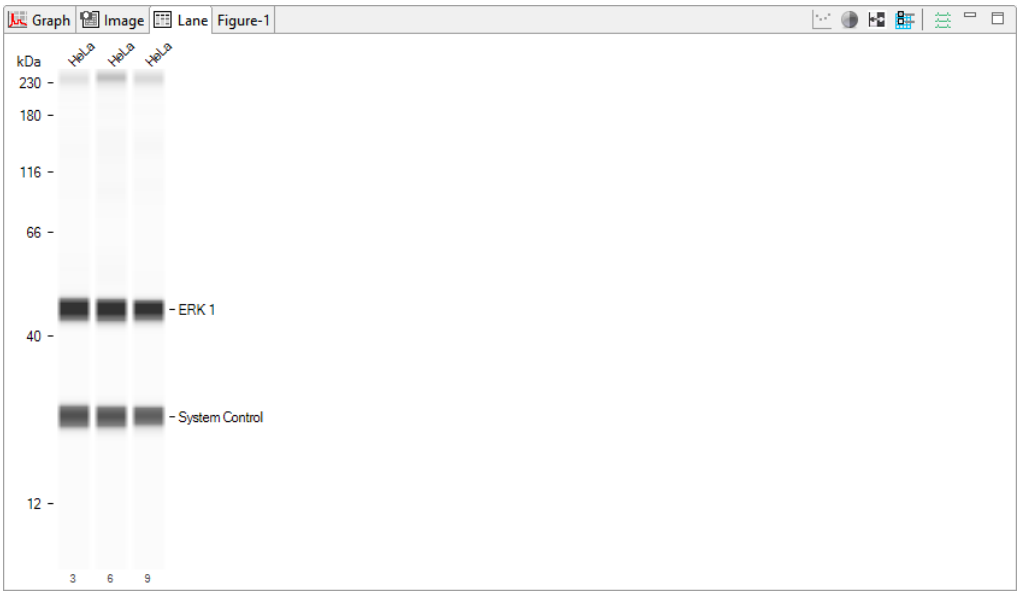



Data for the row(s) selected in the Experiment pane will display as follows:

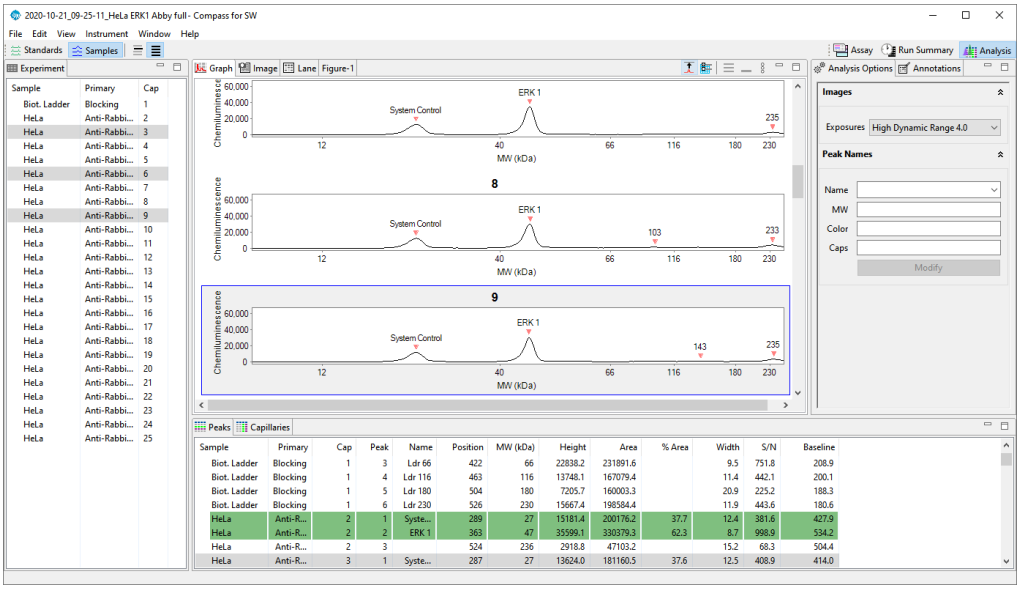
- Electropherograms in the Graph pane display overlaid or stacked (depending on the graph option chosen).
- Results for only the selected row(s) will display in the Peaks and Capillaries tables.
- The selected row(s) are highlighted in the Image pane:



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



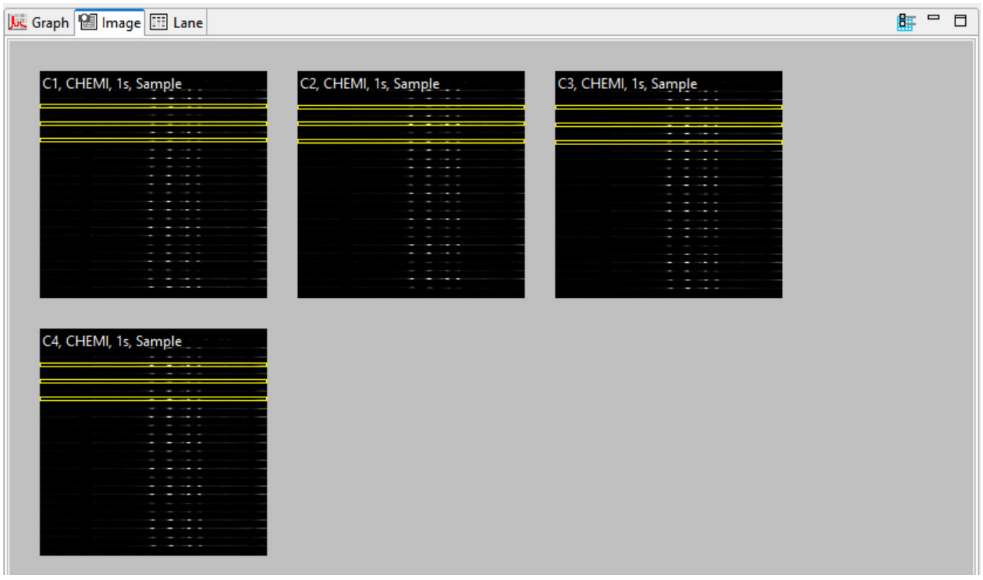

- **To view data in a multiple capillary format** - Click **View All** in the View bar, or select **View** in the main menu and click **All**:



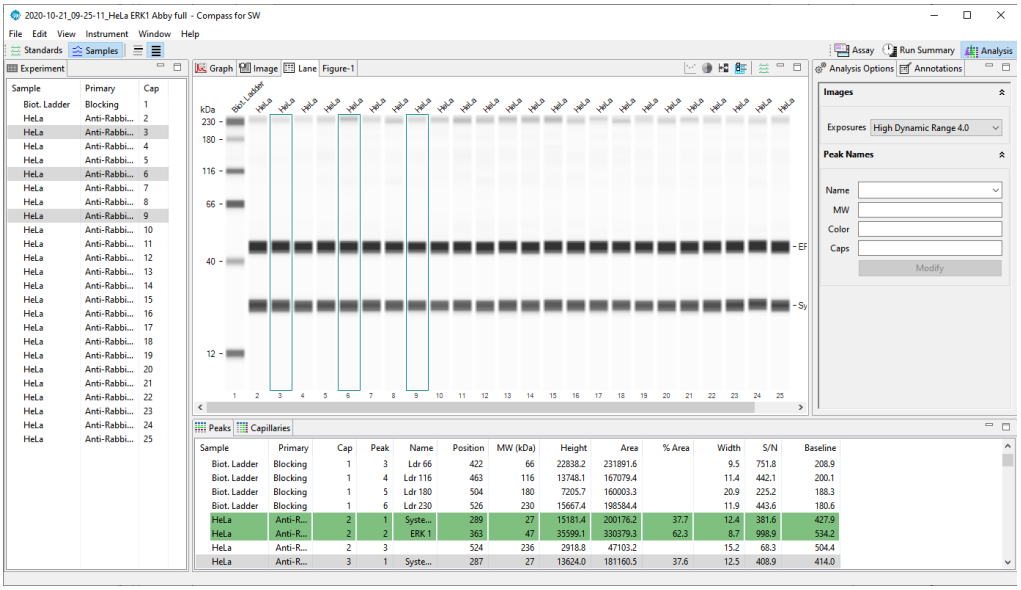

Data for the row(s) selected in the Experiment pane will display as follows:

- Electropherograms corresponding to the selected row(s) are highlighted in the Graph pane.
- Results for selected capillaries are highlighted in the Peaks and Capillaries tables.

- The selected row(s) are highlighted in the Image pane:



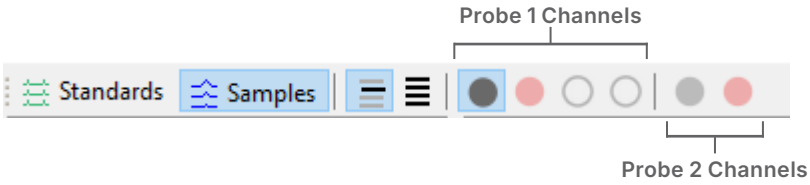

- All selected lanes display in the Lane pane, and lanes corresponding to the selected row(s) are highlighted.



# Viewing RePlex Channel Views

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 FOR JESS AND LEO:**




**NOTE:** NIR, IR, and Protein Normalization Channels are inactive (gray) when viewing a Leo RePlex run.

Detection channels in the View bar:


Overlay chemiluminescent channel (gray)


Overlay near infrared channel (red)

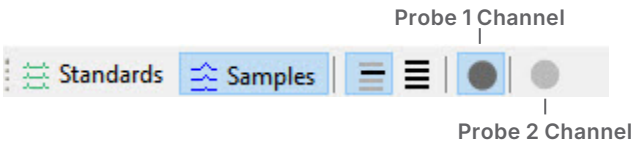

Overlay infrared channel (green)


Unused channel (no color)


Channel data is displayed


Channel data is available but not selected

**CHANNEL DESIGNATIONS FOR ABBY:**



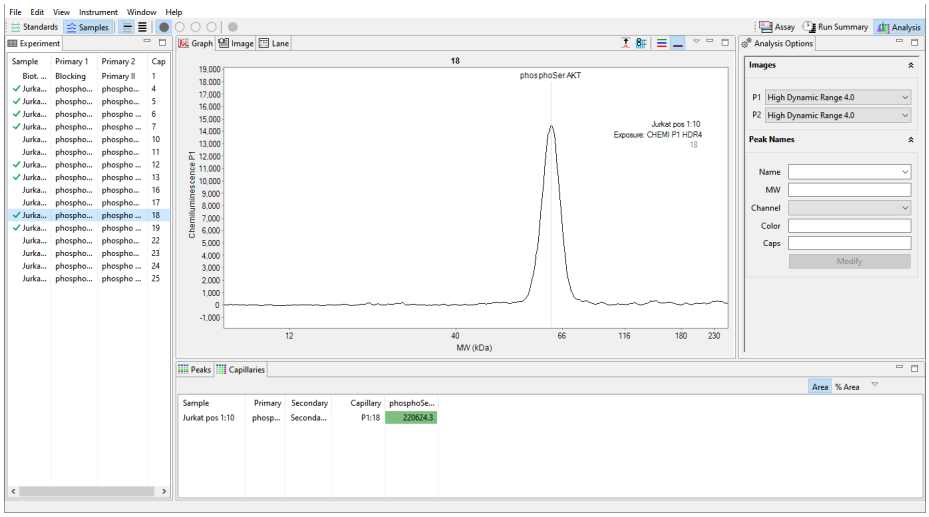
Detection channels in the View bar:

Overlay probe 1 channel

Overlay probe 2 channel

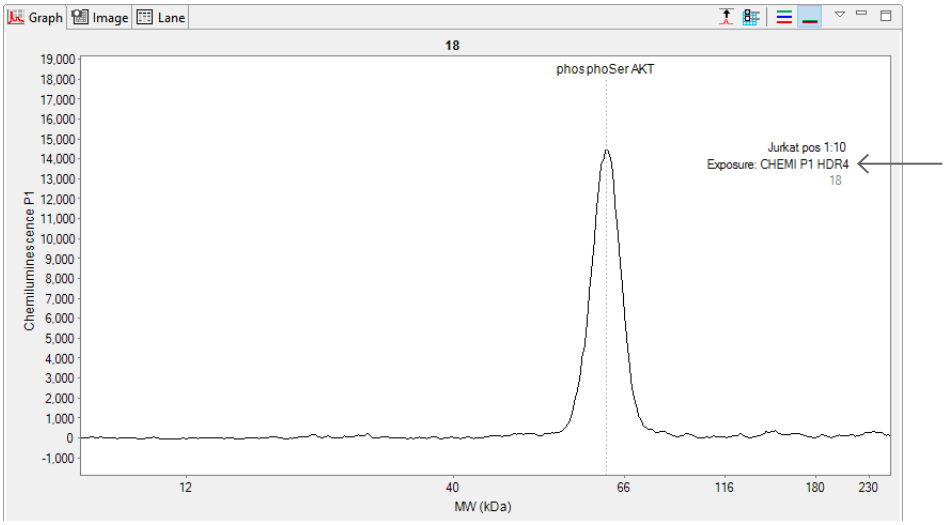
Channel data is available but not selected

The initial view displays Probe 1 data only. In this example showing Jess RePlex Assay data, the Probe 1 channel is chemiluminescence.



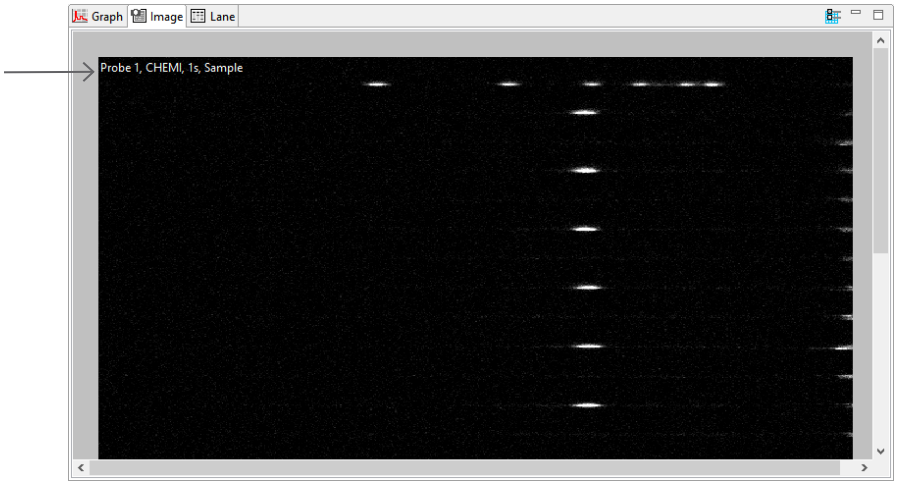

Probe 1 data is labeled in all data views as P1 or Probe 1:

- Graph view:

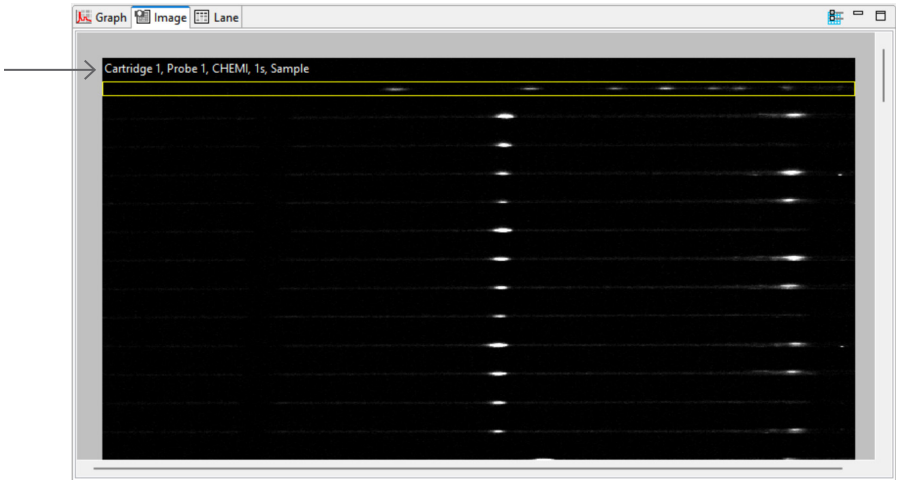


- Image view:

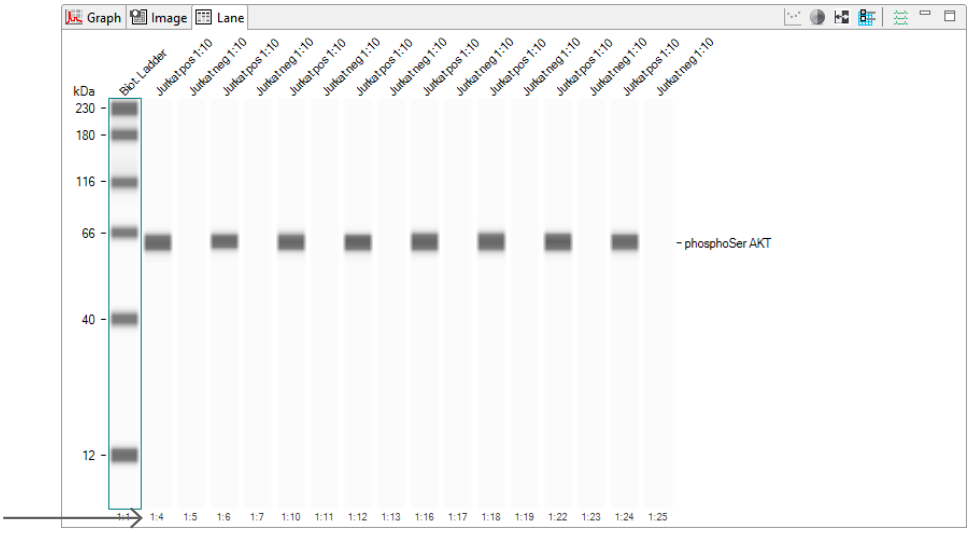
**JESS/ABBY**



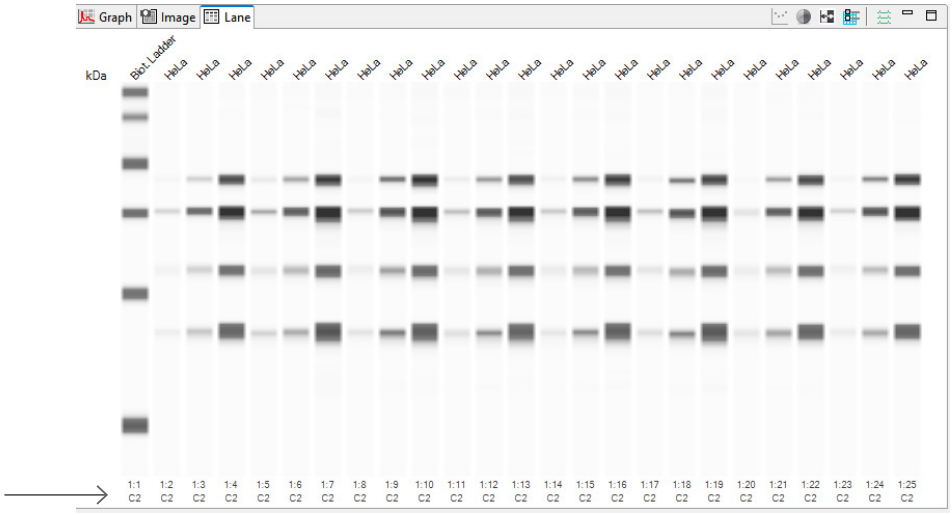

**LEO**



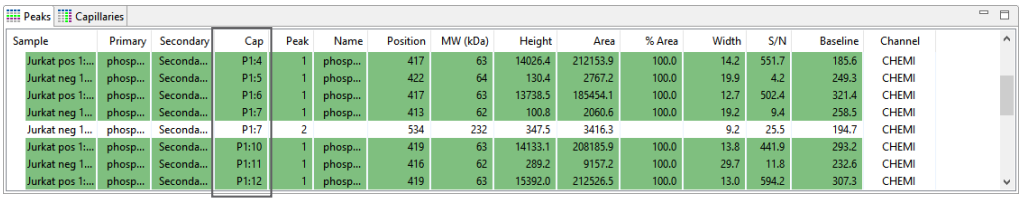
- Lane view
  **Jess and Abby:** Sample lanes are labeled probe number:capillary number



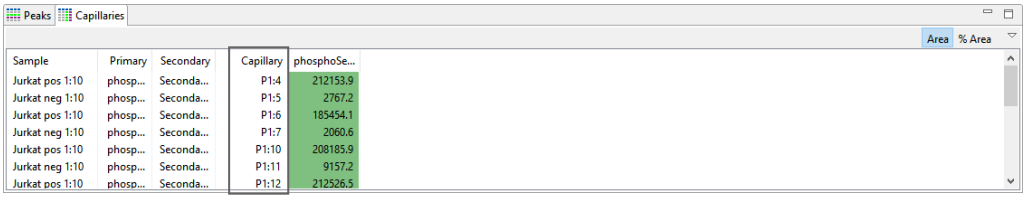

**Leo:** Sample lanes are labeled probe number:capillary number with the cartridge number below.



- Peaks and Capillaries table:
  **Jess and Abby:** Rows are labeled probe number: capillary number

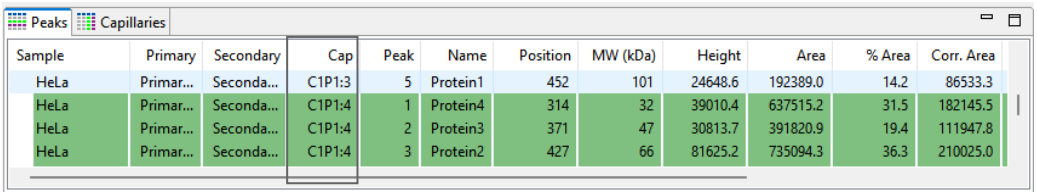


| Sample | Primary | Secondary | Cap | Peak | Name | Position | MW (kDa) | Height | Area | % Area | Width | S/N | Baseline | Channel |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jurkat pos 1:... | phosp... | Seconda... | P1:4 | 1 | phosp... | 417 | 63 | 14026.4 | 212153.9 | 100.0 | 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 |

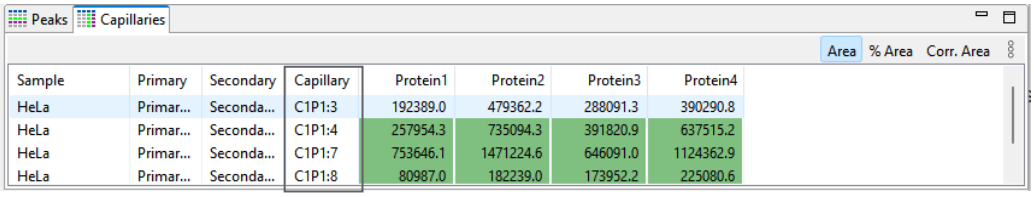


| Sample | Primary | Secondary | Capillary | phosphoSe... |
|---|---|---|---|---|
| Jurkat pos 1:10 | phosp... | Seconda... | P1:4 | 212153.9 |
| Jurkat neg 1:10 | phosp... | Seconda... | P1:5 | 2767.2 |
| Jurkat pos 1:10 | phosp... | Seconda... | P1:6 | 185454.1 |
| Jurkat neg 1:10 | phosp... | Seconda... | 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 |

**Leo:** Rows are labeled cartridge number: probe number: capillary number



| Sample | Primary | Secondary | Cap | Peak | Name | Position | MW (kDa) | Height | Area | % Area | Corr. Area |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HeLa | Primar... | Seconda... | C1P1:3 | 5 | Protein1 | 452 | 101 | 24648.6 | 192389.0 | 14.2 | 86533.3 |
| HeLa | Primar... | Seconda... | C1P1:4 | 1 | Protein4 | 314 | 32 | 39010.4 | 637515.2 | 31.5 | 182145.5 |
| HeLa | Primar... | Seconda... | C1P1:4 | 2 | Protein3 | 371 | 47 | 30813.7 | 391820.9 | 19.4 | 111947.8 |
| HeLa | Primar... | Seconda... | C1P1:4 | 3 | Protein2 | 427 | 66 | 81625.2 | 735094.3 | 36.3 | 210025.0 |

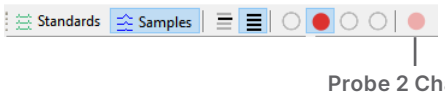


| Sample | Primary | Secondary | Capillary | Protein1 | Protein2 | Protein3 | Protein4 |
|---|---|---|---|---|---|---|---|
| HeLa | Primar... | Seconda... | C1P1:3 | 192389.0 | 479362.2 | 288091.3 | 390290.8 |
| HeLa | Primar... | Seconda... | C1P1:4 | 257954.3 | 735094.3 | 391820.9 | 637515.2 |
| HeLa | Primar... | Seconda... | C1P1:7 | 753646.1 | 1471224.6 | 646091.0 | 1124362.9 |
| HeLa | Primar... | Seconda... | C1P1:8 | 80987.0 | 182239.0 | 173952.2 | 225080.6 |

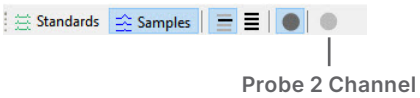
## Viewing Data for Probe 2

To view chemiluminescent or NIR data (Jess only) for Probe 2, select its channel in the view bar.

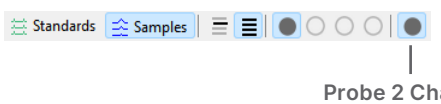
**JESS**



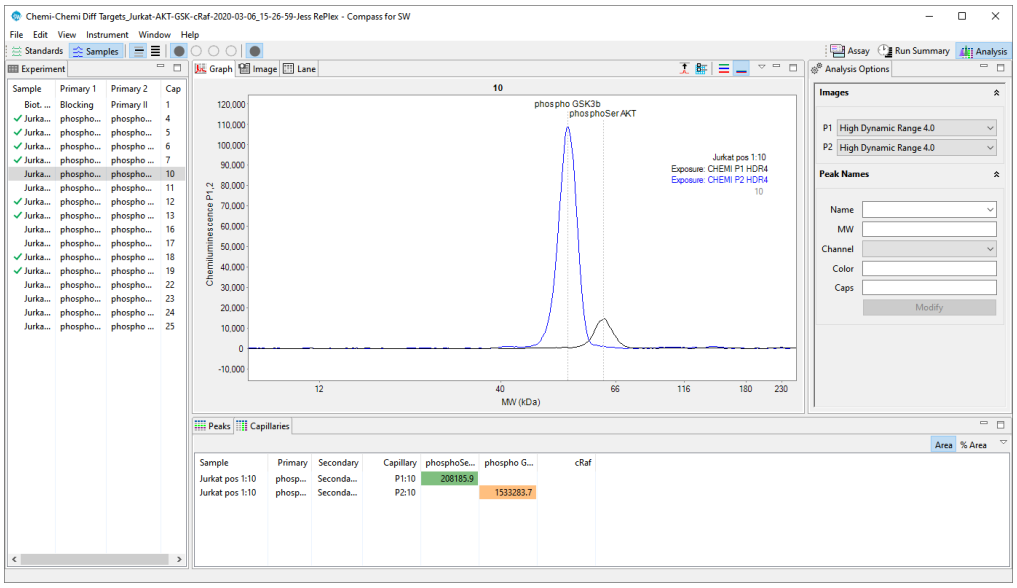

**ABBY**



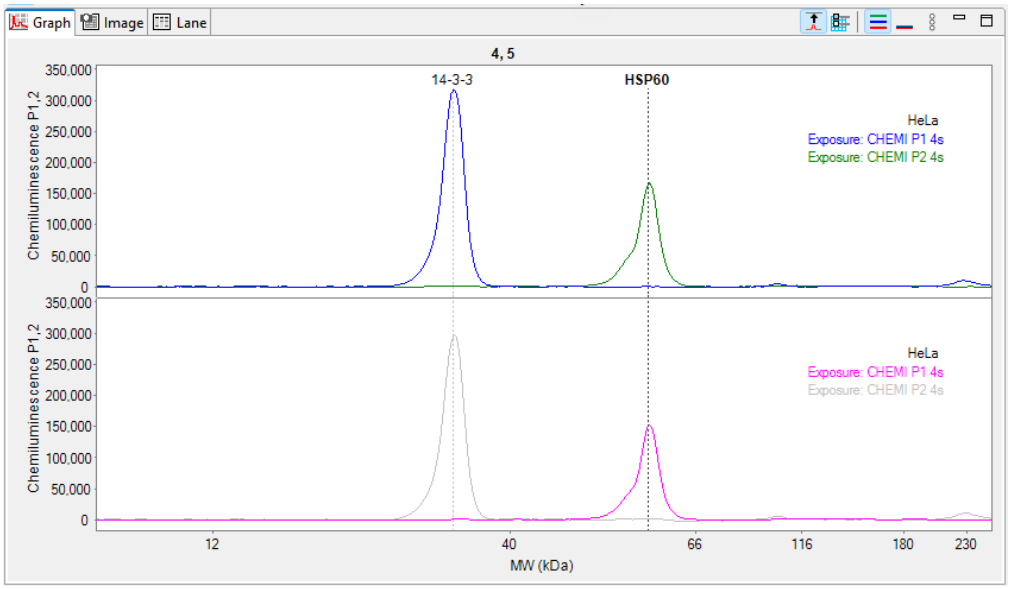

**LEO**



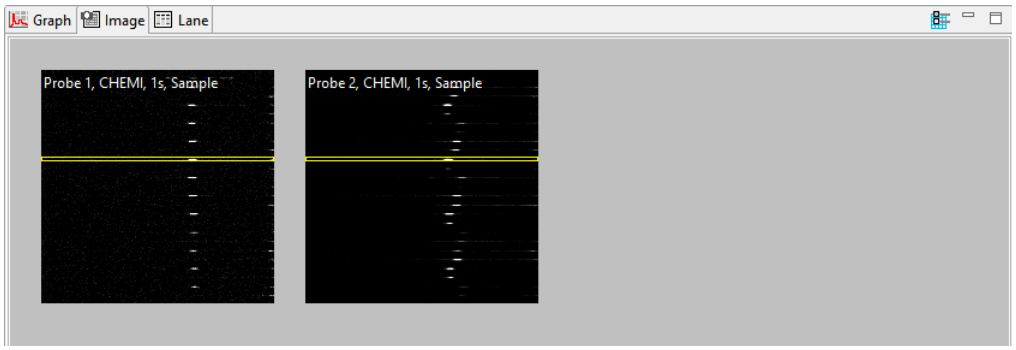
In this example, showing Jess RePlex Assay data, Probe 2 is labeled P2 or Probe 2:




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

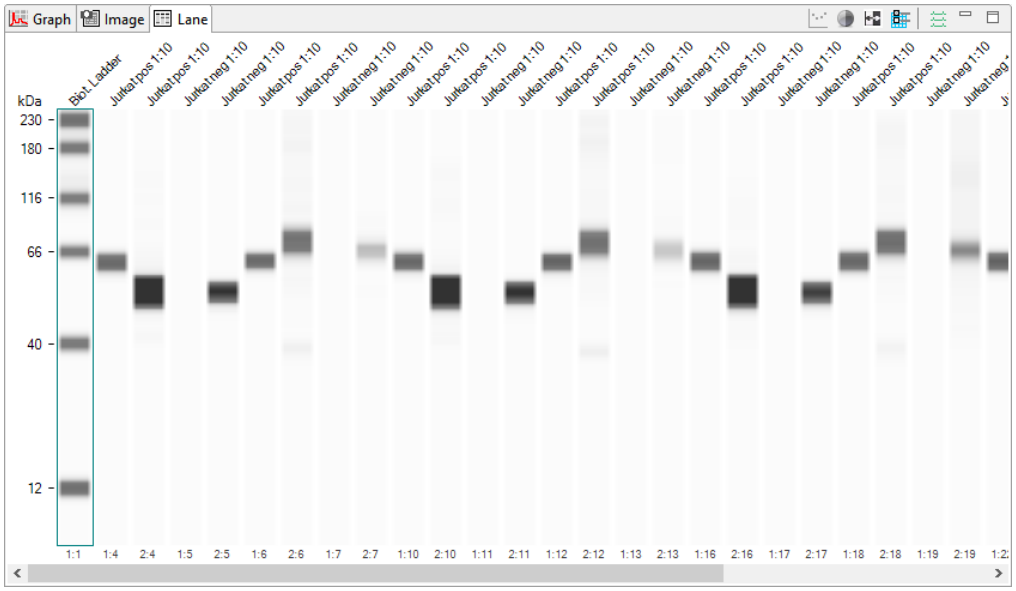


- Image view will display separate images for each probe:

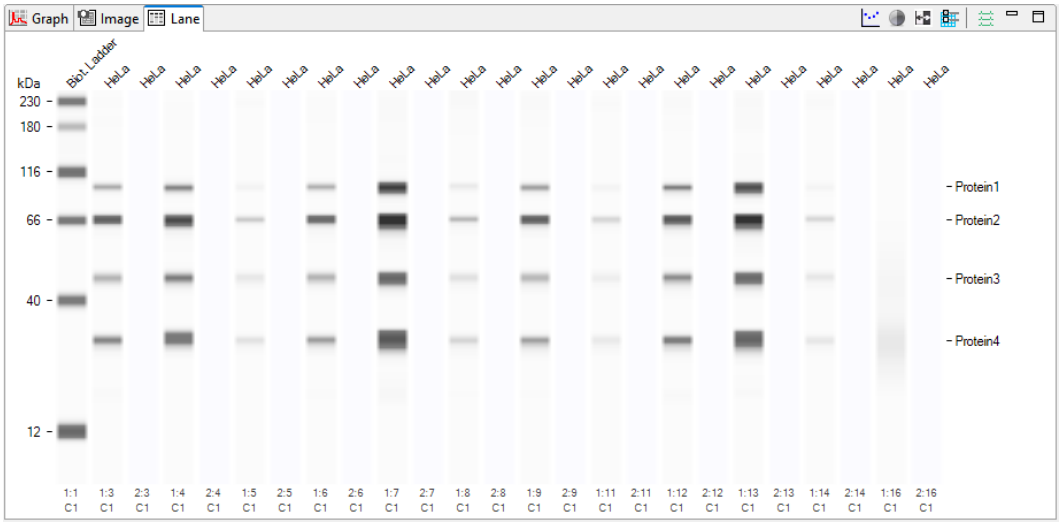



- Lane view will display P1 and P2 lanes side by side:

  **Jess and Abby:** Sample lanes are labeled probe number: capillary number

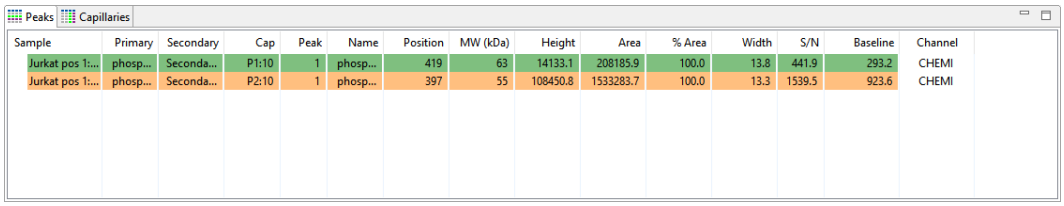


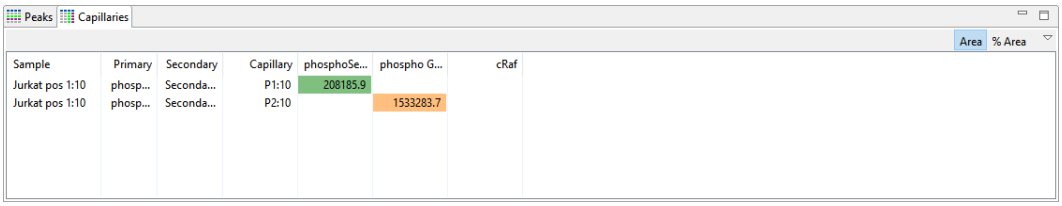
**Leo:** Sample lanes are labeled probe number:capillary number with the cartridge number below



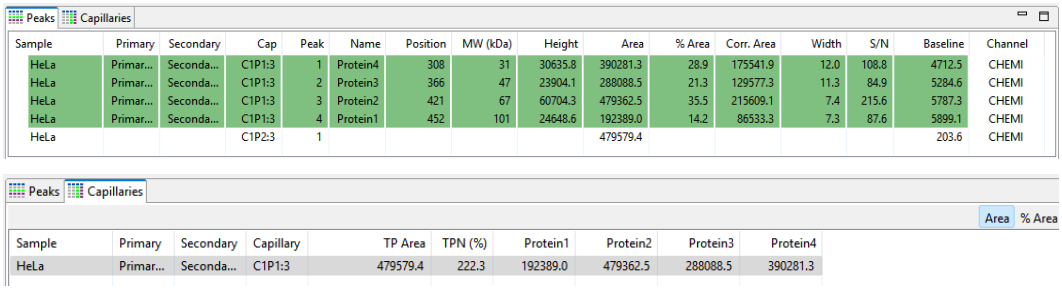

- Peaks and Capillaries tables will display all row data for P1 first, then P2:

  **Jess and Abby:** Rows are labeled probe number : capillary number



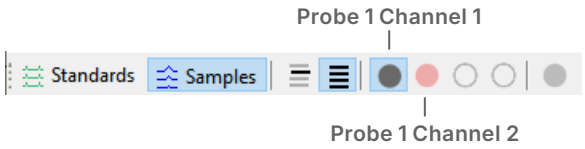




**Leo:** Rows are labeled cartridge number : probe number : capillary number

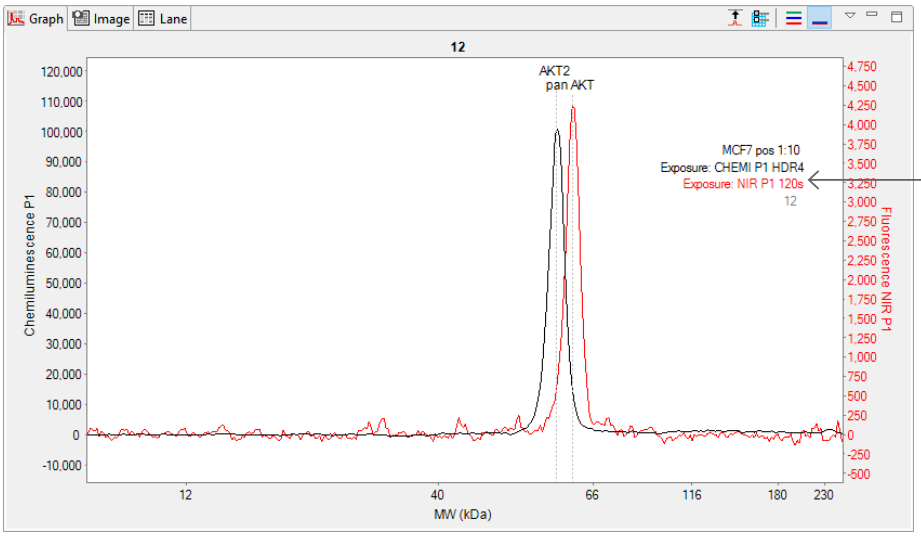


## Viewing Data for a Second Channel in Probe 1 (Jess only)

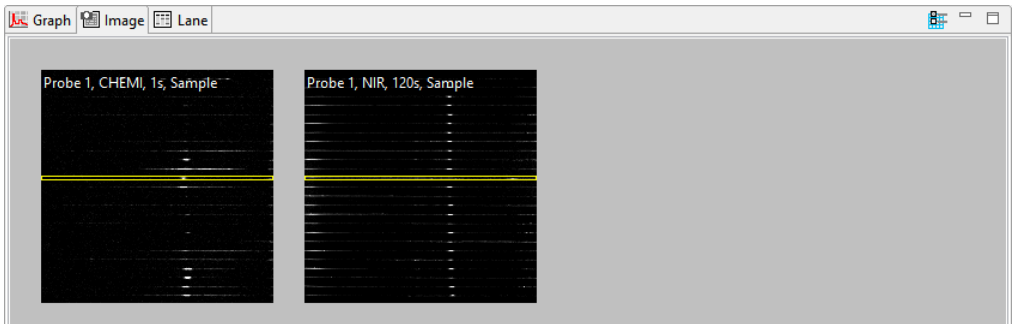
To view the second channel data for Probe 1, for example in a Chemiluminescence and Fluorescence NIR Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection assay, select its channel in the view bar:



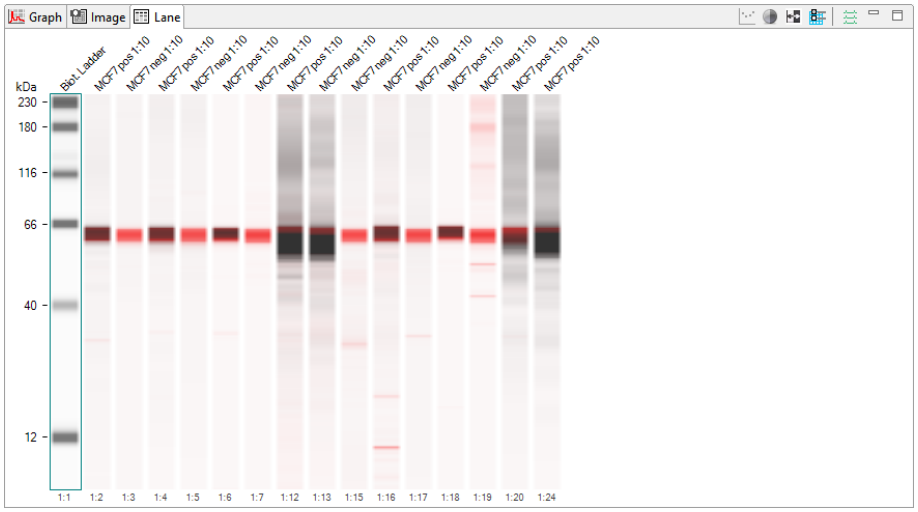

- Graph view will display data stacked or overlaid channel data depending on the option chosen:



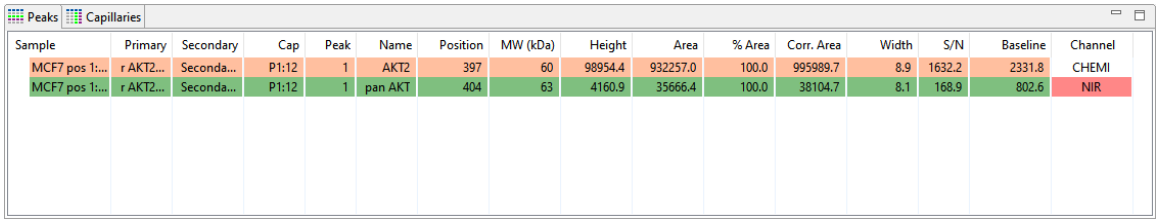
- Image view will display individual images for each channel:

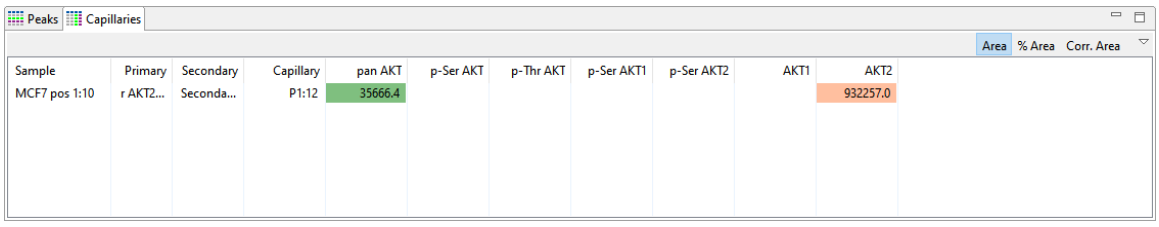



- Lane view will display lanes for each channel overlaid:




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



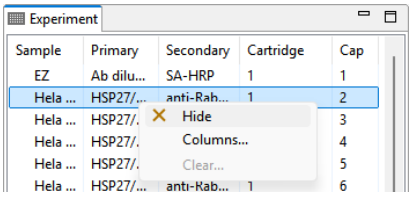



## Hiding Capillary Data

Capillary data can be hidden from view if needed. Hidden caps will not appear in the graph or lane view, will not appear in the Peaks and Capillary tables, and will be excluded from statistical analysis with group data and the exported table files.

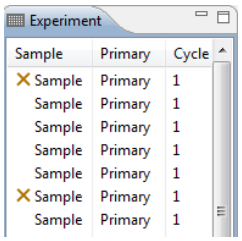
To hide capillaries:

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:



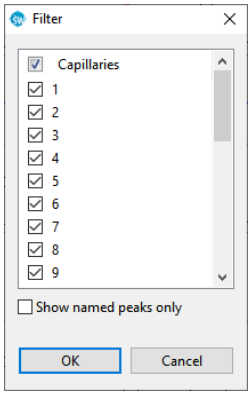

- **To unhide rows** - Select the hidden row(s). Right-click on one of the selected rows and click **Unhide**.

## 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**.



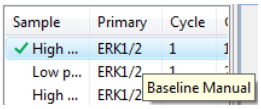

  Only data for the checked capillaries will display in data views and results tables, unchecked capillaries will be hidden.

- **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.

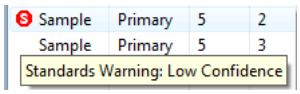
# Run Data Notifications and Warnings

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.

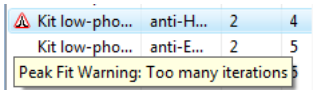



**Standards warning** - Indicates that one of the standards may not be identified properly. This can be resolved by manually identifying the standards. Please refer to "Step 2 – Checking Fluorescent Sizing Standards" on page 193 for details. Rolling the mouse over the icon displays warning details.



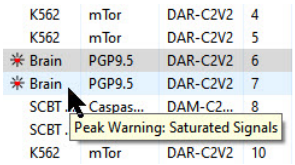

**Manual correction of standards data notification** - Indicates the user modified the standards data manually. Rolling the mouse over the icon displays the type of data modification made.

**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 3 – Checking the Ladder" on page 195 or "Step 4 – Checking Samples" on page 197 for details. Rolling the mouse over the icon displays warning details.




**Peak warning: saturated signals** - Saturation warnings in the Experiment pane indicate there are one or more saturated pixels in the current capillary at the selected exposure setting. If there is a saturation warning when HDR is the selected exposure, it means that a pixel within the capillary was saturated at the shortest exposure time in the image series. Pixels in a peak are considered saturated when they can no longer quantify the data reliably for analysis (i.e., the pixels have hit the threshold value of the amount of light they can absorb). These warnings are displayed for Chemi, NIR, IR, and PN channels and take precedence over all other warnings that are shown for capillaries in the Experiment pane.

When accurate quantitation is needed for samples displaying this warning, we recommend re-running the samples at a higher dilution factor. Rolling the mouse over the icon displays warning details.




# Undo/Redo

Specific actions in Compass for Simple Western can be undone or redone by pressing **Ctrl+Z** and **Ctrl+Y**, or selecting **Undo** or **Redo** from the **Edit** menu. These actions include:

## Assay Changes

- Assay Template
- Assay Protocol
- Assay Layout
- Assay Default Analysis Settings

## Run File Changes

- All Analysis Settings
- All Analysis Options
- Graph - Add/Remove Peaks

- Graph - Add/Remove Baselines
- Graph - Peak Start End
- Graph - Peak Names
- Graph - Force Standard
- Graph - Not a Standard
- Peaks Table - Hide/Unhide
- Peaks Table - Remove Peaks
- Peaks Table - Peak Names
- Lane - Contrast Adjustment
- Lane - Name Peak
- Lane - Samples, Overlay Standards: Not a Standard
- Lane - Samples, Overlay Standards: Force Standard
- Lane - Standards: Not a Standard
- Lane - Standards: Force Standard
- Experiment - Sample Clear Manual settings
- Experiment - Hide/Unhide caps
- Experiment - Standards Clear Manual settings
- Run file - Assay template changes
- Import Analysis Settings
- Import Template
- Template changes

# Checking Your Results

**NOTE:** Visit the **Bio-Techne Academy** for more information about Simple Western size assay data analysis.

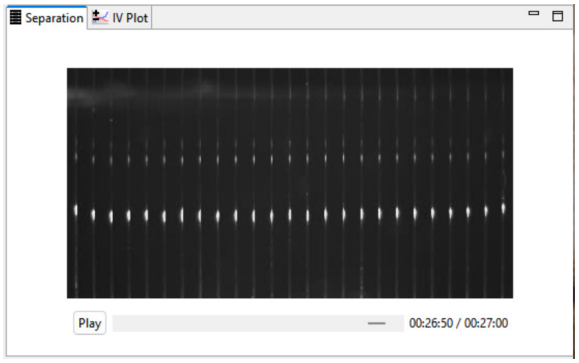
Compass for Simple Western detects proteins and fluorescent standards and reports results automatically with no user-interaction. However, we recommend you review the data as outlined in this section as good general practice to ensure accurate results. If a data warning is presented in the Experiment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

## Step 1 – Review the Fluorescent Sizing Standards Movie

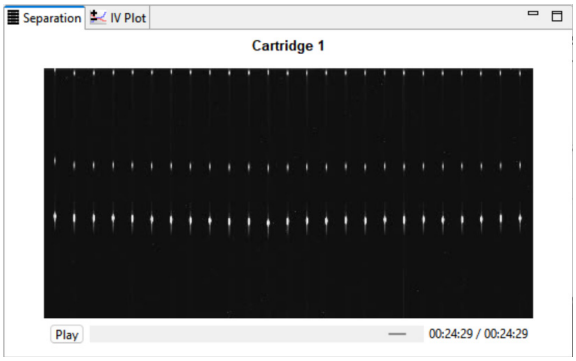
All capillaries should have three fluorescent sizing standards for 12-230 kDa size assays or two fluorescent standards for 66-440 kDa and 2-40 kDa size assays. To verify the standards separated in the capillary correctly:

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).

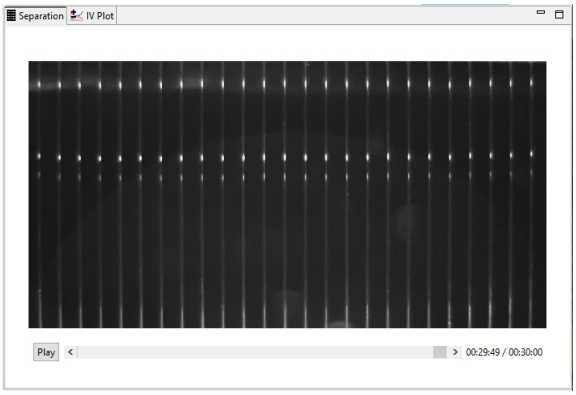
**2-40 kDa FLUORESCENT STANDARDS ON JESS**




**12–230 kDa FLUORESCENT STANDARDS ON LEO**




**66–440 kDa FLUORESCENT STANDARDS ON ABBY**



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

## Step 2 – Checking Fluorescent Sizing Standards

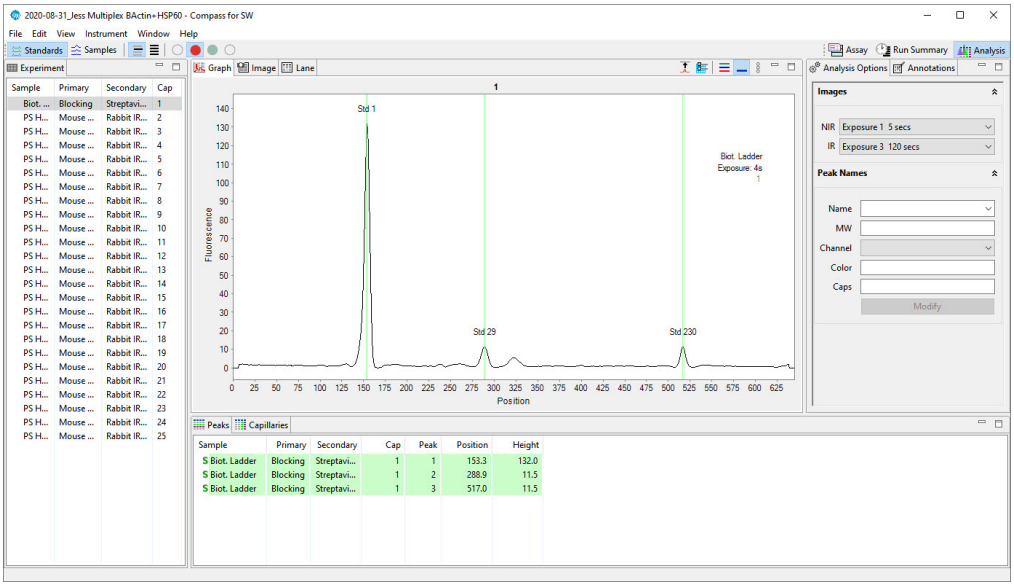
To verify the standards are identified correctly:

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 **View Selected** in the View bar.

b. Click on the first row in the Experiment pane, then click the **Graph** tab. Check that the electropherogram has 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 26 (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**.

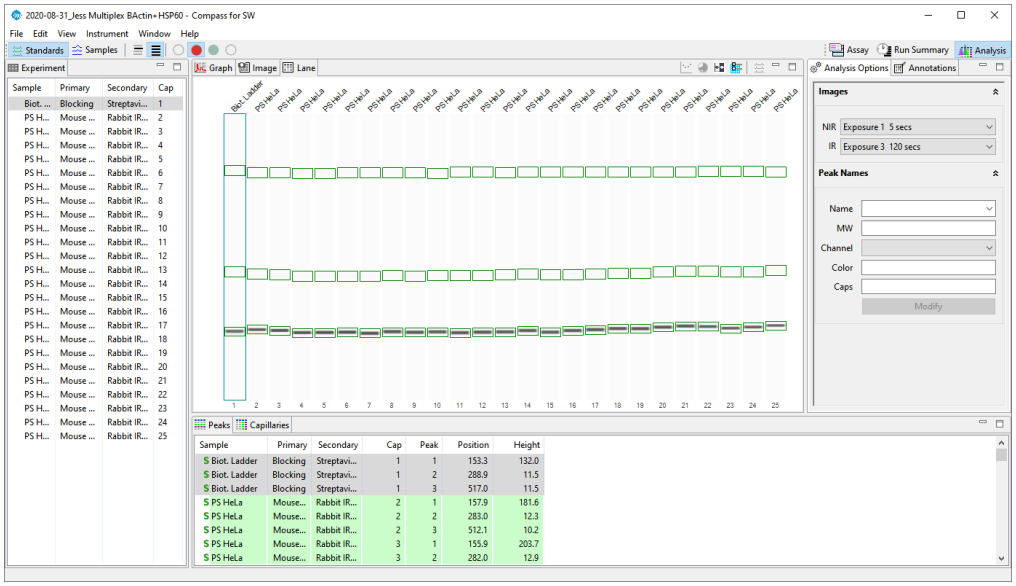
c. Repeat the previous steps for the remaining rows in the Experiment pane to make sure all standards are identified correctly.

**Lane Pane:**

**NOTE:** We recommend checking Fluorescent Sizing Standards using the Graph pane.

a. Click **View All** 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 by rolling the cursor over the individual bands: Std 1, Std 29 and Std 230 (for 12-230 kDa size assays) or Std 57 and Std 280 (for 66-440 kDa size assays) or Std 1 and Std 26 (for 2-40 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.

**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.

## Step 3 – Checking the Ladder

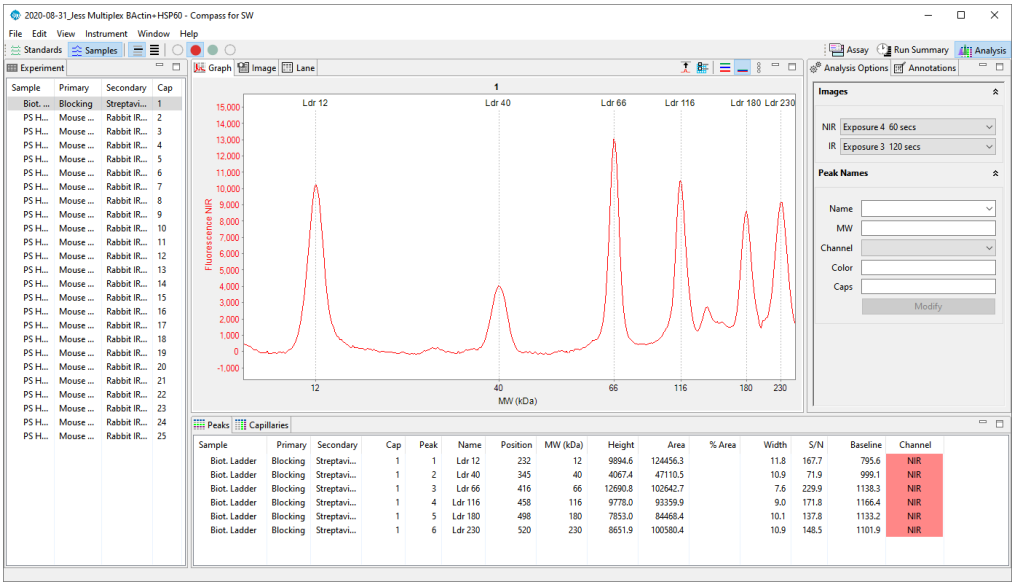
Depending on the size assay you are running, the biotinylated ladder should have the following sizing standards: 12, 40, 66, 116, 180 and 230 kDa (for 12-230 kDa size assays), 66, 116, 200, 280 and 440 kDa (for 66-440 kDa assays) or 2, 5, 12, 26, and 40 kDa (for 2-40 kDa assays). To verify the ladder 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 **View Selected** 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:

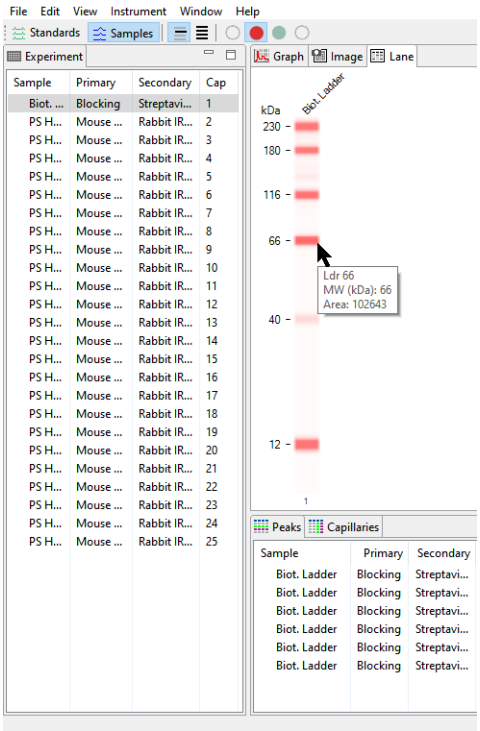
- **If an incorrect peak is identified as a ladder peak** - Right-click the peak in the electropherogram or Peaks table and select **Remove peak**. Compass for Simple Western should correctly reassign the remaining peaks as ladder standards.
- **To set an unidentified peak as a ladder peak** - Right-click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

**Lane Pane:**

a. Click either **View Selected** or **View All** 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 4 – 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.[1,2]

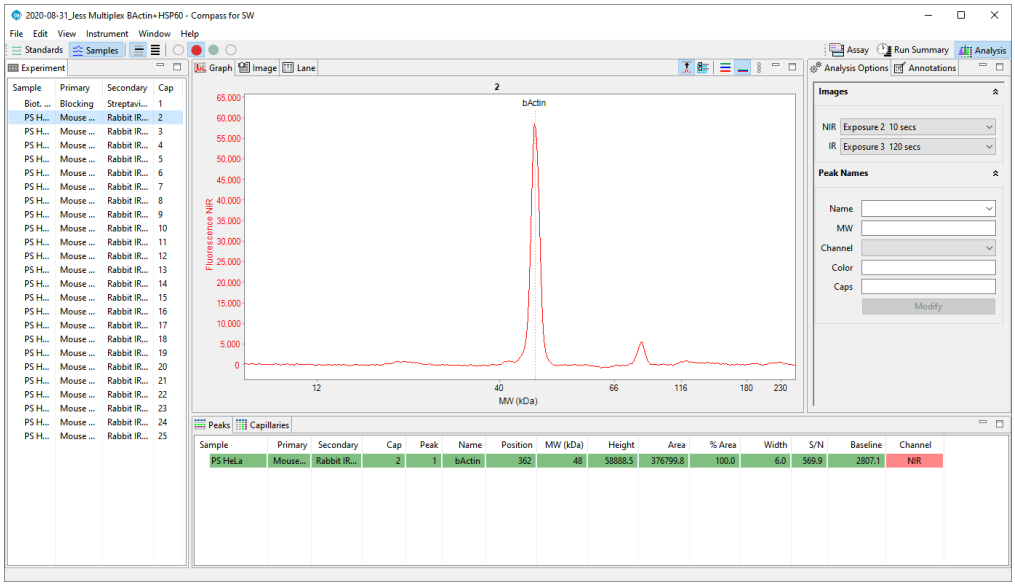
To verify that sample proteins are identified correctly:

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 **View Selected** 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.

[1] *Electrophoresis. 2021 Aug;42(14-15):1521-1531. doi: 10.1002/elps.202100068.*
[2] *Electrophoresis. 2021 Feb;42(3):206-218. doi: 10.1002/elps.202000199.*

- **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.

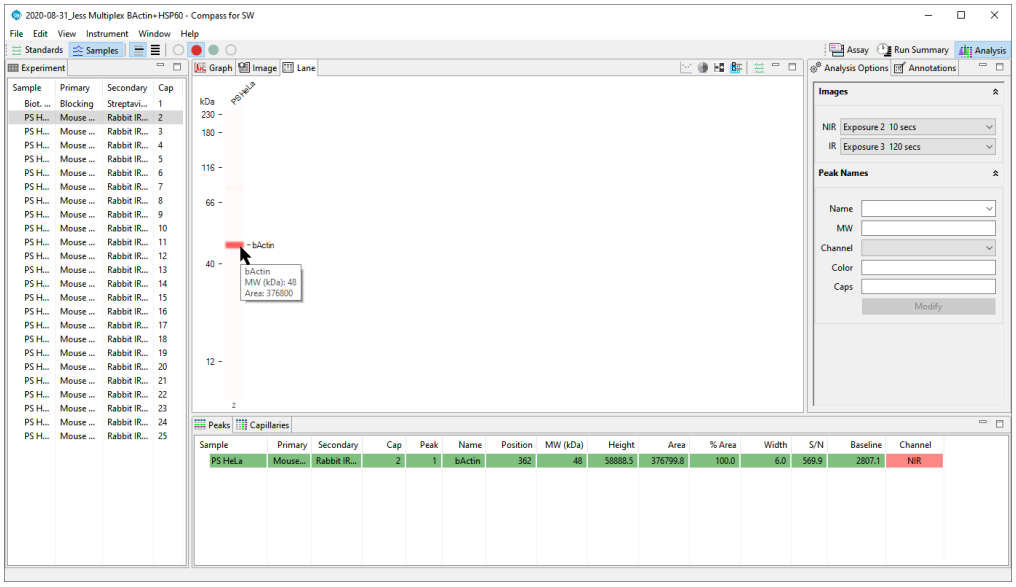
> **NOTE:** To remove sample peak assignments that were made manually and go back to the original view of the data, right-click in the electropherogram, and click **Clear**.

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 **View Selected** or **View All** 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 293.

# Group Statistics

The Grouping View is used to analyze replicates by calculating the mean, standard deviation, coefficient of variation (%CV), and standard error of the mean of named proteins (see “Peak Names Settings” on page 293 for detailed information on entering named proteins). Statistics for each protein are also plotted for easy comparison between samples, antibodies, and proteins.

## Using Groups

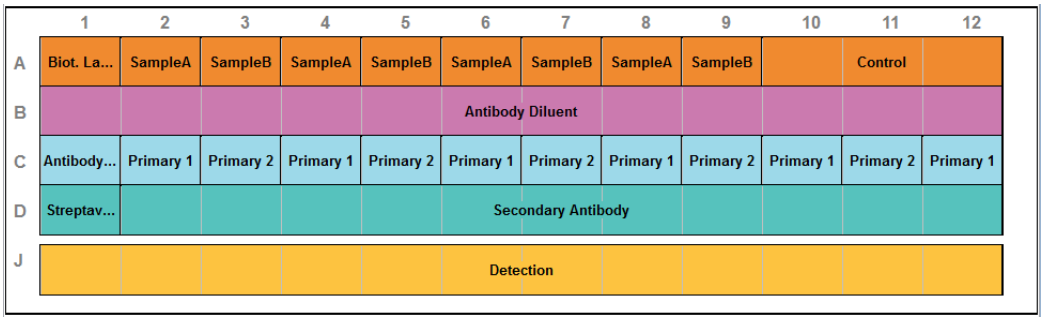
The default group settings groups capillaries with the same sample and primary antibody name. Groups can be adjusted to include parameters like secondary antibodies, attributes, probes, channels (Jess only), and cartridges (Leo only).

1. To use the grouping feature, you will need to annotate the plate in the Assay template. To do this:

   a. Click the **Assay** tab and go to the Template (Jess/Abby) or Sample Template (Leo) pane.

   b. Annotate the parameters used to group (i.e. sample names, primary antibody names, attributes) as described in “Step 5 - Add Assay Plate Annotations (Optional)” on page 52. Be sure to enter the same sample and/or 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.

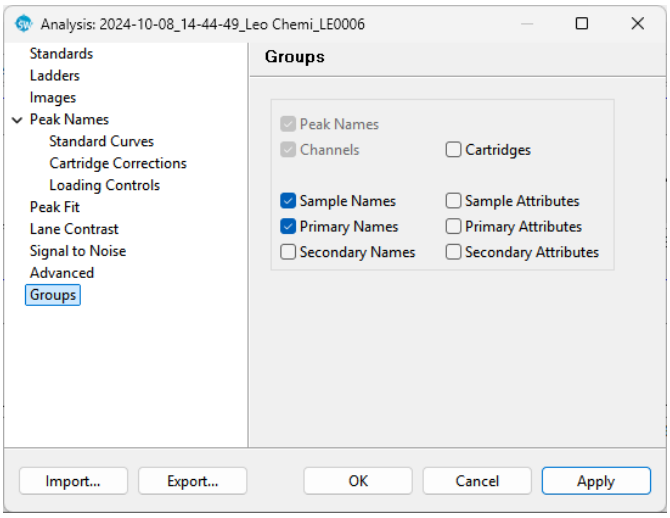



   Each of the two samples were run with each of the two antibodies. This creates four groups for the combination of two samples and two antibodies.

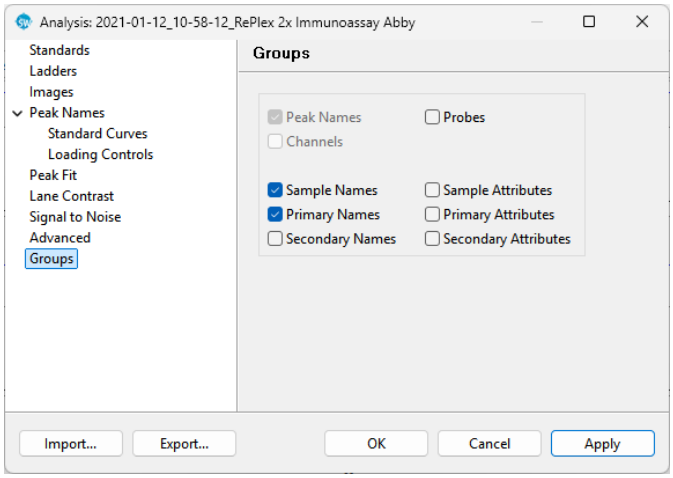
2. To set a grouping option

   a. Go to **Edit** > **Analysis** and select **Groups**.

b. Check the boxes next to the parameters you want to use.

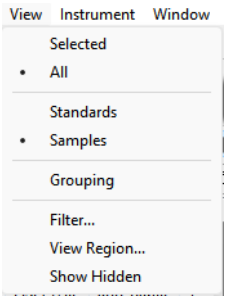



For a RePlex assay:




c. Click **Apply** and then select **OK**.

3. Group your results and view the associated statistics by selecting **View** and clicking on **Grouping**.

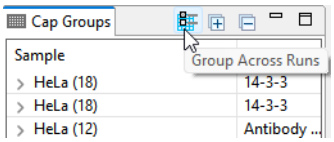


# Grouping Across Runs

Groups will be applied to capillaries within a run by default. Groups can be applied across runs with matching detection channels and when analysis settings are the same.

**To group across runs:**

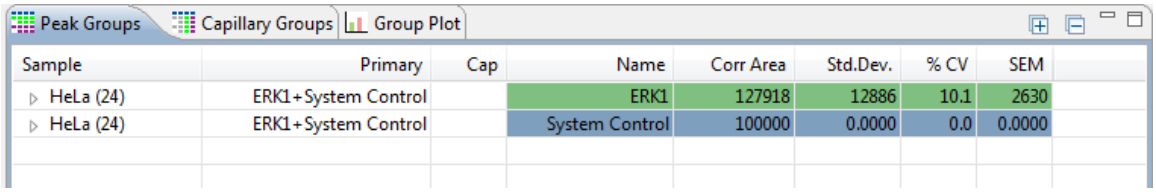
1. Open multiple runs in Compass for Simple Western. See “Opening Multiple Run Files” on page 153.
2. Group your results based on the instructions in “Using Groups” on page 199.
3. Click on the **Group Across Runs** icon in the Cap Groups pane.




# Viewing Statistics

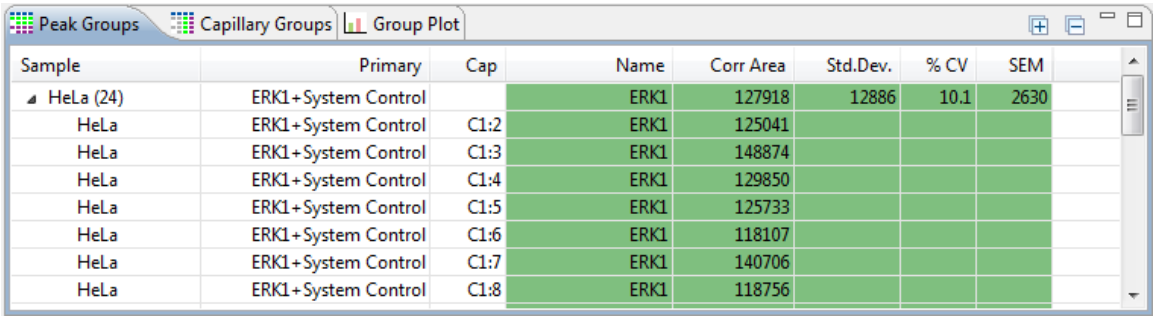
## Peak and Capillary Groups

The Peak Groups pane reports statistics for each named protein in every group. Each group shows the statistics for named proteins which includes peak area average, standard deviation (Std.Dev.), coefficient of variation (%CV), and standard error of the mean (SEM). The number in parenthesis after the sample name indicates the number of capillaries in the group.



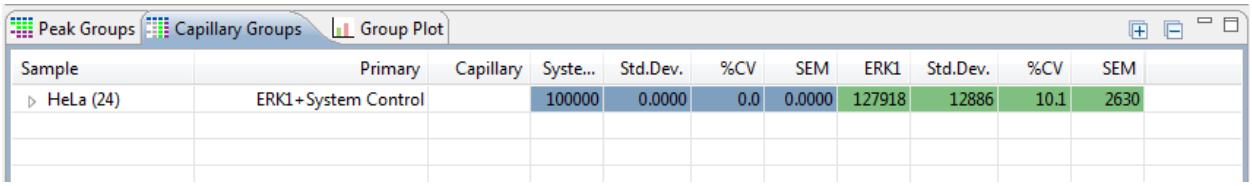

| Sample | Primary | Cap | Name | Corr Area | Std.Dev. | % CV | SEM |
|---|---|---|---|---|---|---|---|
| HeLa (24) | ERK1+System Control | | ERK1 | 127918 | 12886 | 10.1 | 2630 |
| HeLa (24) | ERK1+System Control | | System Control | 100000 | 0.0000 | 0.0 | 0.0000 |

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.

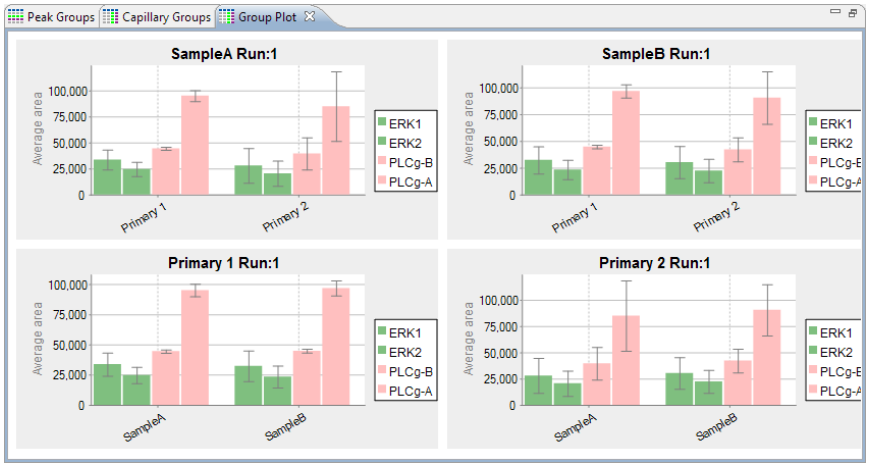



| Sample | Primary | Capillary | Syste... | Std.Dev. | %CV | SEM | ERK1 | Std.Dev. | %CV | SEM |
|---|---|---|---|---|---|---|---|---|---|---|
| HeLa (24) | ERK1+System Control | | 100000 | 0.0000 | 0.0 | 0.0000 | 127918 | 12886 | 10.1 | 2630 |

### Group Plots

**NOTE:** Group Plots are not available for runs performed on Leo systems.

The mean peak area 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.

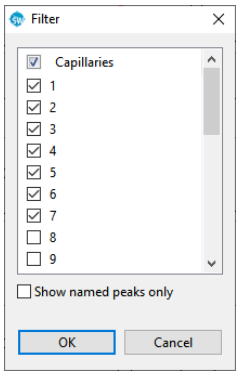



## Hiding or Removing Capillaries in Group Analysis

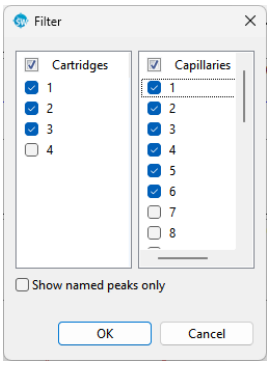
Hidden capillaries are not included in groups. Hiding capillaries provides an easy way to reject individual capillaries from the statistical analysis. See “Hiding Capillary Data” on page 188 for details on how to do this.

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

**JESS/ABBY**




**LEO**



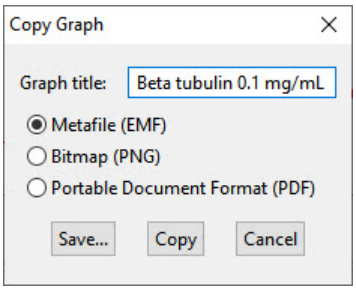

Uncheck the box next to the capillaries or cartridges you wish to remove and click **OK**. This data will now be removed from the grouped view statistics.

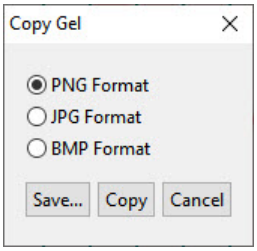
# Copying Data Views and Results Tables

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 the **Graph** or **Lane** tab, or on an image in the Image pane. In the Image pane, make sure to select the entire image by clicking the top or bottom edge of the image. This adds a yellow border around the image to indicate it's selected.
2. Select **Edit** in the main menu and click **Copy**, or right-click and select **Copy**.
3. If you selected copy from the Graph or Lane pane, one of the following windows will display, then click **Copy**. If you selected copy from the Image pane skip to the next step.



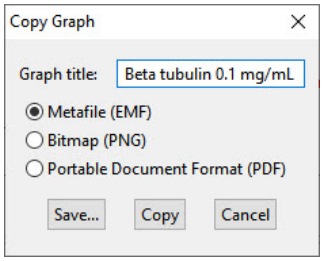

4. Open a document (Microsoft® Word®, Excel®, PowerPoint®, etc.). Right-click in the document and select **Paste**. A graphic of the copied data view will be pasted into the document.

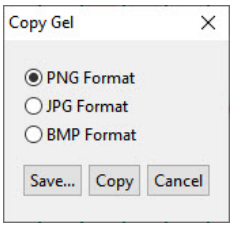
## Copying Results Tables

1. Select one or multiple rows in the Peaks or Capillaries pane.
2. Select **Edit** in the main menu and click **Copy**, or right-click and select **Copy**.
3. 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 or Lane View as an Image File

1. Click the **Graph** or **Lane** tab.
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 for graph, PNG, JPG or BMP for lane) 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

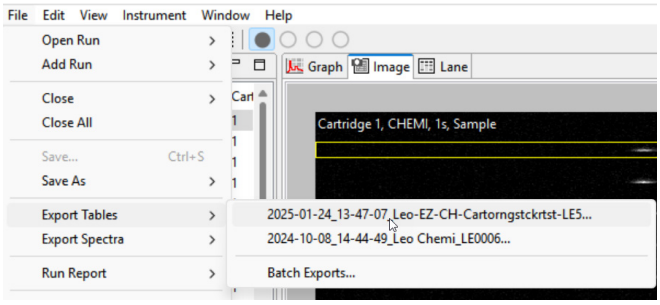
**To export the information in the Peaks and Capillaries tables when one run is open:**

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 341.

**To export the information in the Peaks and Capillaries tables for a specific run when multiple runs are open:**

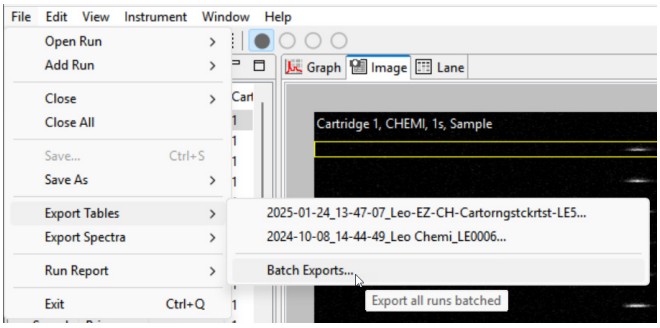
1. Click **File** in the main menu.
2. Select the run you want to export result tables from in the **Export Tables** submenu.




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

**To export the information in the Peaks and Capillaries tables for all runs when multiple runs are open:**

1. Click **File** in the main menu.
2. Select the **Batch Report...** in the **Export Tables** submenu.

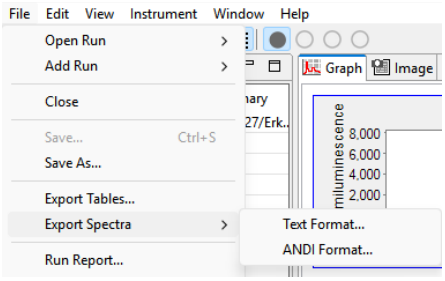



3. Select a directory to save the files to and click **OK**. Data will exported in .txt format. An additional file named “Batch Export” lists the order of run files exported.

## Raw Sample Electropherogram Data

**To export raw sample plot data:**

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



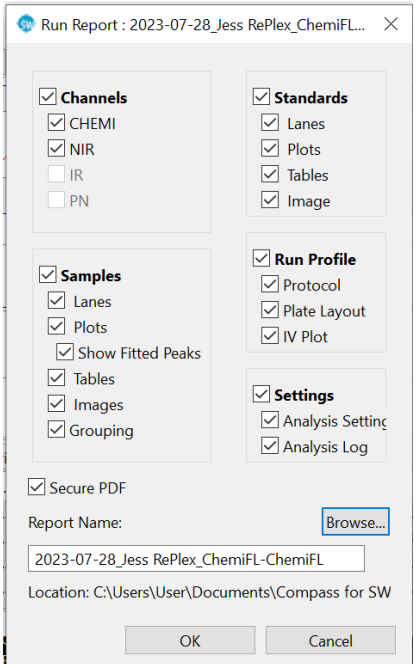

- **To export data in .txt format** - Select **Text Format**. Plots will be exported in one file for all capillary sample data. If a standard curve is performed, standard curve trace data will be exported in a separate file.
- **To export data in .cdf format** - Select **ANDI Format**. Plots will be 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 is displayed.



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 by default.
4. Report PDFs generated by Compass for Simple Western are secured by default, which means they can be viewed and printed but not modified or renamed. Uncheck **Secure PDF** if you don't want to generate a secure report.
5. 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

**JESS/ABBY**

Run

| | |
|---|---|
| Run | 2024-05-08_12-45-20_Jess-FL model test-JS3913-YF |
| Path | C:\Users\User\Compass runs |
| Assay | Jess(16) |
| Kit Info | Regular: 12-230 kDa |
| | |
| Instrument | Jess : Jess JS3913 - js3913 |
| Firmware Version | 2.1.2021.09.01.00.26.14.bc9a11c63 |
| | |
| Plate S/N | 1079202154 |
| Cartridge S/N | |
| Cartridge Expires | N/A |
| Started | Wed 12:46 PM May 8, 2024 PDT |
| Completed | Wed 3:52 PM May 8, 2024 PDT |
| Error | None |

**LEO**

Run

| | |
|---|---|
| Run | 2024-10-08_14-44-49_Leo Chemi_LE0006 |
| Path | C:\Users\User\Compass runs |
| Assay | Leo Chemi |
| Kit Info | Regular: 12-230 kDa |
| | |
| Instrument | Leo : le0006 - le0006 |
| Firmware Version | 1.0.0.2024-10-04-22-25-18.7c117aa |
| | |
| Started | Tue 2:45 PM Oct 8, 2024 PDT |
| Completed | Tue 5:32 PM Oct 8, 2024 PDT |

- Cartridge and Plate (Leo only)

Cartridges and Plates

| | Barcode | Expires |
|---|---|---|
| Sample Plate | 20167060252 | |
| | | |
| Reagent Plate 1 | 20251000019070144 | Oct 2025 |
| Reagent Plate 2 | 20251000019070135 | Oct 2025 |
| Reagent Plate 3 | 20251000019070009 | Oct 2025 |
| Reagent Plate 4 | 20251000019070010 | Oct 2025 |
| | | |
| Cartridge 1 | 20251154933010009 | Nov 2025 |
| Cartridge 2 | 20251154933010010 | Nov 2025 |
| Cartridge 3 | 20251154933010011 | Nov 2025 |
| Cartridge 4 | 20251154933010012 | Nov 2025 |

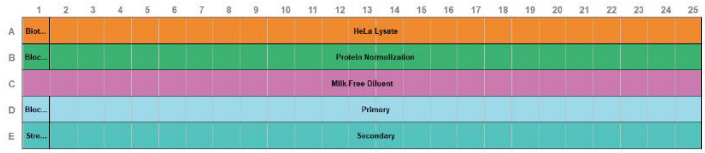
- Protocol details

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

Plate Layout



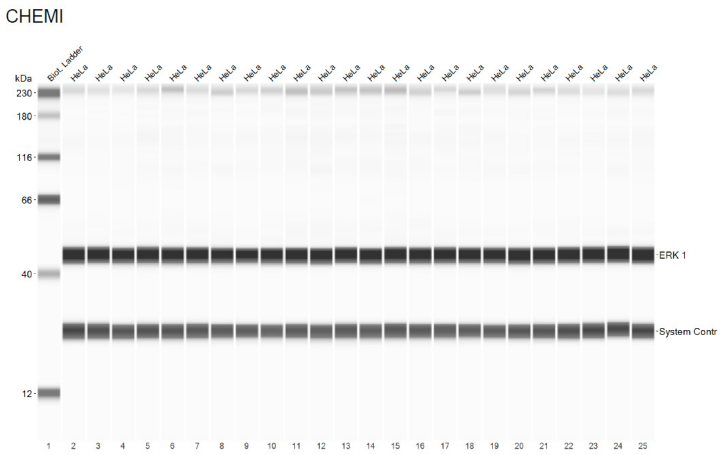

- Analysis settings

| | |
|---|---|
| Hidden Capillaries | None |
| Images | |
| Exposures for All Cartridges | yes |
| CHEMI Luminescence | High Dynamic Range 4.0 |
| Lane Contrast | Slider |
| Cartridge 1 CHEMI White Level | 3048.3 |
| Cartridge 1 CHEMI Black Level | 82570.8 |
| Cartridge 2 CHEMI White Level | 3107.7 |
| Cartridge 2 CHEMI Black Level | 87287.3 |
| Cartridge 3 CHEMI White Level | 3019.2 |
| Cartridge 3 CHEMI Black Level | 91430.7 |
| Cartridge 4 CHEMI White Level | 2983.9 |
| Cartridge 4 CHEMI Black Level | 83273.4 |
| Peak Fit | fit |
| Apply To | Default |
| Range Min (MW (kDa)) | 1.0 |
| Range Max (MW (kDa)) | 250.0 |
| Range View | Analysis |
| Baseline Type | Spline |
| Baseline Threshold | 1.0 |
| Baseline Window (pixels) | 15.0 |
| Baseline Stiffness | 1.0 |
| Peak Find Threshold | 200.0 |
| Peak Find Width (pixels) | 9.0 |
| Peak Find Area Calculation | Gaussian Fit |
| Peak Name | Peak Group 1 |
| Apply To | All |
| HSP27 (MW (kDa)) | 33 |
| HSP27 Range (%) | 10 |
| HSP27 Channel | CHEMI |
| Erk1 (MW (kDa)) | 44 |

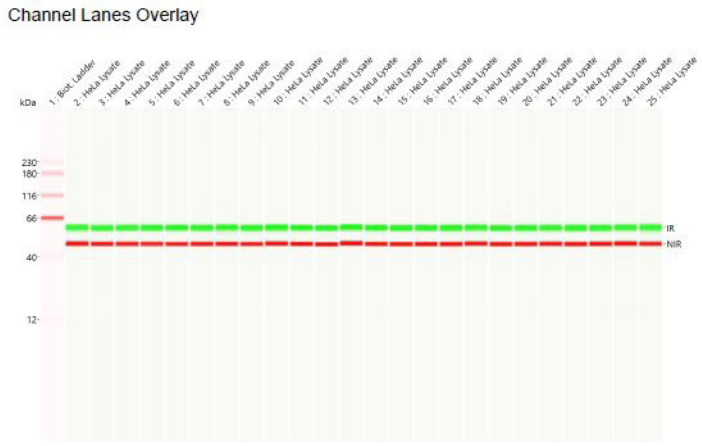
| | |
|---|---|
| Erk1 Range (%) | 10 |
| Erk1 Channel | CHEMI |
| HSP70 (MW (kDa)) | 67 |
| HSP70 Range (%) | 10 |
| HSP70 Channel | CHEMI |
| HSP90 (MW (kDa)) | 100 |
| HSP90 Range (%) | 10 |
| HSP90 Channel | CHEMI |
| Ladder Settings | Ladder, Cartridge: per Cartridge, Cap: 1, Chemiluminescent/Fluorescent Channel: CHEMI |
| Apply To | Default |
| Ladder Peak 1 (MW (kDa)) | 12.0 |
| Ladder Peak 1 Position (pixels) | 210 |
| Ladder Peak 2 (MW (kDa)) | 40.0 |
| Ladder Peak 2 Position (pixels) | 330 |
| Ladder Peak 3 (MW (kDa)) | 66.0 |
| Ladder Peak 3 Position (pixels) | 410 |
| Ladder Peak 4 (MW (kDa)) | 116.0 |
| Ladder Peak 4 Position (pixels) | 460 |
| Ladder Peak 5 (MW (kDa)) | 180.0 |
| Ladder Peak 5 Position (pixels) | 500 |
| Ladder Peak 6 (MW (kDa)) | 230.0 |
| Ladder Peak 6 Position (pixels) | 530 |
| Signal To Noise | |
| Algorithm | Signal-to-Noise Ratio |
| Noise Region | Full |
| Region Range | kDa |
| Standards | Biotinylated Ladder (12kDa-230kDa) |
| Apply To | Default |
| Standard 1 (MW (kDa)) | 1 |
| Standard 1 Position (pixels) | 120 |
| Standard 1 Fit | no |
| Standard 2 (MW (kDa)) | 29 |
| Standard 2 Position (pixels) | 270 |
| Standard 2 Fit | yes |

| | |
|---|---|
| Standard 3 (MW (kDa)) | 230 |
| Standard 3 Position (pixels) | 530 |
| Standard 3 Fit | yes |
| Advanced | Advanced |
| Apply To | Default |
| Standards Peak Width (pixels) | 15 |
| Standards Allowable Drift (pixels) | 100 |
| Sample Peak Fit Starting Width Ratio | 0.5 |
| Image Median Filter Threshold Ratio | 0.5 |
| Image Median Filter Threshold Limit (counts) | 1000 |
| Groups | |
| Peak Names | yes |
| Channels | yes |
| Cartridges | no |
| Sample Names | yes |
| Sample Attributes | no |
| Primary Names | yes |
| Primary Attributes | no |
| Secondary Names | no |
| Secondary Attributes | no |

- Lane views:

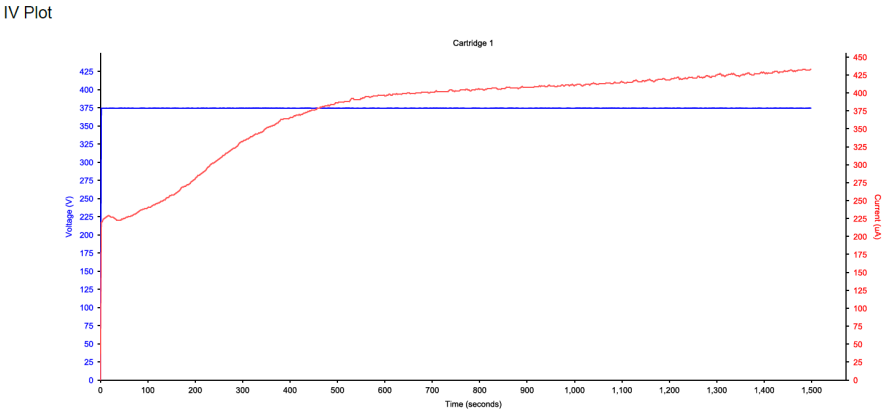


- Lane views (Channel Lanes Overlay for Jess Only):

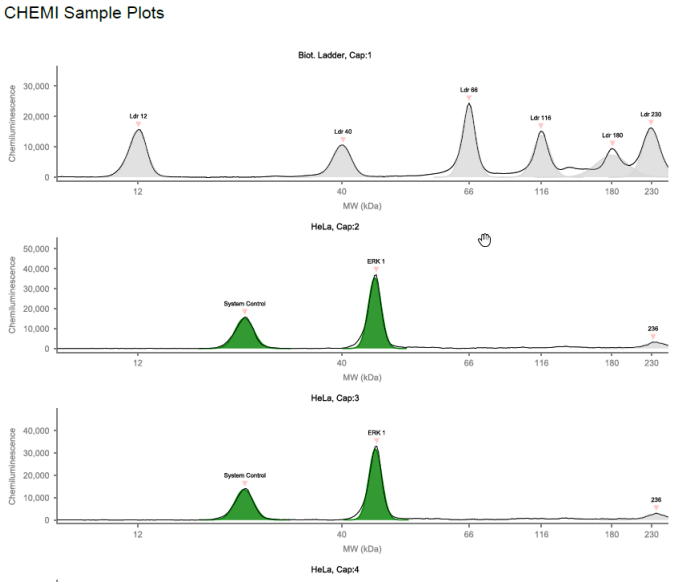



**NOTE:** The channel lanes overlay is also divided in the report as individual chemiluminescence, NIR, IR, Protein Normalization (PN), Total Protein (TP) channels and Standard lanes.

- IV plot:

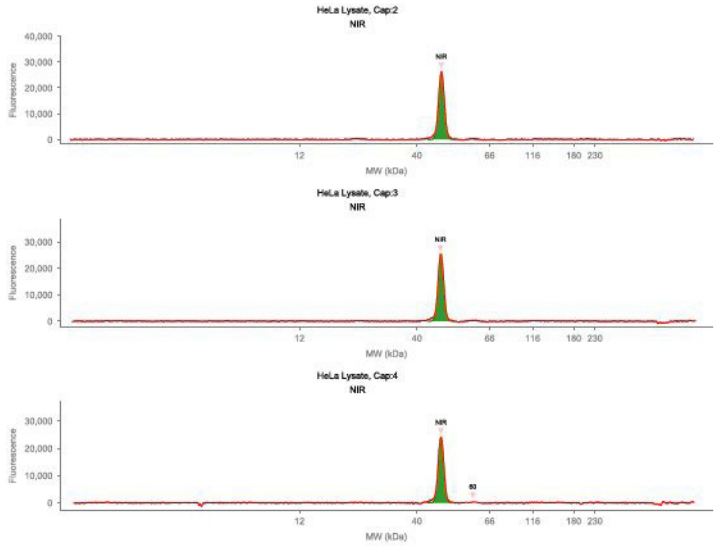


- Sample plots for each capillary:




You can also include the fluorescent standards graphs for each capillary and the standard peaks position 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 and the standard peaks position in pixels.

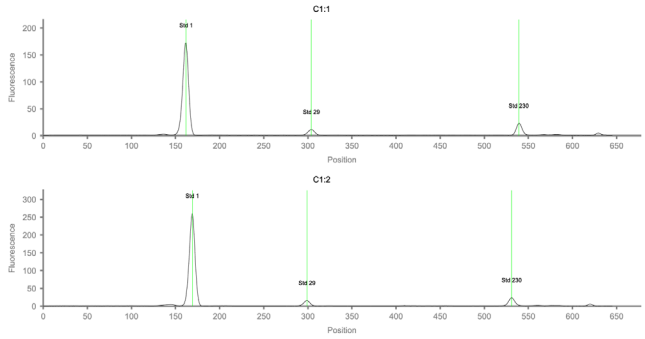
- Peak tables:

CHEMI Sample Peaks

| Sample | Primary | Secondary | Cap | Peak | Name | Position | MW (kDa) | Height | Area | % Area | Width | S/N | Baseline |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EZ | Ab diluent 2 | SA-HRP | C1:1 | 1 | Ldr 12 | 243 | 12 | 2693.2 | 43594.5 | | 15.2 | 1281.3 | 19.7 |
| EZ | Ab diluent 2 | SA-HRP | C1:1 | 2 | Ldr 40 | 360 | 40 | 3385.5 | 50995.6 | | 14.2 | 1610.7 | 39.7 |
| EZ | Ab diluent 2 | SA-HRP | C1:1 | 3 | Ldr 66 | 433 | 66 | 2472.7 | 34767.1 | | 13.2 | 1176.4 | 48.9 |
| EZ | Ab diluent 2 | SA-HRP | C1:1 | 4 | Ldr 116 | 476 | 116 | 4724.6 | 61659.1 | | 12.3 | 2247.8 | 53.0 |
| EZ | Ab diluent 2 | SA-HRP | C1:1 | 5 | Ldr 180 | 517 | 180 | 3190.5 | 43846.1 | | 12.9 | 1517.9 | 55.8 |
| EZ | Ab diluent 2 | SA-HRP | C1:1 | 6 | Ldr 230 | 539 | 230 | 3493.1 | 48225.0 | | 13.0 | 1661.9 | 56.9 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:2 | 1 | HSP27 | 325 | 33 | 33760.3 | 372657.3 | 28.0 | 10.4 | 316.9 | 3666.2 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:2 | 2 | Erk1 | 373 | 47 | 44910.5 | 502787.2 | 37.7 | 10.5 | 421.5 | 4114.0 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:2 | 3 | HSP70 | 425 | 66 | 31443.7 | 276392.1 | 20.7 | 8.3 | 295.1 | 4456.9 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:2 | 4 | HSP90 | 455 | 100 | 21242.2 | 180780.6 | 13.6 | 8.0 | 199.4 | 4543.4 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:3 | 1 | HSP27 | 324 | 33 | 33164.0 | 369870.9 | 28.1 | 10.5 | 359.3 | 3412.0 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:3 | 2 | Erk1 | 373 | 47 | 45158.4 | 481482.7 | 36.6 | 10.0 | 489.2 | 3885.7 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:3 | 3 | HSP70 | 425 | 66 | 31294.9 | 273090.2 | 20.8 | 8.2 | 339.0 | 4291.7 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:3 | 4 | HSP90 | 454 | 100 | 22563.1 | 190954.5 | 14.5 | 8.0 | 244.4 | 4440.5 |

- Standard Plots for each capillary:

Standard Plots



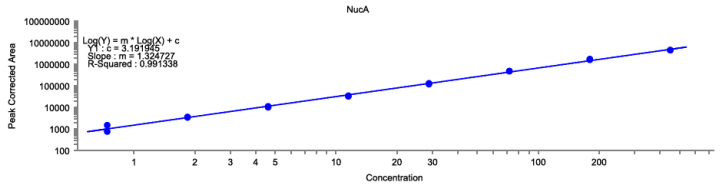

- Standard Peaks by pixel for each capillary

Standard Peaks (pixels)

| Sample | Primary | Secondary | Cap | Std 1 | Std 29 | Std 230 |
|---|---|---|---|---|---|---|
| EZ | Ab diluent 2 | SA-HRP | C1:1 | 161.2 | 303.9 | 539.4 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:2 | 168.6 | 298.8 | 531.1 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:3 | 166.9 | 298.0 | 531.0 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:4 | 166.0 | 297.7 | 530.2 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:5 | 163.6 | 295.8 | 530.0 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:6 | 163.9 | 295.7 | 529.2 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:7 | 161.3 | 294.7 | 530.0 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:8 | 160.7 | 293.8 | 529.3 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:9 | 158.7 | 293.6 | 530.2 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:10 | 157.3 | 291.9 | 528.5 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:11 | 158.1 | 291.8 | 529.2 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:12 | 155.4 | 290.6 | 528.4 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:13 | 154.2 | 290.7 | 529.1 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:14 | 153.7 | 288.8 | 528.3 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:15 | 152.5 | 288.7 | 528.4 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:16 | 153.1 | 288.7 | 528.4 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:17 | 152.2 | 288.7 | 529.3 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:18 | 151.3 | 287.9 | 529.2 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:19 | 149.9 | 287.8 | 530.0 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:20 | 151.2 | 287.7 | 529.1 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:21 | 149.0 | 287.8 | 530.1 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:22 | 150.0 | 286.9 | 529.1 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:23 | 148.9 | 286.9 | 529.2 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:24 | 149.9 | 287.0 | 529.4 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C1:25 | 152.7 | 290.8 | 531.1 |
| EZ | Ab diluent 2 | SA-HRP | C2:1 | 152.4 | 299.1 | 540.2 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C2:2 | 160.8 | 297.0 | 534.2 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C2:3 | 159.6 | 296.1 | 534.2 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C2:4 | 158.9 | 295.9 | 533.4 |
| Hela 0.25mg/ml | HSP27/Erk1/HSP70/HSP90 | anti-Rabbit-HRP | C2:5 | 158.5 | 295.0 | 533.4 |

- Standard Curves:

Standard Curves




| Curve: NucA (CHEMI) | |
|---|---|
| Log(Y) = m * Log(X) + c | NucA (CHEMI) |
| Y1 (c) | 3.191945 |
| Slope (m) | 1.324727 |
| R-Squared | 0.991338 |
| | |

- Grouping by peak groups:

Grouping

Peak Groups

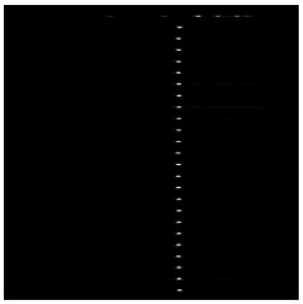
| Sample | Primary | Name | Area | Std. Dev. | % CV | SEM | Channel |
|---|---|---|---|---|---|---|---|
| EZ (4) | Ab diluent 2 | Ldr 12 | 49351.7 | 4689 | 9.5 | 2345 | CHEMI |
| EZ (4) | Ab diluent 2 | Ldr 40 | 59223.7 | 5747 | 9.7 | 2874 | CHEMI |
| EZ (4) | Ab diluent 2 | Ldr 66 | 39195.4 | 3516 | 9.0 | 1758 | CHEMI |
| EZ (4) | Ab diluent 2 | Ldr 116 | 67545.6 | 4137 | 6.1 | 2068 | CHEMI |
| EZ (4) | Ab diluent 2 | Ldr 180 | 45981.9 | 2304 | 5.0 | 1152 | CHEMI |
| EZ (4) | Ab diluent 2 | Ldr 230 | 53608.2 | 3941 | 7.4 | 1970 | CHEMI |
| Hela 0.25mg/ml | HSP27/Erk1/ HSP70/HSP90 | HSP27 | 389119.6 | 21125 | 5.4 | 2156 | CHEMI |
| Hela 0.25mg/ml | HSP27/Erk1/ HSP70/HSP90 | Erk1 | 528506.1 | 24240 | 4.6 | 2474 | CHEMI |
| Hela 0.25mg/ml | HSP27/Erk1/ HSP70/HSP90 | HSP70 | 334761.8 | 34690 | 10.4 | 3541 | CHEMI |
| Hela 0.25mg/ml | HSP27/Erk1/ HSP70/HSP90 | HSP90 | 219752.6 | 29101 | 13.2 | 2970 | CHEMI |

| Sample | Primary | Name | % Area | Std. Dev. | % CV | SEM | Channel |
|---|---|---|---|---|---|---|---|
| EZ (4) | Ab diluent 2 | Ldr 12 | | | | | CHEMI |
| EZ (4) | Ab diluent 2 | Ldr 40 | | | | | CHEMI |
| EZ (4) | Ab diluent 2 | Ldr 66 | | | | | CHEMI |
| EZ (4) | Ab diluent 2 | Ldr 116 | | | | | CHEMI |
| EZ (4) | Ab diluent 2 | Ldr 180 | | | | | CHEMI |
| EZ (4) | Ab diluent 2 | Ldr 230 | | | | | CHEMI |

- Sample images:

Sample Images

NIR, 10s, Sample



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

- Analysis log:

Analysis Log

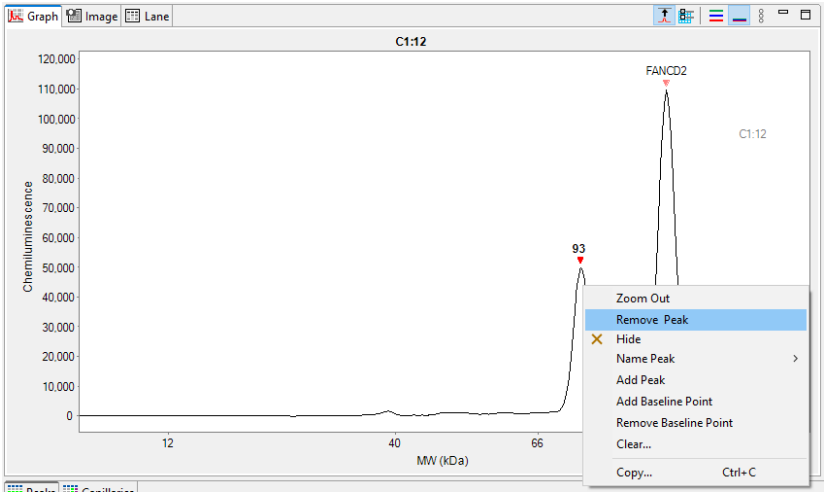
| Date | User Name | Message | Comment |
|---|---|---|---|
| 2018-04-27 15:43:52 | | Started run: 2018-04-27_15-43-31_Jess Alpha 2 SL Assay: Jess.assay | |
| 2018-04-30 17:23:53 | | Saved analysis and template changes from Compass for SW v4.0.0-0423 | |
| | | Added Peak Names Apply Settings "apply Peak 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.

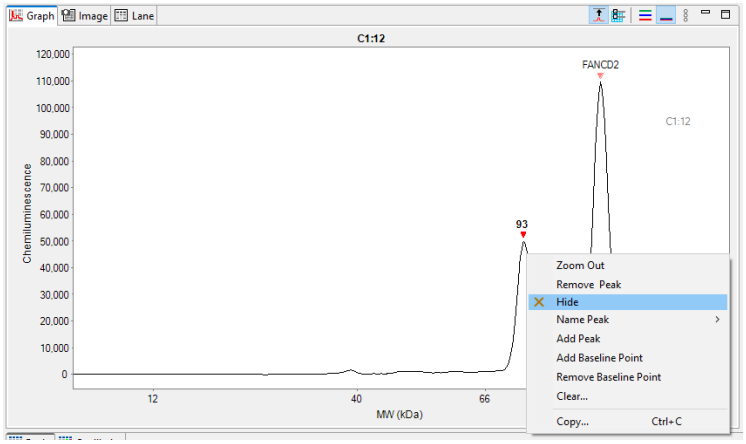
**NOTES:**
To remove sample peak assignments that were made manually and go back to the original view of the data, right-click in the electropherogram and click **Clear**.

Virtual blot data in the Lane pane will also update to reflect changes made in the Graph pane.

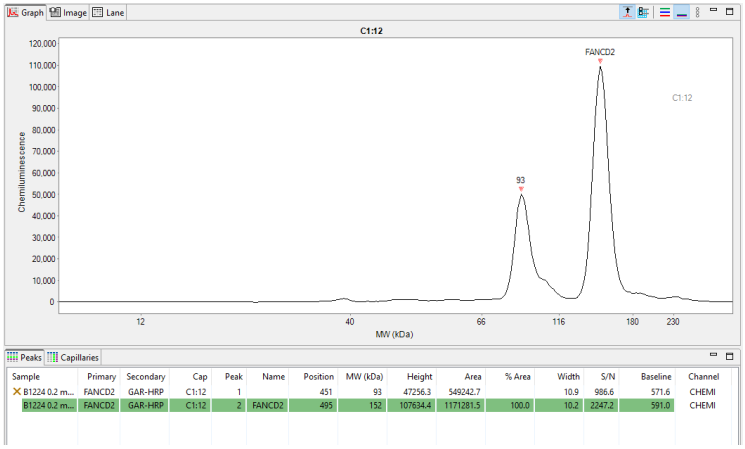
## Hiding Sample Data

You can hide the results for a sample protein in the results tables without completely removing it from the results calculated by Compass for Simple Western. 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 select **Hide**. Compass for Simple Western will hide the peak data in the results tables.



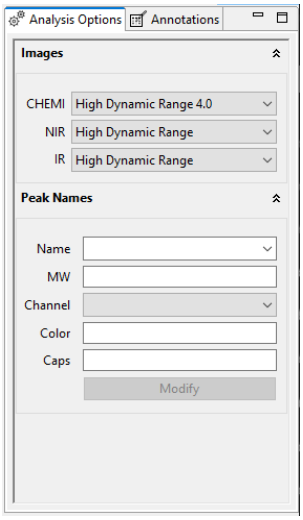
5. To view hidden peak data, click **View** in the main menu and click **Show Hidden**. Hidden peak data will display in the results table and be marked with an **X**.




6. To unhide a peak, right-click on the peak in the electropherogram or Peaks table and select **Unhide**.

## Changing Peak Names for Sample Data

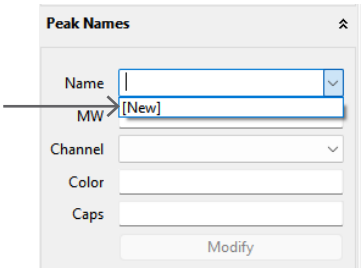
If Compass for Simple Western did not automatically name a sample protein associated with a specific primary antibody, this can be adjusted manually. You can do this in the Analysis Options pane.



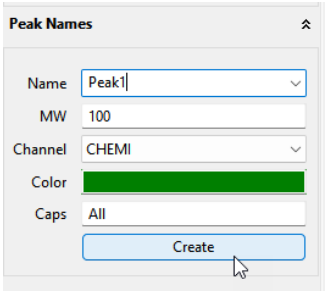

**NOTES:**
For details on how to specify peak name settings, see “Peak Names Settings” on page 293

Virtual blot data in the Lane pane will also update to reflect changes made in the Graph pane.

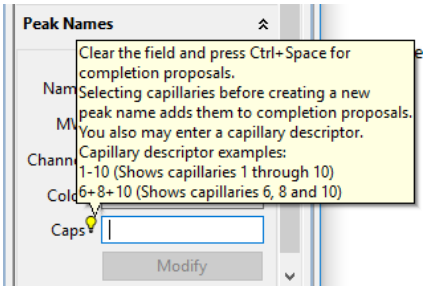
The New option in the Name drop-down box creates a new default Peak Name using the current channel and capillaries that are selected.



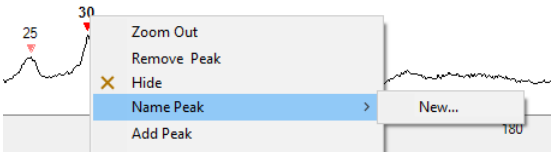

Rename the peak and modify the parameters, then click **Create**.

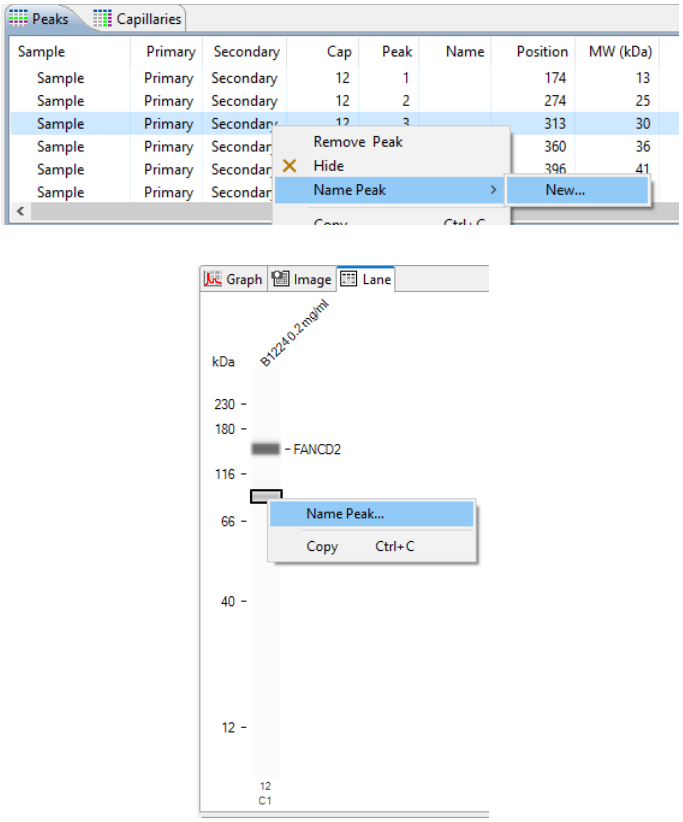



The tooltip next to the Caps field has useful information on acceptable nomenclature when designating capillaries to apply a Peak Name to:

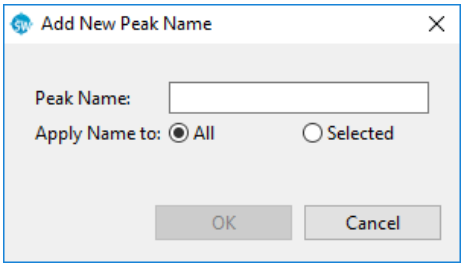



Alternatively, right-click the peak in the electropherogram, Peaks table, or Lane view. Then click **Name Peak** and either select a name in the list or create a new peak name by selecting **New...**. Compass for Simple Western will change the peak name in the electropherogram and results tables and adjust peak names for other sample proteins accordingly.

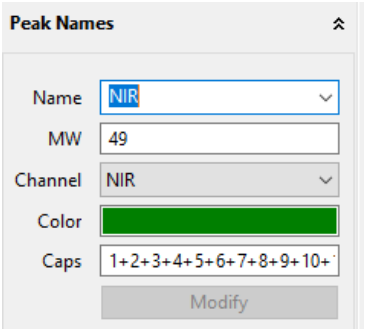





When creating a new peak name, enter the appropriate name in the Peak Name box:



For a named peak, you can change the name, molecular weight, the channel, its associated color, or designate corresponding capillaries. Click the desired peak in the Graph view, band in the Lane view, or row in the Peaks table. Then in the Peak Names menu of the Analysis Options pane, make the desired modification and click **Modify**:

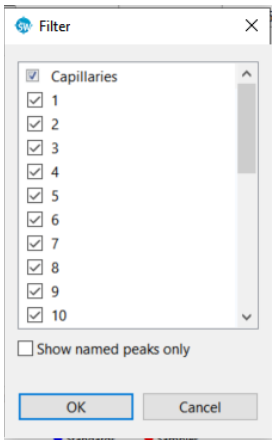



## Displaying Sample Data for Named Peaks Only

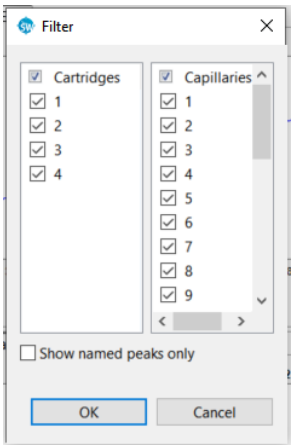
You can adjust the sample data to display results only for user-specified named peaks. To do this:

1. Click **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**.

**JESS/ABBY**




**LEO**




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.

# Changing the Virtual Blot View

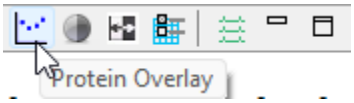
Options in the Lane pane let you change the contrast or invert the virtual blot, visualize baseline corrected data, change lane labels or overlay standards data on sample lanes.

The Lane pane toolbar has the following options:

- Protein Overlay (only when a Total Protein assay or Protein Normalization was run)
- Contrast Adjustment
- Invert
- Lane Options
- Overlay Standards Data

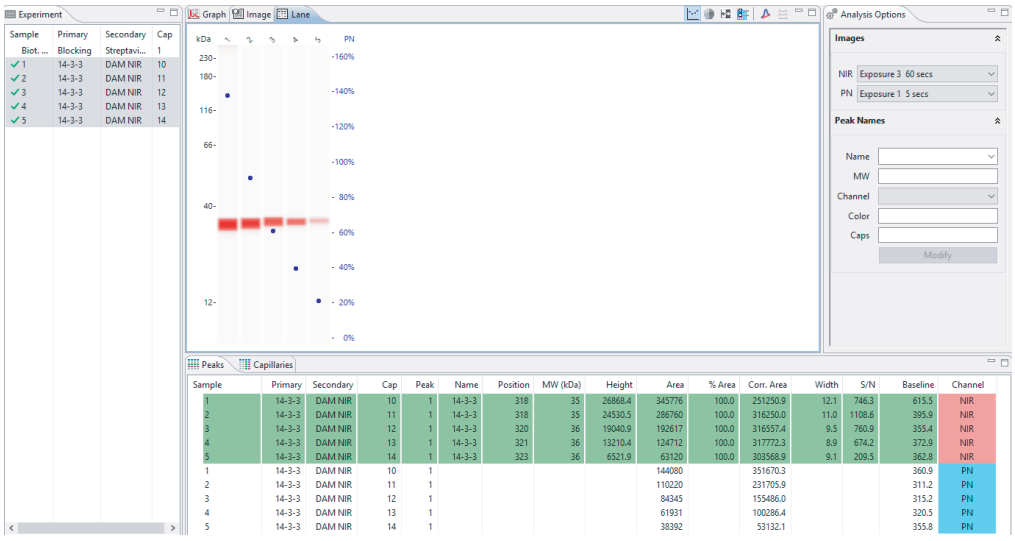
## Overlaying Protein Normalization or Total Protein Data

To view Total Protein or Protein Normalization data, make sure the Protein Normalization channel (Jess only), chemiluminescence channel in a Stellar assay with Total Protein (Jess only), or Total Protein in Probe 2 of RePlex Assays is selected first, then click the **Protein Overlay** icon.

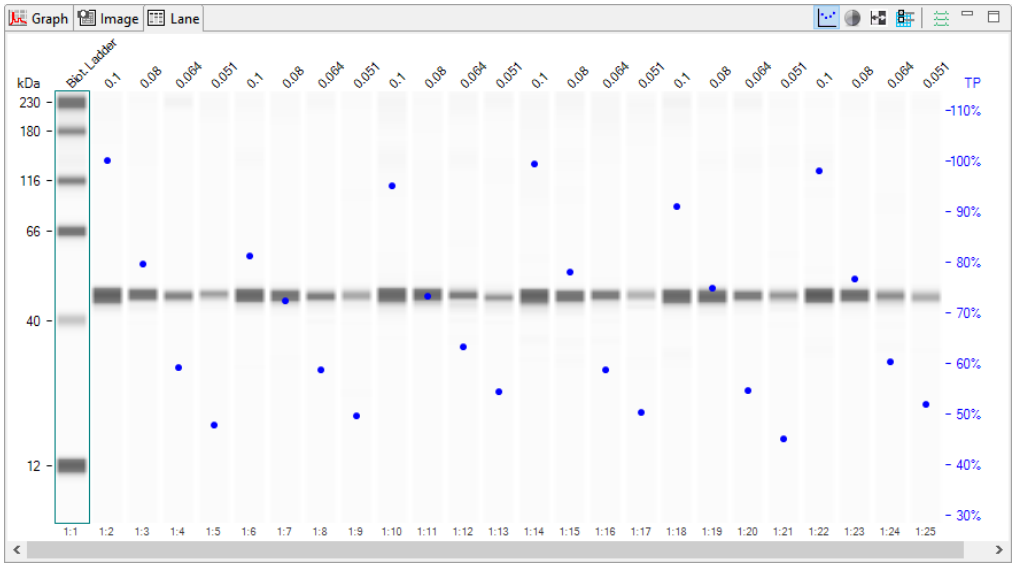



Normalized data will appear.

**PROTEIN NORMALIZATION NORMALIZED DATA ON JESS**



### TOTAL PROTEIN NORMALIZED DATA ON ABBY




Protein Normalization or Total Protein data is shown as peak area counts, or peak area count percentages relative to a selected reference capillary on the secondary y-axis in lane view.

**NOTE:** Normalization must be enabled in the Analysis settings to display peak count percentage values.

For Protein Normalization runs (Jess only), RePlex runs with Total Protein (Jess/Abby/Leo), and Stellar runs with Total Protein (Jess only), Compass automatically displays the normalized peak area for detected peaks in Chemiluminescence, NIR, and/or IR channels in the Corr. Area column in the Peaks table. Additionally, if Protein Normalization is on (Jess only), the total peak area used for normalization is listed in the Corr. Area column.

Peaks | Capillaries

| Sample | Primary | Secondary | Cap | Peak | Name | Position | MW (kDa) | Height | Area | % Area | Corr. Area | Width | S/N | Baseline | Channel |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 14-3-3 | DAM NIR | 10 | 1 | 14-3-3 | 318 | 35 | 26868.4 | 345776 | 100.0 | 251250.9 | 12.1 | 746.3 | 615.5 | NIR |
| 2 | 14-3-3 | DAM NIR | 11 | 1 | 14-3-3 | 318 | 35 | 24530.5 | 286760 | 100.0 | 316250.0 | 11.0 | 1108.6 | 395.9 | NIR |
| 3 | 14-3-3 | DAM NIR | 12 | 1 | 14-3-3 | 320 | 36 | 19040.9 | 192617 | 100.0 | 316557.4 | 9.5 | 760.9 | 355.4 | NIR |
| 4 | 14-3-3 | DAM NIR | 13 | 1 | 14-3-3 | 321 | 36 | 13210.4 | 124712 | 100.0 | 317772.3 | 8.9 | 674.2 | 372.9 | NIR |
| 5 | 14-3-3 | DAM NIR | 14 | 1 | 14-3-3 | 323 | 36 | 6521.9 | 63120 | 100.0 | 303568.9 | 9.1 | 209.5 | 362.8 | NIR |
| 1 | 14-3-3 | DAM NIR | 10 | 1 | | | | | 144080 | | 351670.3 | | | 360.9 | PN |
| 2 | 14-3-3 | DAM NIR | 11 | 1 | | | | | 110220 | | 231705.9 | | | 311.2 | PN |
| 3 | 14-3-3 | DAM NIR | 12 | 1 | | | | | 84345 | | 155486.0 | | | 315.2 | PN |
| 4 | 14-3-3 | DAM NIR | 13 | 1 | | | | | 61931 | | 100286.4 | | | 320.5 | PN |
| 5 | 14-3-3 | DAM NIR | 14 | 1 | | | | | 38392 | | 53132.1 | | | 355.8 | PN |

For RePlex runs with Total Protein in Probe 2, total peak area used for normalization is listed in the Area column when the Probe 2 channel is on as shown in the Peaks table below. For Stellar runs with Total Protein, total peak area used for normalization is listed in the Area column when the chemiluminescence channel is on.

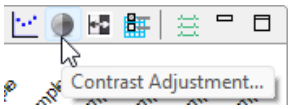
Peaks | Capillaries

| Sample | Primary | Cap | Peak | Name | Position | MW (kDa) | Height | Area | % Area | Corr. Area | Width | S/N | Baseline |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.1 | anti-E... | P1:22 | 1 | ERK1 | 369 | 47 | 4479.2 | 46091.6 | 100.0 | 47071.2 | 9.7 | 151.8 | 602.6 |
| 0.08 | anti-E... | P1:23 | 1 | ERK1 | 369 | 47 | 3277.2 | 34388.6 | 100.0 | 44910.2 | 9.9 | 82.6 | 542.8 |
| 0.064 | anti-E... | P1:24 | 1 | ERK1 | 370 | 47 | 1915.2 | 22337.3 | 100.0 | 37130.0 | 11.0 | 70.2 | 626.2 |
| 0.051 | anti-E... | P1:25 | 1 | ERK1 | 372 | 47 | 1763.8 | 19067.5 | 100.0 | 36890.0 | 10.2 | 39.5 | 502.4 |
| 0.1 | | P2:2 | 1 | | | | | 231565.8 | | | | | 136.1 |
| 0.08 | | P2:3 | 1 | | | | | 184228.1 | | | | | 129.6 |
| 0.064 | | P2:4 | 1 | | | | | 136757.9 | | | | | 121.6 |
| 0.051 | | P2:5 | 1 | | | | | 110361.7 | | | | | 121.6 |

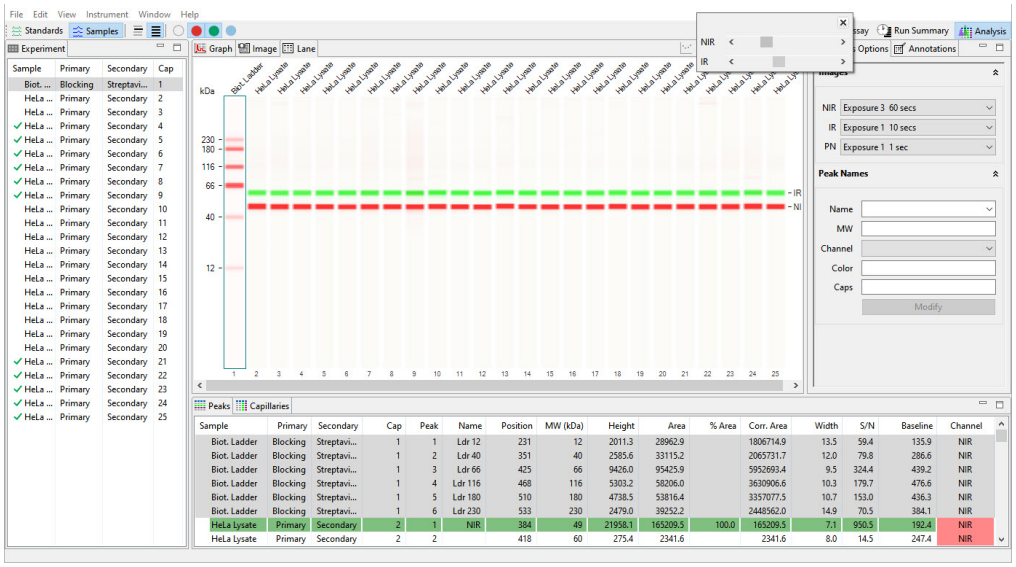
Normalization is enabled by default. For more details on enabling or disabling Normalization, or to choose a different reference capillary see “Normalization” on page 290.

## Adjusting the Contrast

1. Click the **Contrast Adjustment** button.

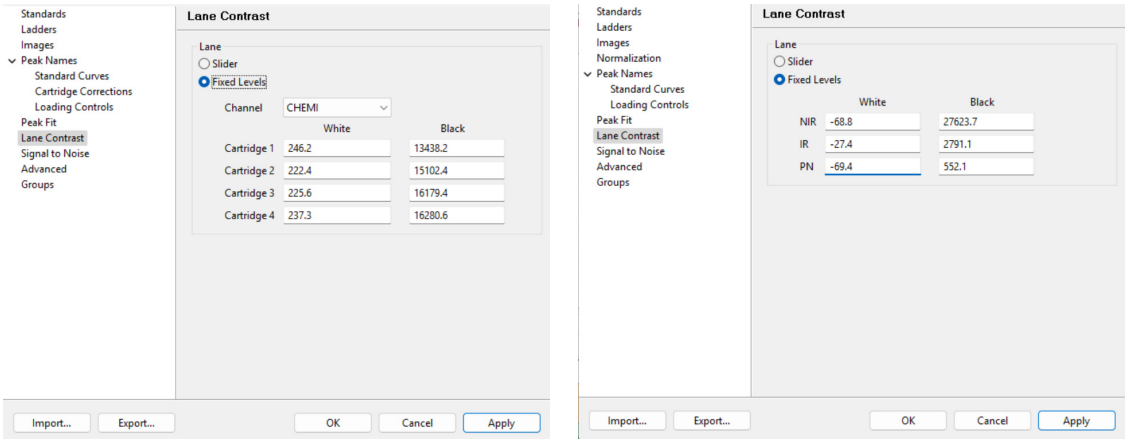



2. The contrast tool will display:



3. 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.

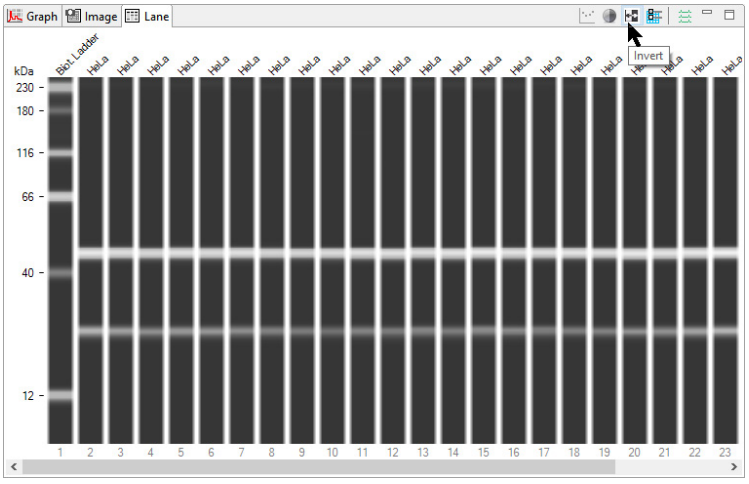
4. To lock the slider, select **Edit** > **Analysis**, and choose the **Lane Contrast** page. Select **Fixed Levels**. The numbers displayed refer to the current White and Black levels for the chemiluminescence or fluorescence (Jess only) run being analyzed. Change these settings and click **Apply** to see how they impact the contrast on the lane view. Once you're satisfied with the changes, click **OK**.




- When you select Fixed Level, the slider won't be available in the lane view.
- The Lane Contrast setting can 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.

# Lane Options

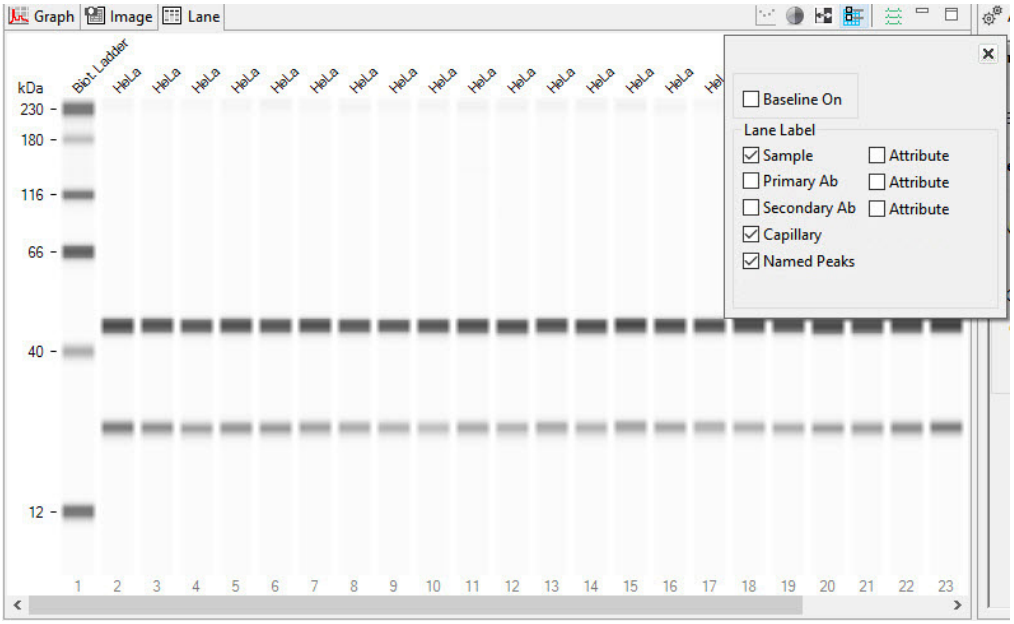
**NOTE:** If you save the run file after updating Graph, Lane, and Table options in the Analysis screen, those selections will override the Default Analysis settings and will automatically display the next time you open the run file. See “Step 8 - Modify Default Analysis View (Optional)” on page 57 for more information.

## Selecting Lane Labels

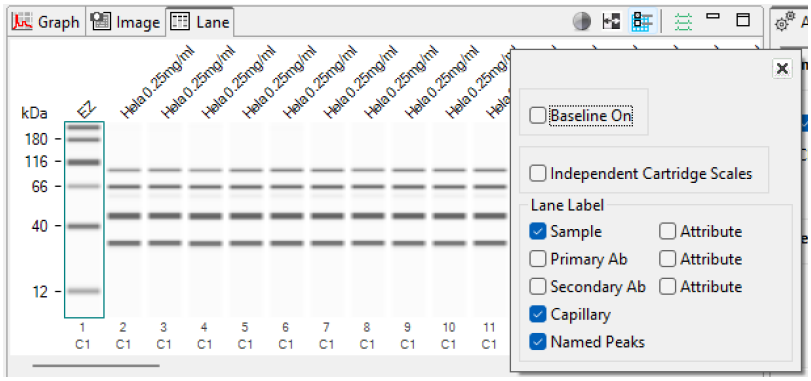
The labels shown above the lanes in the virtual blot can be customized. To do this:

1. Click the **Lane Options** button. The label box will display. Lane labels for a Chemiluminescence Immunoassay and a RePlex Assay are shown:

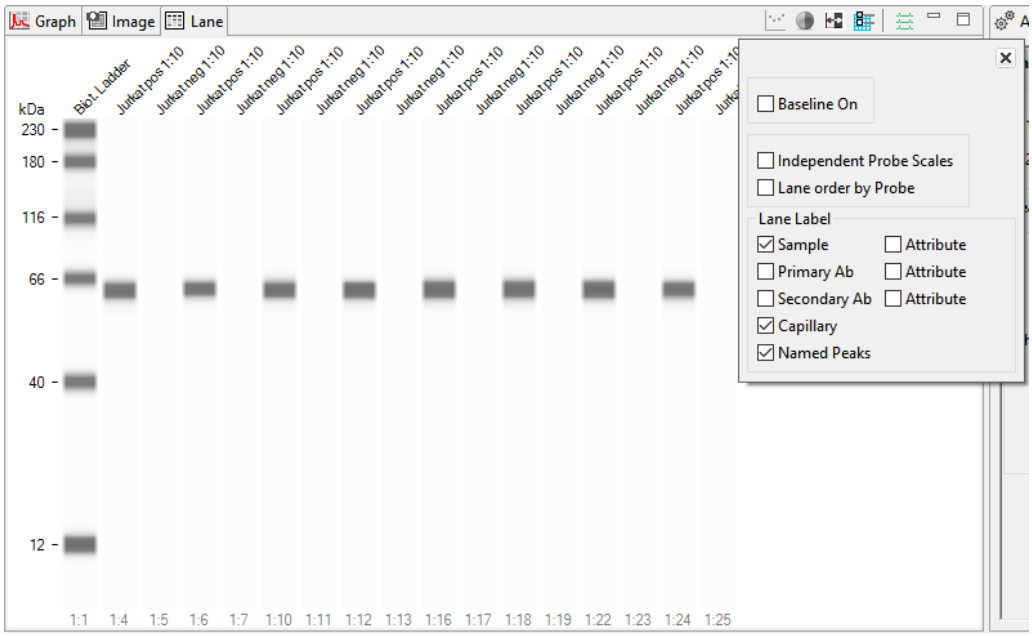
#### CHEMILUMINESCENCE IMMUNOASSAY ON JESS/ABBY



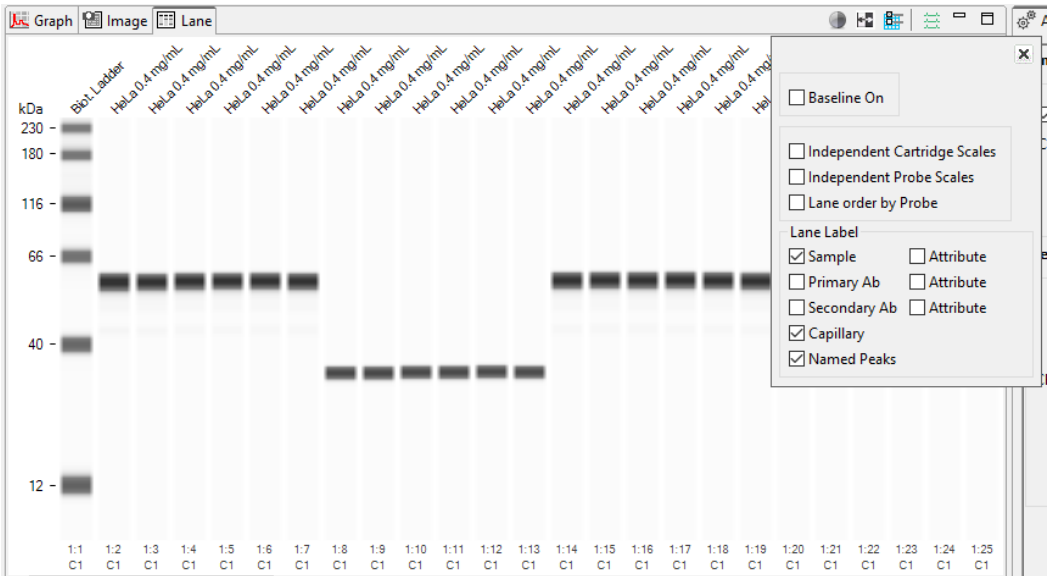
### CHEMILUMINESCENCE IMMUNOASSAY ON LEO




### REPLEX ASSAY ON JESS/ABBY



**REPLEX ASSAY ON LEO**




2. The following lane display options are available. Check one or multiple label boxes, and uncheck those you don't want to display. To remove labels completely, uncheck all boxes.

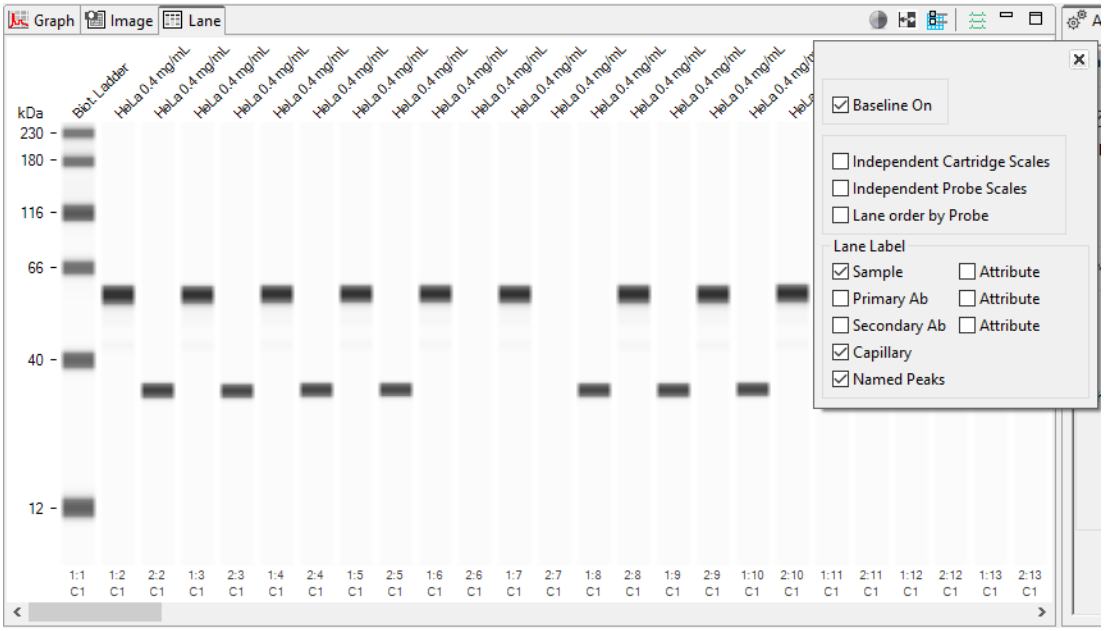
   - **Baseline On** - Checking this box applies the baseline correction to the view.
   - **Independent Cartridge Scales (Leo only)** - Checking this box lets you have independent control of contrast scales for cartridges in a run when using the Contrast Adjustment button.
   - **Independent Probe Scales (RePlex Assays only)** - Checking this box lets you 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.
   - **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**
     - **Jess/Abby Immunoassays (non-RePlex)**: Capillary number.
     - **Jess/Abby RePlex Assays**: Probe and capillary number. For example, 1:4 indicates Probe 1, capillary 4.
     - **Leo Immunoassays (non-RePlex):** Capillary and cartridge number.

- **Leo RePlex Assay**: Probe and capillary number, followed by cartridge number. For example, 1:4 C1 indicates Probe 1, capillary 3, cartridge 1.

- **Attributes** - Attribute text. If attribute information was entered for any of the rows or wells in the assay template (Assay screen), up to three can be selected and displayed as lane labels.

## Viewing the Corrected Sample Baseline

1. Click the **Lane Options** button and select the **Baseline On** box (active for sample data only). This will apply the automatic baseline correction.

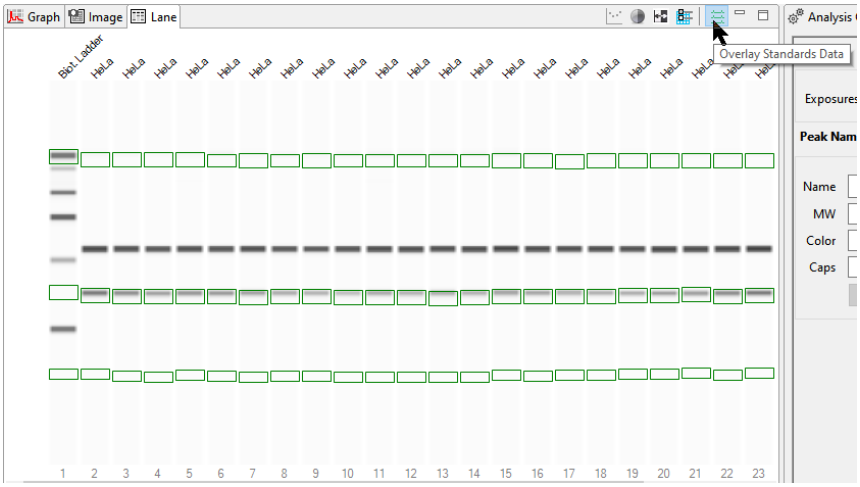



2. Deselect the **Baseline On** box to return to the default, uncorrected baseline view.

## 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 standards data, in green boxes, and raw sample data will display:

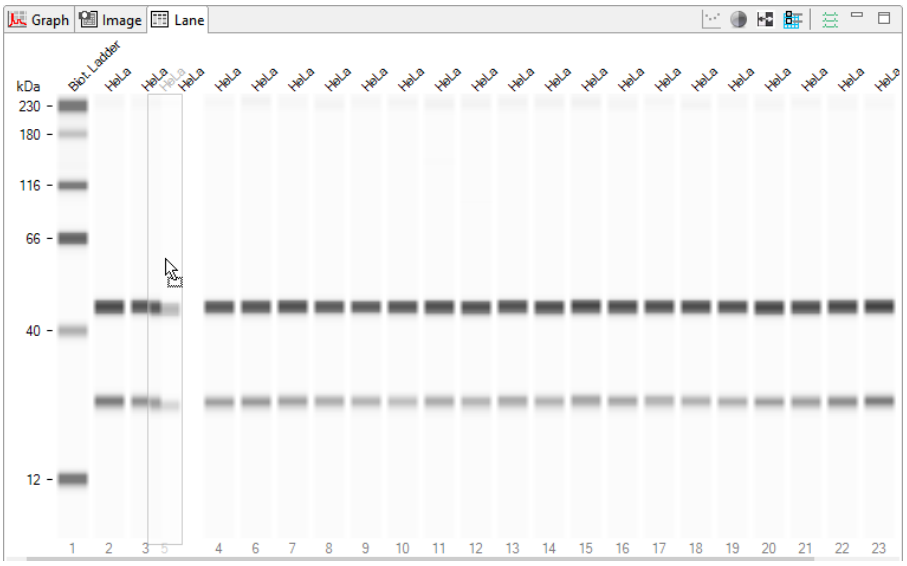



2. Click the **Overlay Standards Data** button again to return to the default, auto-aligned view.

## 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.

**NOTE:** If you change the channel selection or exposure, the lanes will revert back to the default order.

# Creating Annotated Lane View Figures

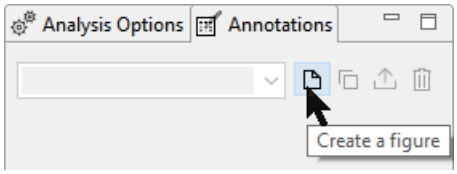
The Annotations pane lets you create annotated figures of the Compass Lane view for use in presentations or publications.


**NOTES:**
You can only create annotated figures from one run file at a time. The Annotations pane is not active when multiple run files have been opened using **File** > **Add Run**.

The Annotations pane can't be used when Access Control is enabled for 21 CFR Part 11 compliance.

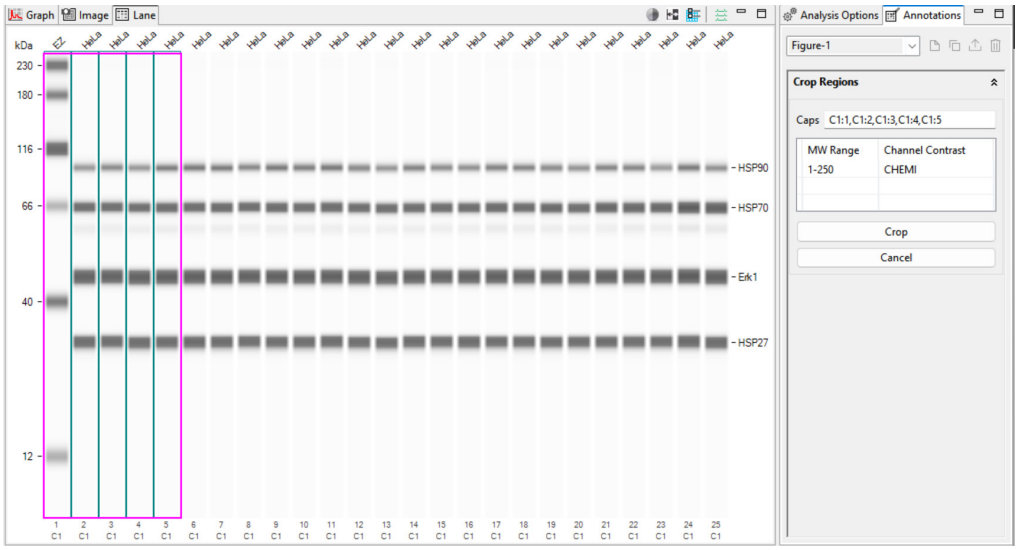
To create a figure:

1. Select the lanes you want to create a figure from. For RePlex runs, select the probe/channel(s) to include.
2. Adjust the lane contrast as needed.
3. Click the **Create a figure** icon in the Annotations pane.



The Lane pane shows a figure preview in a pink bounding box. The Annotations pane displays the crop region information for the bounding box, which includes capillaries for the selected lanes, the MW range of the assay type used for the run file, and contrast settings.

#### CHEMILUMINESCENCE IMMUNOASSAY

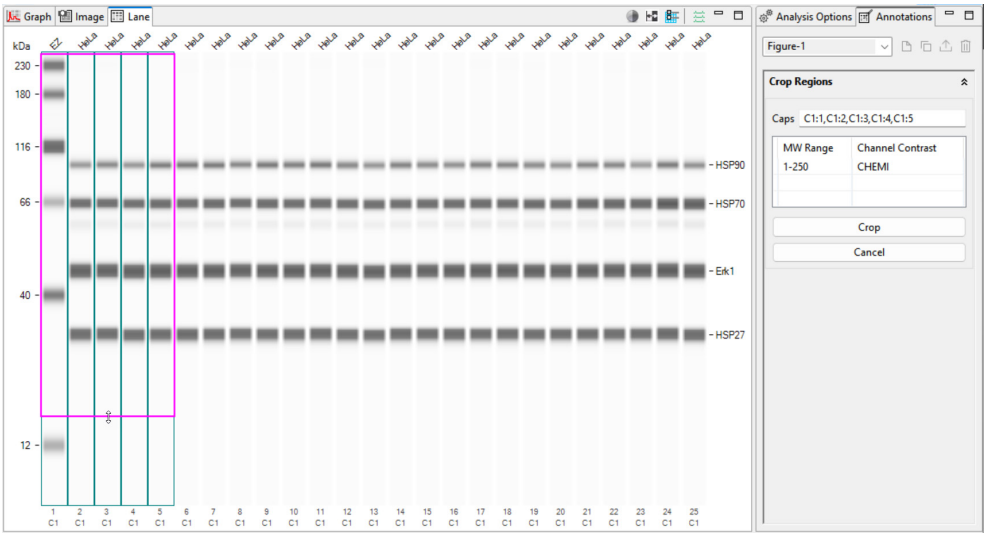



#### REPLEX ASSAY WITH TWO CHANNELS SELECTED


In the preview you can:

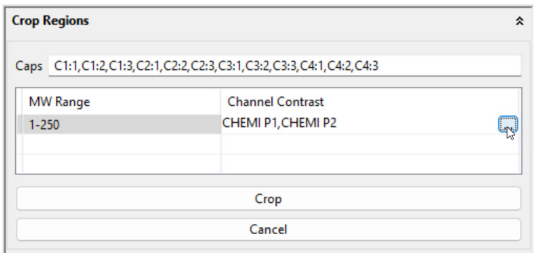
- **Adjust the lane/capillary order** - Click on a lane and drag it to the position you want it to be in, or type the capillary order in the Caps field. Separate each number with a comma.

- **Adjust the MW Range** - Drag the pink bounding box until the desired range is displayed in the table, or just click in the table cell and type a range.
- **Adjust the contrast** - see “Adjusting Lane Contrast” on page 239.




**For probe specific (Jess, Abby, and Leo) and cartridge specific (Leo only) contrast adjustment:**

a. After the figure has been created, click in the Channel Contrast column and click on the ... next to the available channels for the crop region.

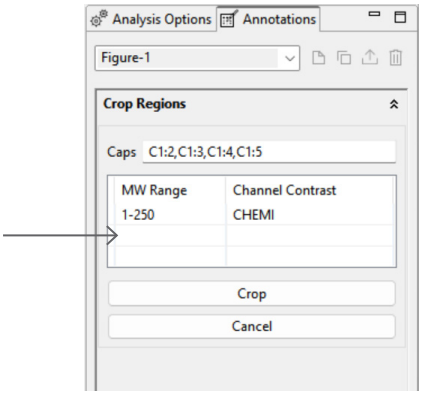


b. Select the Custom Contrast checkbox in the Custom Contrast dialog window.

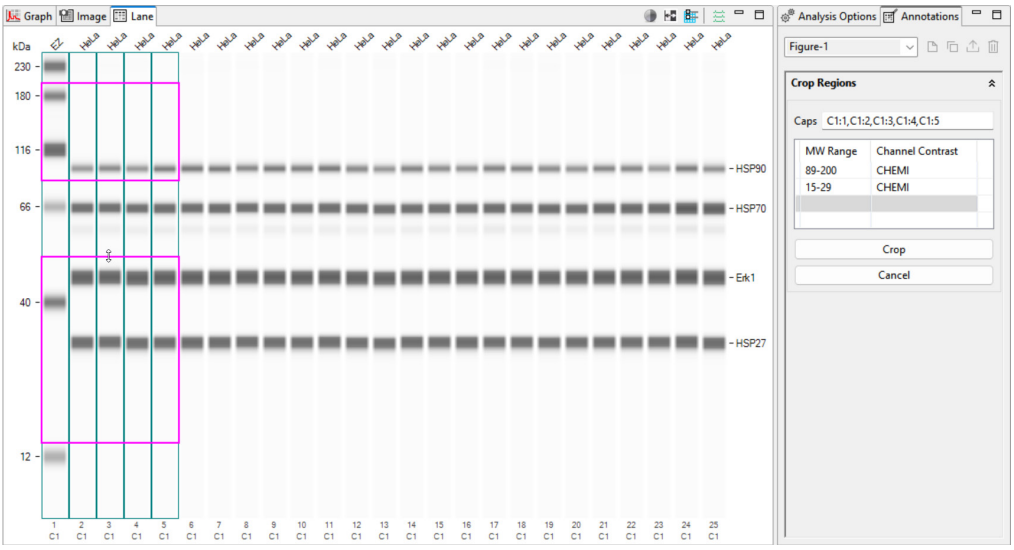

c. Move the sliders for the specific channel, cartridge (C1, C2, etc.), or probe (P1 or P1) to adjust the lane contrast.

d. Click **OK** to save the changes.

- **Add another crop region** - Click in the first blank cell in the MW Range column.

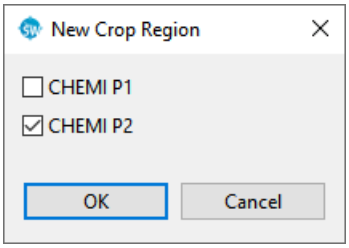


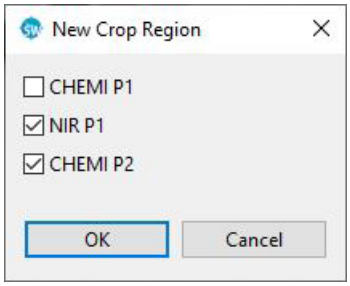
A second bounding box will display on the figure preview.



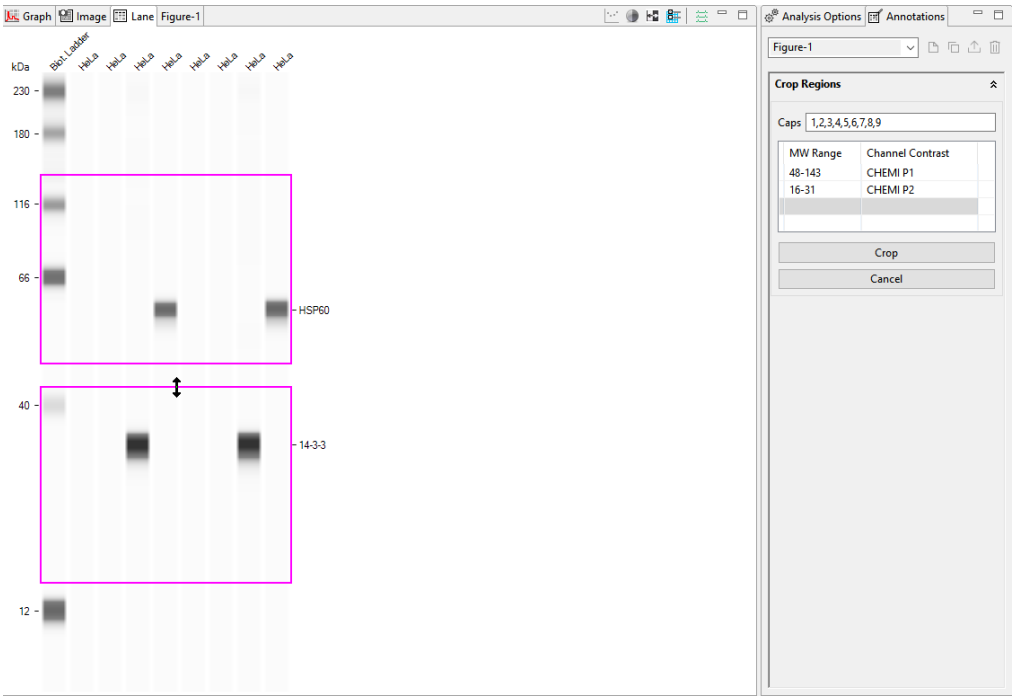

Drag the pink bounding box for the second crop region until the desired range is displayed in the table, or just click in the table cell and type a range.

- **Add a channel/probe for RePlex assays** - This lets you create a figure that stacks multiple channel/ probe views. Click in the first blank cell in the MW Range column. Select the channel checkbox(es) and click **OK**.



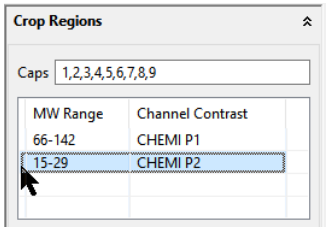



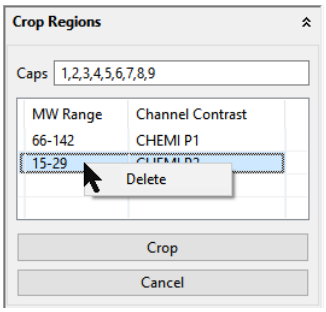
A second bounding box will display in the figure preview:



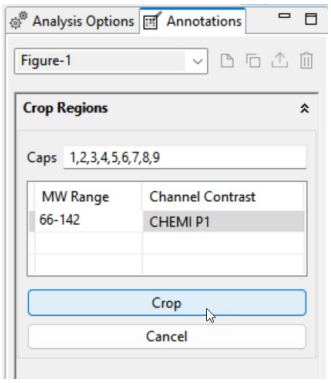

Drag the pink bounding box for the second crop region until the desired range is displayed in the table, or just click in the table cell and type a range.

- **To delete a crop region** - click the cell on the left of the MW Range column to highlight the row, then right-click the column, and select **Delete**.



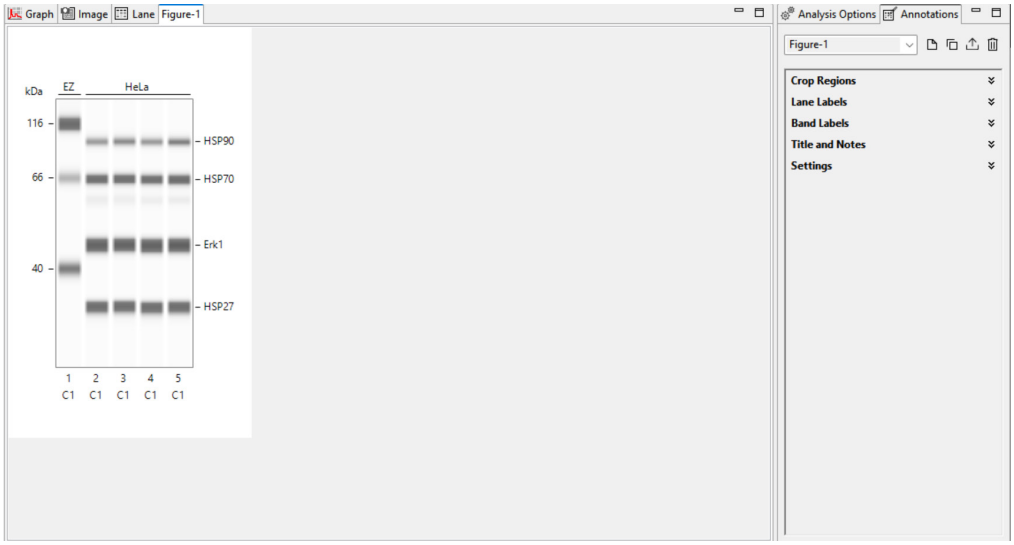



4. Click **Crop** to create the figure file.

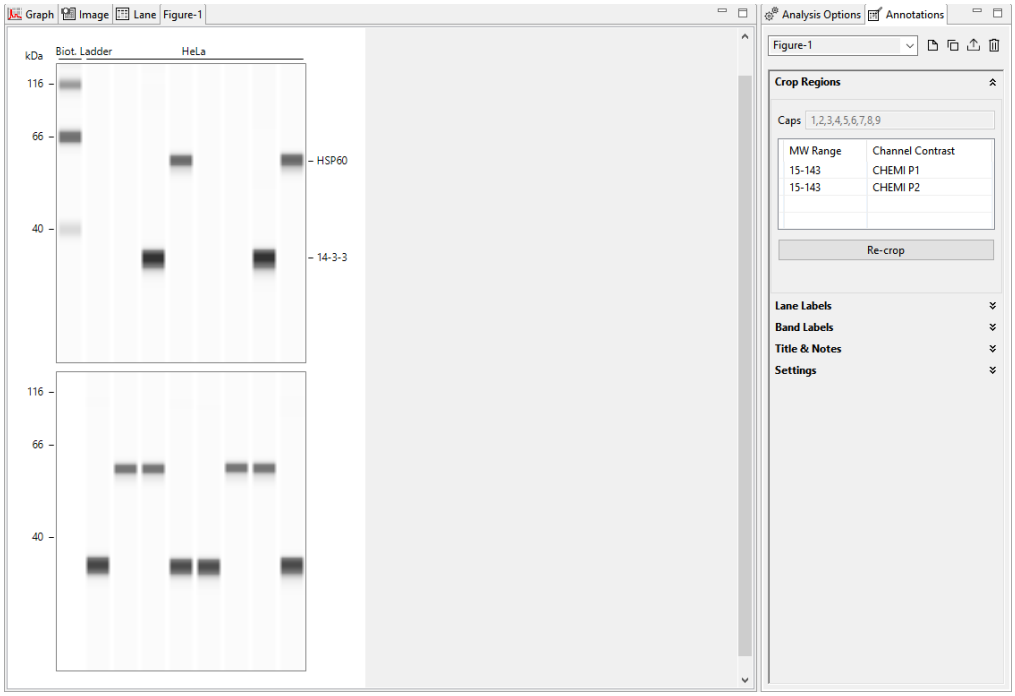



The figure displays in a new tab.

#### CHEMILUMINESCENCE IMMUNOASSAY WITH ONE CROP REGION



**REPLEX ASSAY WITH TWO STACKED CROP REGIONS FOR EACH PROBE**

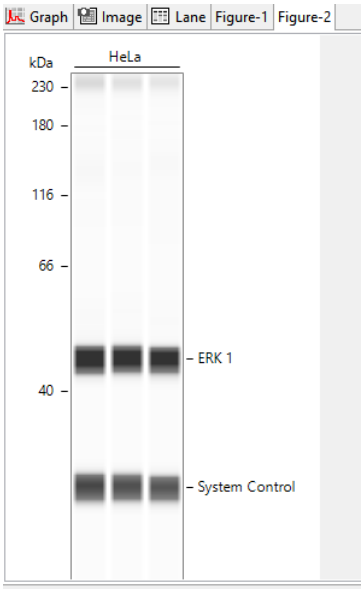



After the figure is cropped, the information in Caps and the table can't be adjusted. If changes are needed, click **Re-crop**. This returns you to the prior view where you can reorder lanes, adjust the MW range, adjust the contrast, or add a new crop region.

**NOTES:**
After you create a figure, saving the run file saves the figure with the run data and the figure will display the next time you open the file.

If you don't see the data you want to include in the figure preview, you can click on the **Lane** tab to review all run data and select/deselect channels.

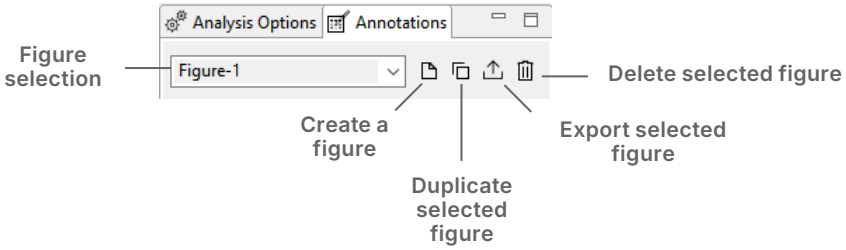
5. **Optional**: If you'd like to create a new figure, repeat the prior steps. Each new figure will display in its own tab:




**NOTE:** If Analysis settings are modified in the run file, for example peak name changes or changes that affect peak area, those changes are automatically applied to figures created from the run file.

## Annotations Pane Toolbar

These options are available in the Annotations pane toolbar:




**To export a figure:**

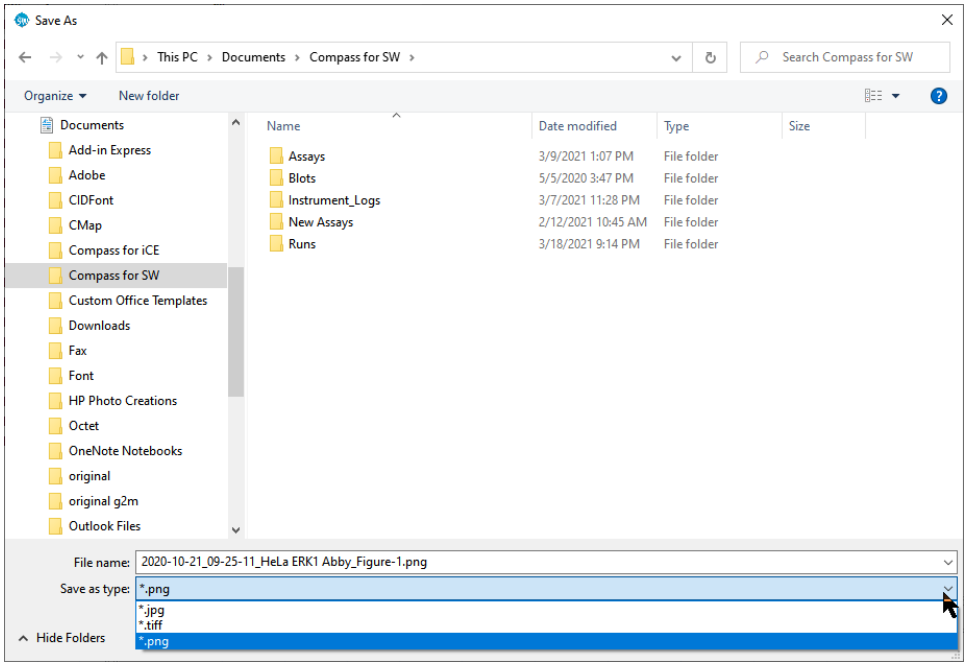
Click the **Export selected figure** icon or right-click on the figure and select **Export**. Browse to the directory you want to save the file to and update the file name as needed. File names must use these guidelines:

- Names must be between 1 and 20 characters
- Names must not already be in use in the same run file

- Use Unicode characters only:
  - Letters and numbers
  - Spaces
  - Hyphen, minus or underline
  - $

Click the down arrow in **Save as Type** to choose a file format.

- .png and .jpg are best for use in external programs like Microsoft® Word® or PowerPoint®.
- .tiff is exported at 300 dpi and is the recommended format for submitting data to journals for publication.




**To duplicate the current figure:** Click the **Duplicate selected figure** icon. A duplicate of the figure currently selected will display in a new tab.

**To create another figure:** Follow the steps outlined earlier in this section. When you click the **Create a figure** icon, the new figure will display in a new tab.

**To switch between annotating multiple figures:** Click the **Figure selection** drop-down and select the figure you'd like to annotate.

**To copy a figure:** Right-click on the figure and select **Copy**. Open a document (Microsoft® Word®, Excel®, PowerPoint®, etc.). Right-click in the document and select **Paste**.

**To delete a figure:** Click the **Delete selected figure** icon.

**To rename a figure:** Select the figure in the Figure selection drop-down, then click on the text and type a new name.

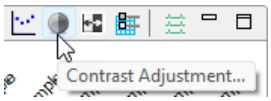
# Adjusting Lane Contrast

After you create a figure, you can adjust the lane contrast in the figure two ways:

**Contrast adjustment in the Lane pane:**

This option lets you change the contrast for the Lane View and the figure at the same time. Note that contrast adjustments can be channel-specific and probe-specific depending on which Lane View options are selected.

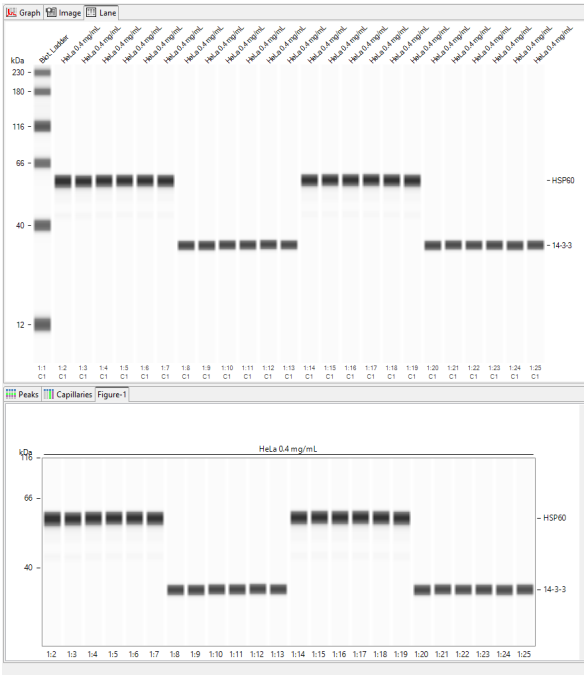
Click the **Contrast Adjustment** icon in the Lane pane.



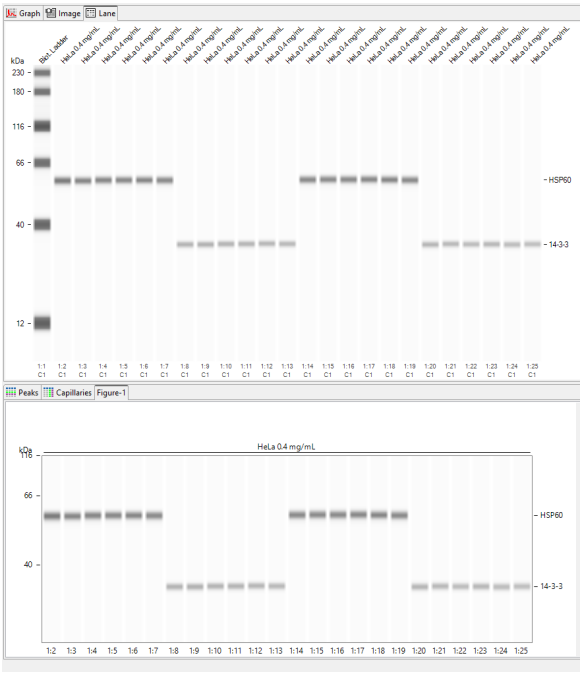

Click the bar and slide it left or right until the desired contrast is reached. The virtual blot lane contrast will change as you do this, but not the figure. The contrast adjustment will be applied to the figure when you release the mouse.

The following examples show Lane View and figure contrast before after adjusting the contrast using Contrast Adjustment:

**BEFORE**




**AFTER**



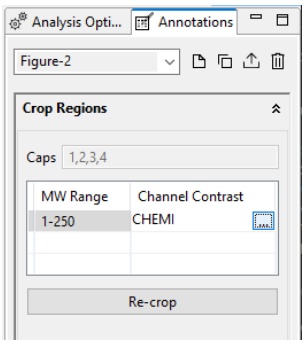
**Custom contrast in the Annotations pane:**

This option lets you change the contrast in the figure only.

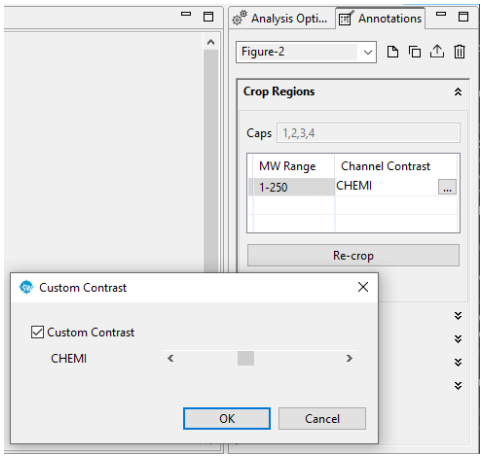
**NOTES:**
Custom contrast changes made in the Annotations pane are applied to the figure only. They are not applied to the run data or in Lane View.

If Custom Contrast is selected in the Annotations pane, figure contrast can't be changed using the Contrast Adjustment tool in the Lane View.

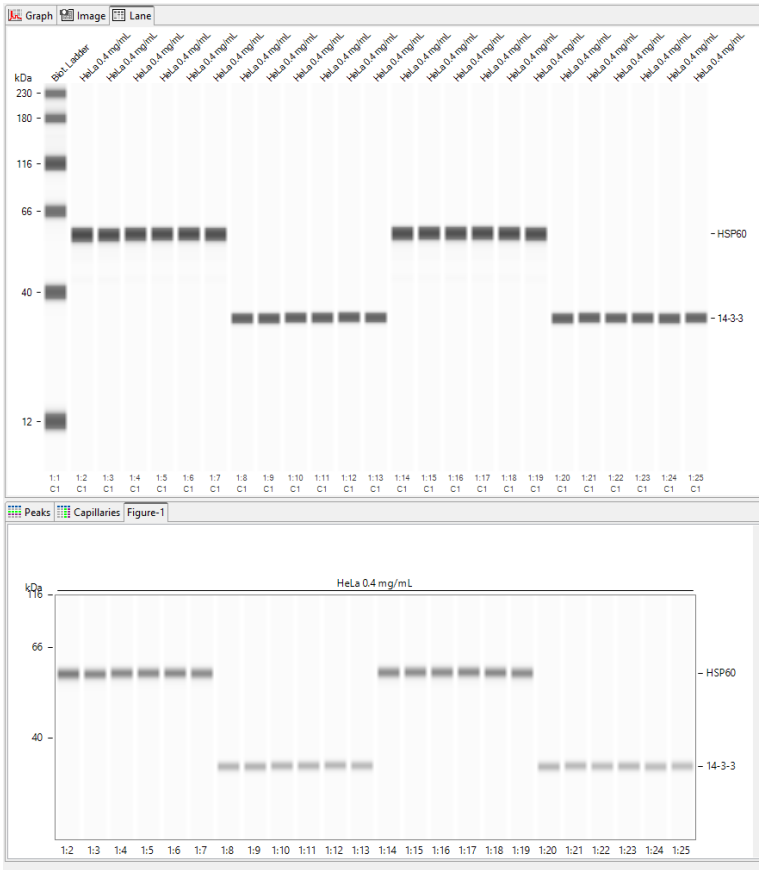
1. In the Annotations pane, click the down arrow next to **Crop Regions** to expand the section. Click the cell in the Contrast column for the crop region you'd like to adjust, then select the **...** button:




2. Select the **Custom Contrast** checkbox.



3. Click the bar and slide it left or right until the desired contrast is reached. Only the lane contrast in the figure, not Lane View, will change as you do this.

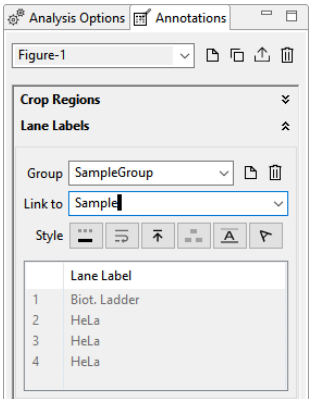


## Adding Lane Labels

This option lets you add lane labels on the figure, and change how they display.

**NOTE:** Lane labels cannot be added when Access Control is enabled.

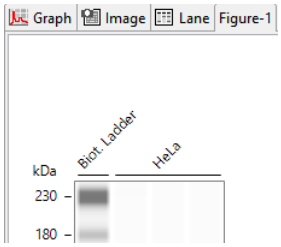
1. Click the down arrow next to **Lane Labels** to expand the section.




You can create different lane label groups based on assay attributes or create your own custom group.

**NOTE:** Assay attributes for the run file can be viewed in the Assay screen Template pane.

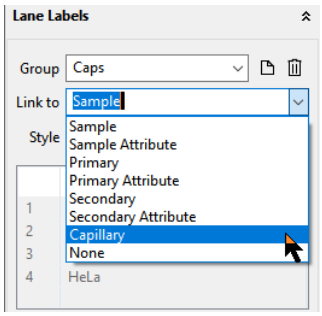
Compass automatically creates a default SampleGroup which is linked to the Sample name in the assay template. For more information on assay plate annotations, see “Step 5 - Add Assay Plate Annotations (Optional)” on page 52. This information for the run data is displayed in the table and above the lanes in the figure.



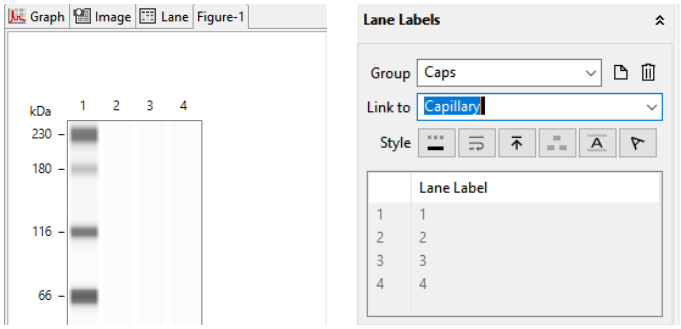
2. Edit the default lane label group as needed:

   a. Click in the **Group** field and enter a new name.

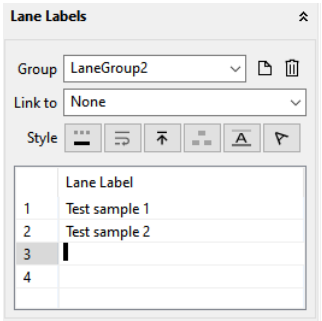
   b. Select an attribute using the Link to drop-down menu.




In this example, Capillary was selected as the attribute (for RePlex assays, this attribute is Probe:Capillary). This displays capillary numbers above the lanes in the figure. This information is also displayed in the Lane table.

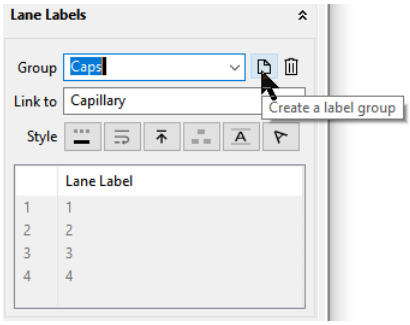



You can also create a custom group by selecting **None** from the Link to drop-down menu. Selecting None allows you to create custom names for each Lane Label in the table.



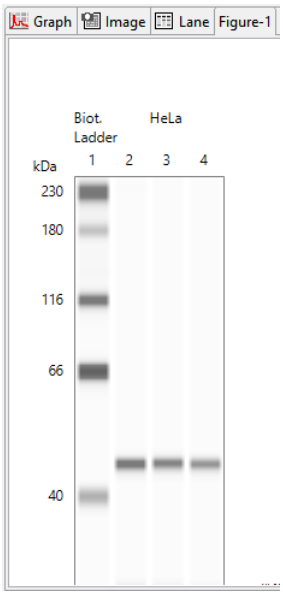
3. Create new groups as needed. Lane labels for all groups will be displayed on the figure.

   a. Click the **Create a label group** icon.

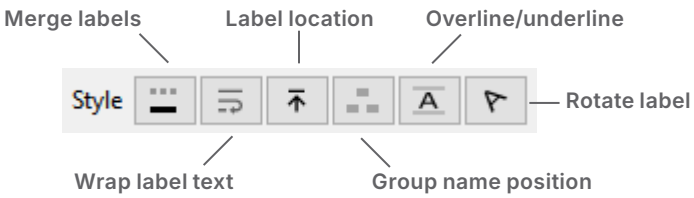



   b. Click the **Group** field and enter a name.

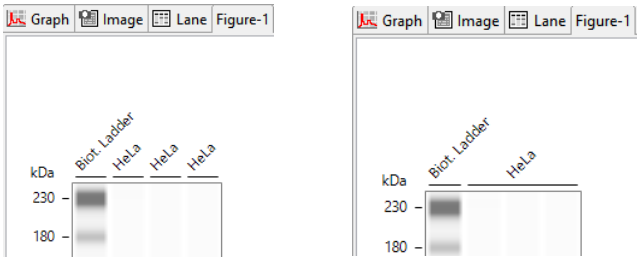
   c. Select an attribute using the Link to drop-down menu. In this example, two lane groups are displayed on the figure, one for capillaries and for samples:



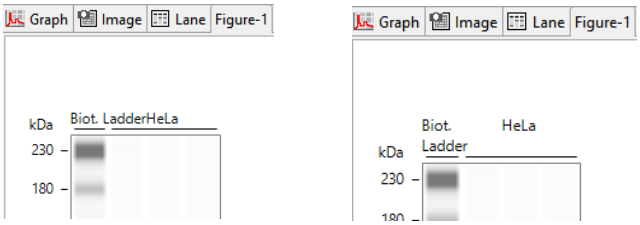

4. Change the lane label styles using the Style icons. Styles are applied per group. If you're using more than one lane label group, first select the group you want to change in the Group drop-down.



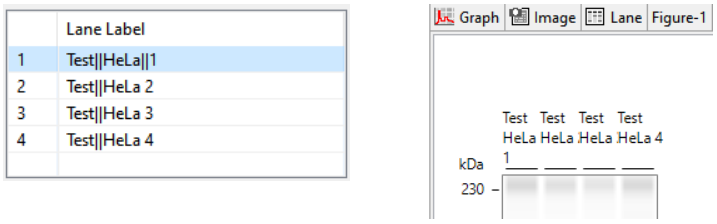
**Merge labels:** Toggling this icon switches between having individual labels on every lane or merging labels across lanes with the same Lane Label name.




**Wrap label text:** Toggling this icon switches between the label text displaying on one line or wrapping onto the next line.



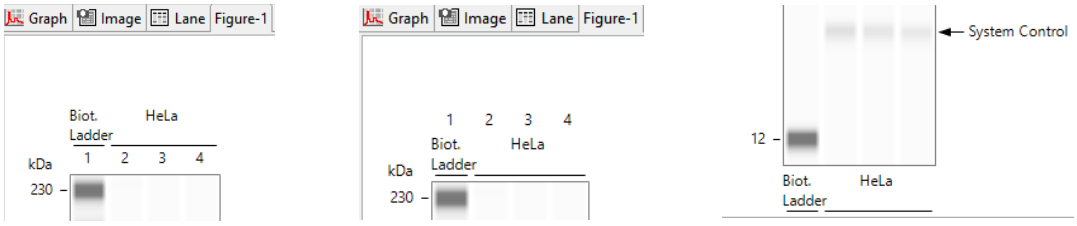

When this option is active, || displays in the label text in the table to indicate where the text will wrap. If you've created a custom group, you can add additional breaks by typing || next to a word.



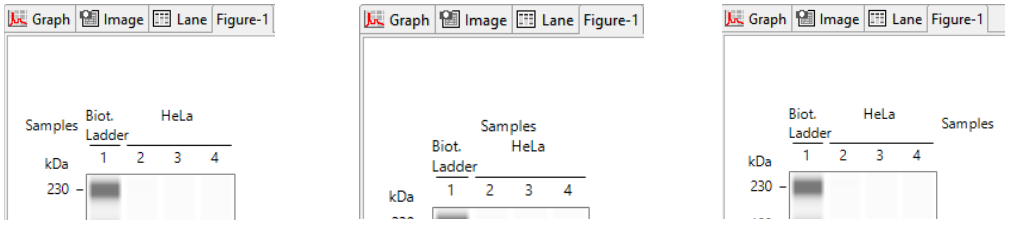
| | Lane Label |
|---|---|
| 1 | Test\|\|HeLa\|\|1 |
| 2 | Test\|\|HeLa 2 |
| 3 | Test\|\|HeLa 3 |
| 4 | Test\|\|HeLa 4 |



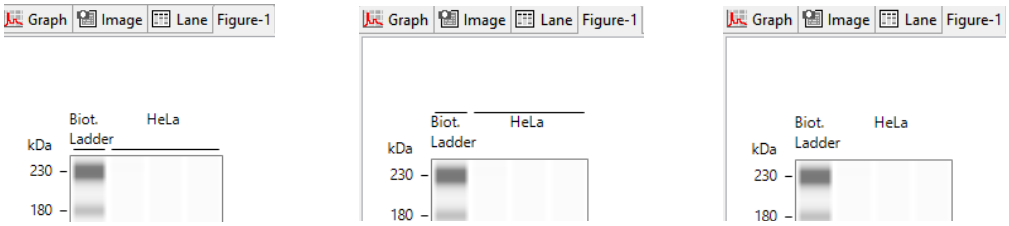
**Label location:** Toggling this icon moves the labels to under the lanes and back to the top. The default position is above the lanes. If more than one set of group labels is displayed, toggling the icon will move the currently selected group's labels under other group labels first before moving it under the lanes.



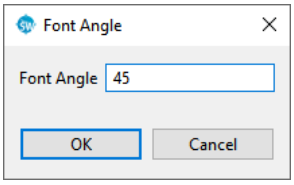
**Group name position:** Toggling this icon displays the group name on the left, top and right of the lane labels, or turns the display off. The default is off.



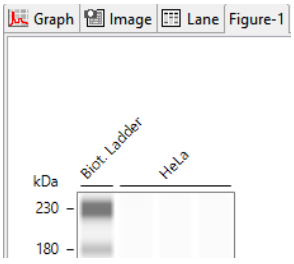

**Underline/Overline:** Toggling this icon switches between displaying a line under the labels, above the labels, or off.




**Rotate label:** This lets you change the font angle of the labels. Click the **Rotate label** icon and enter a font angle. The default setting is 0 (horizontal). You can enter values from 0 to 90 degrees.




Lane labels will rotate accordingly:

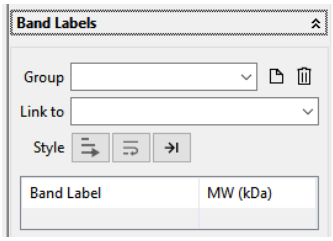



## Adding Band Labels

**NOTE:** Band labels cannot be added when Access Control is enabled.

This option lets you add band labels on the figure and change how they display.

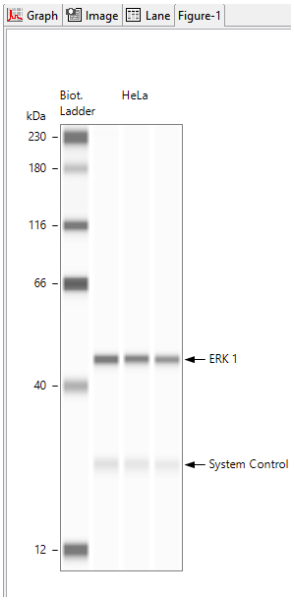
1. Click the down arrow next to **Band Labels**.




You can create different band label groups based on the Peak Names you created in the Analysis Settings for your run or create your own custom groups.

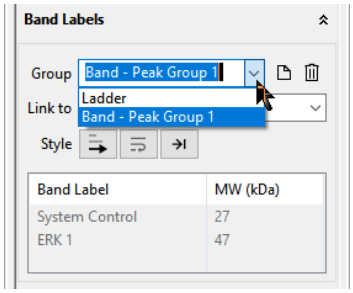
**NOTE:** The Peak Name groups associated with the run data can be viewed in the Analysis Screen in the Analysis Options pane.

Compass automatically creates default band label groups based on the Ladder and Peak Name groups in the run data, and these labels automatically display on the figure. In this example, the default groups included a Ladder, and a group with ERK1 and System Control.



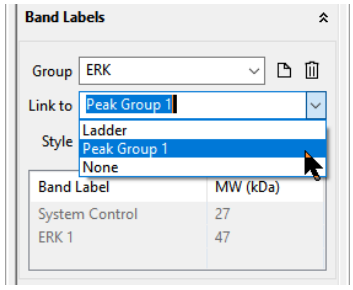
2. Edit the default groups as needed:

   a. Click in the **Group** field and select a group.

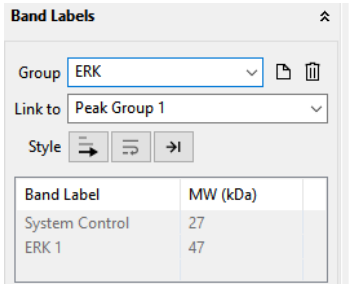



   b. Change the name of the group as needed.

   c. Select a Peak Name group using the Link to drop-down menu.

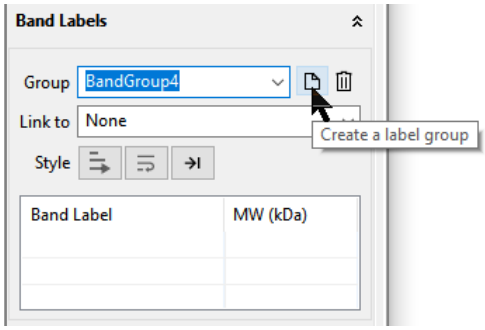



The peak names for the linked group will display in the table.



3. Create new groups as needed. Band labels for all groups will be displayed on the figure.

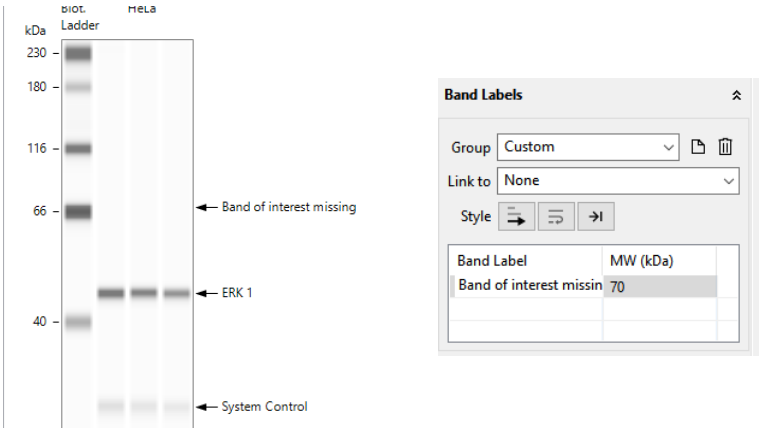
   a. Click the **Create a label** group icon.



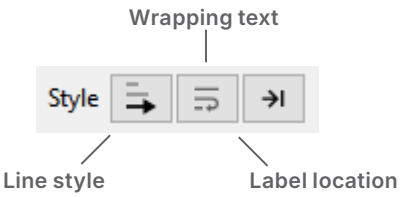

   b. Click the **Group** field and enter a name.

   c. Select a Peak Name group using the Link to drop-down menu.

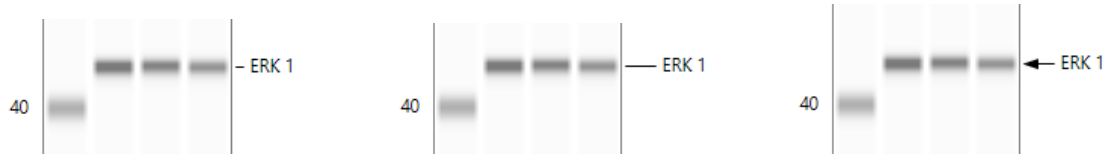
      You can also create a custom band label group by selecting **None** as the Link to option. This lets you add new label names directly into the table. Click in the table cells and enter a name and a MW for each band. In this example, a custom group was added to indicate where a band was expected but not detected in the sample:



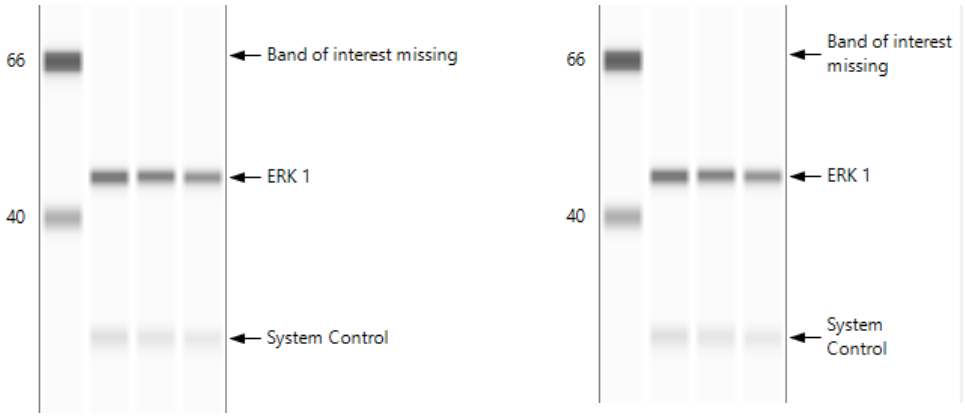

4. Change the band label styles using the Style icons. Styles are applied per group. If you're using more than one group, first select the group you want to change in the Group drop-down.



**Line style:** Toggling this icon changes the line style next to the band label between no line, a short line, a long line and a line with an arrow.

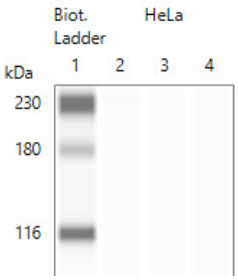



**Wrap label text:** Toggling this icon switches between the label text displaying on one line or it wrapping to the next line.

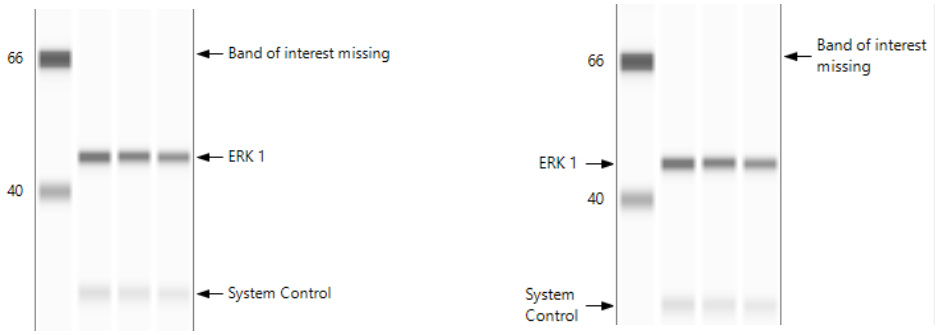



When this option is active, || displays in the label text in the table to indicate where the text will wrap. You can add additional breaks by typing || next to a word.

| Band Label | MW (kDa) |
|---|---|
| Band of\|\|interest\|\|missing | 70 |
| | |
| | |



**Label location:** Toggling this icon moves the labels back and forth between the left and right side of the lanes. The default setting is on the right.

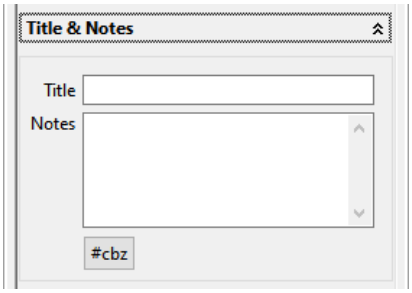



## Adding a Title and Notes

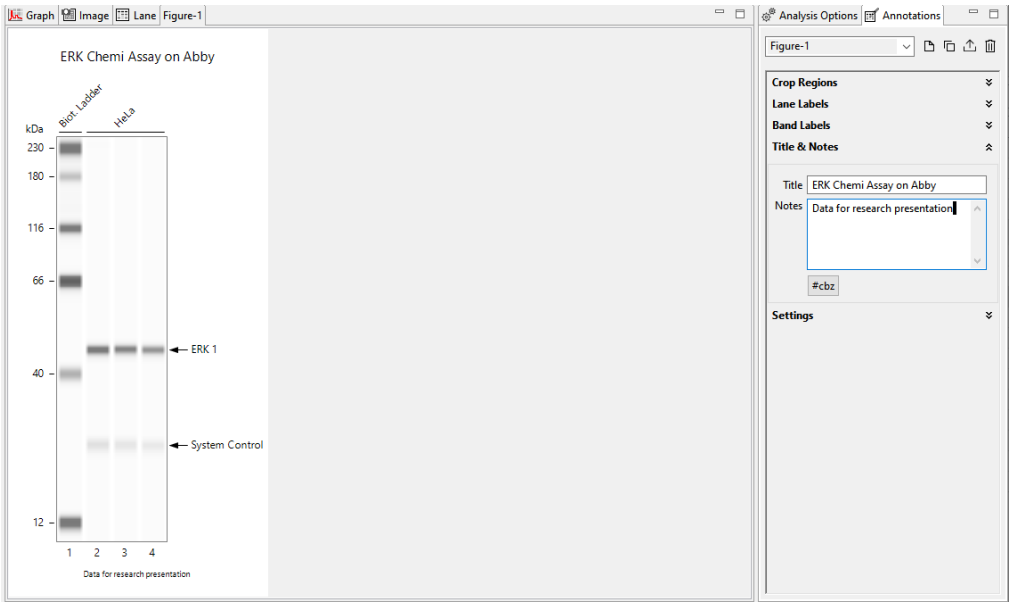
**NOTE:** Titles and notes cannot be added when Access Control is enabled.

You can add a title, notes and insert the run file name on the figure.

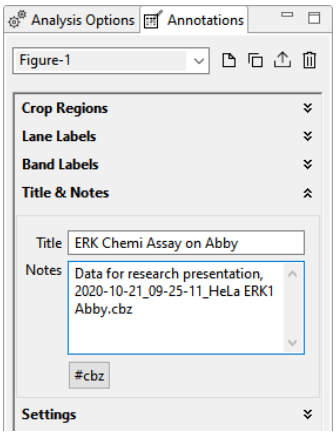
1. Click the down arrow next to Title & Notes to expand the section.

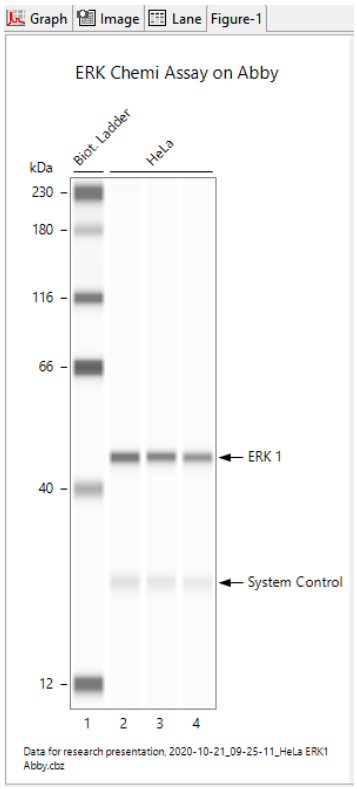


2. Enter a title and any notes. Text for both will display on the figure as you type. The title displays at the top of the figure, notes display under the lanes.




3. Clicking **#cbz** will insert the run file name in the Notes text box:



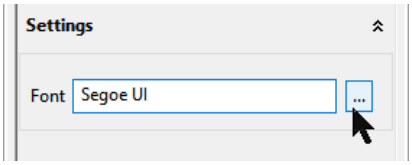



# Changing the Font

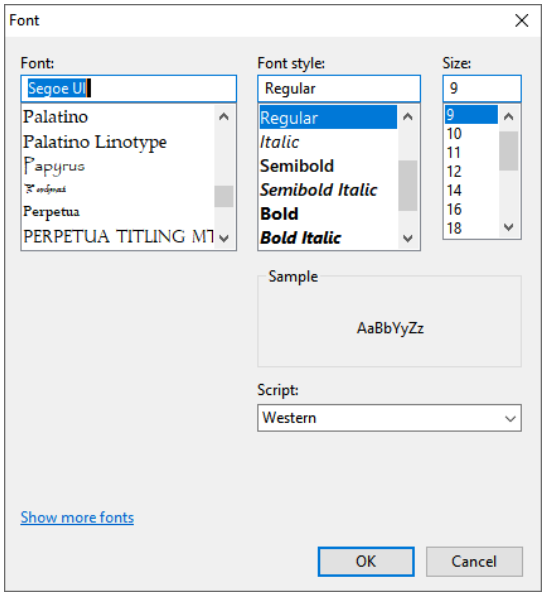
**NOTE:** Fonts cannot be changed when Access Control is enabled.

You can change the font, font size, and style used for figure annotations.

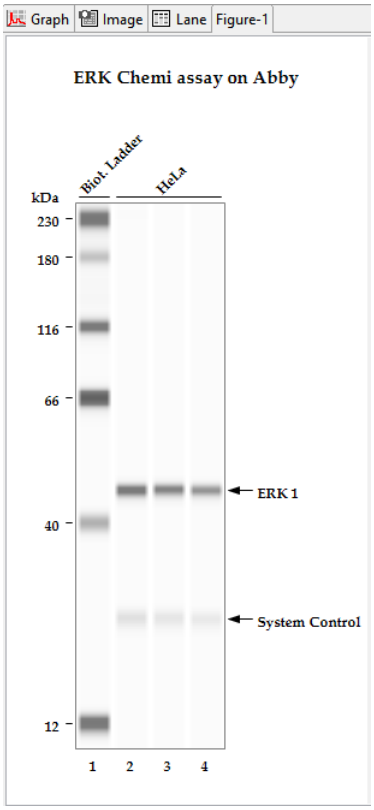
1. Click the down arrow next to Settings to expand the section and click the **...** button.




2. Select the desired font, font style, and size and click **OK**. The default font is Segoe UI.



Font changes are applied to all text on the figure. The font for labels, titles, and notes can't be changed individually. In this example, the selected font is Palatino bold:




# Changing the Electropherogram View

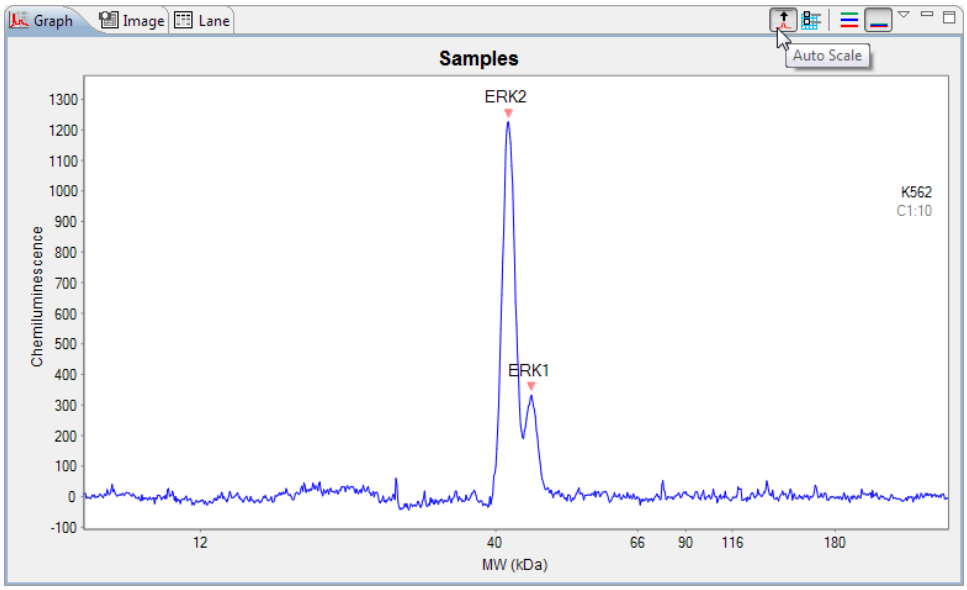
Options in the Graph pane let you zoom and scale electropherograms, overlay or stack plots, and change the peak and plot information displayed.

The Graph pane toolbar has the following options:

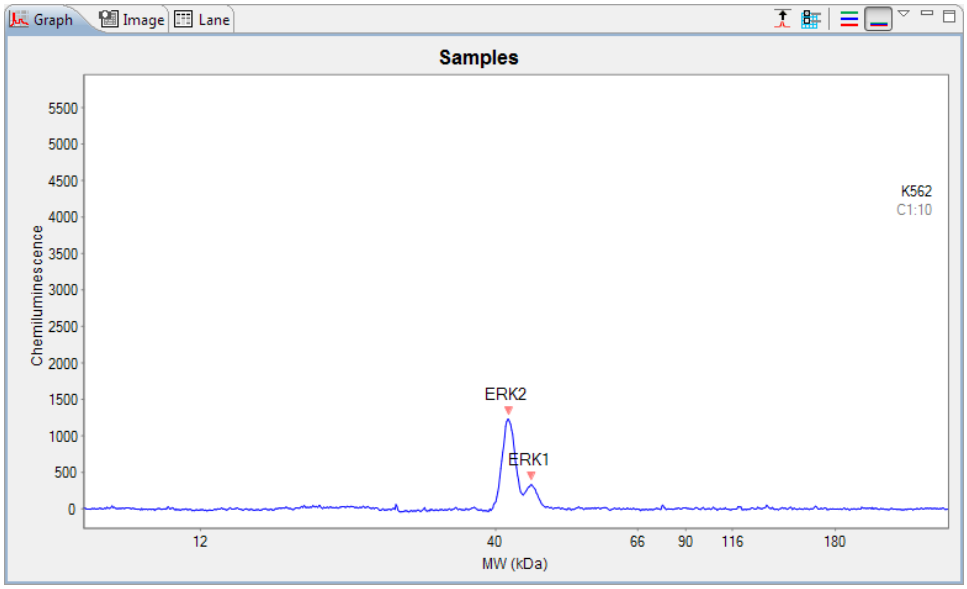
- Auto Scale
- Graph Options
- Stack the Plots
- Overlay the Plots

# Autoscaling the Electropherogram

Click the **Autoscale** button to scale the y-axis to the largest peak in the electropherogram.



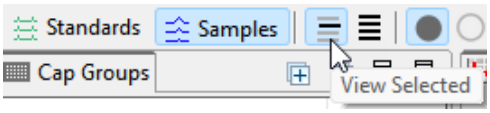

Click the **Autoscale** button again to return to default scaling.



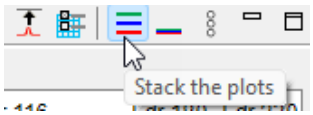
## Stacking Multiple Electropherograms

Electropherogram data for multiple capillaries can be stacked vertically in the Graph pane for comparison. To do this:

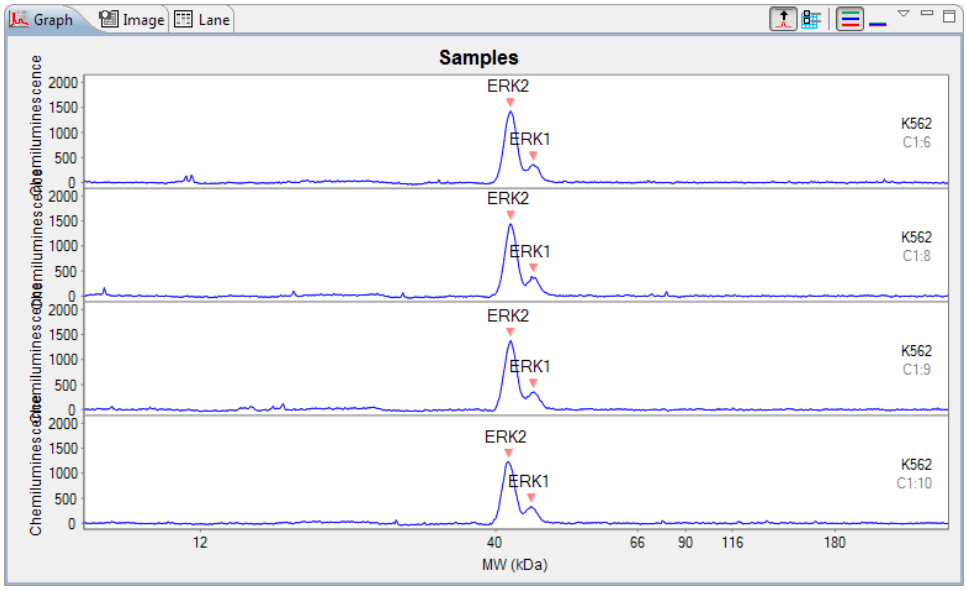
1. Click the **View Selected** icon. You can also select **View** in the main menu and click **Selected**.




2. Select multiple rows in the Experiment pane.
3. Click the **Stack the Plots** button.




4. The individual electropherograms for each row selected will stack in the Graph pane.



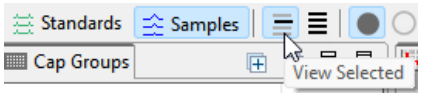

You can customize the colors used for the stacked plot display. For more information see “Selecting Custom Plot Colors for Graph Overlay” on page 342.

**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, an error message displays if you try to change Custom Preferences in any instance except the first instance opened.

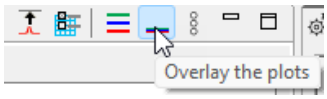
## Overlaying Multiple Electropherograms

The electropherogram data for multiple capillaries can also be overlaid on top of each other for comparison in the Graph pane. To do this:

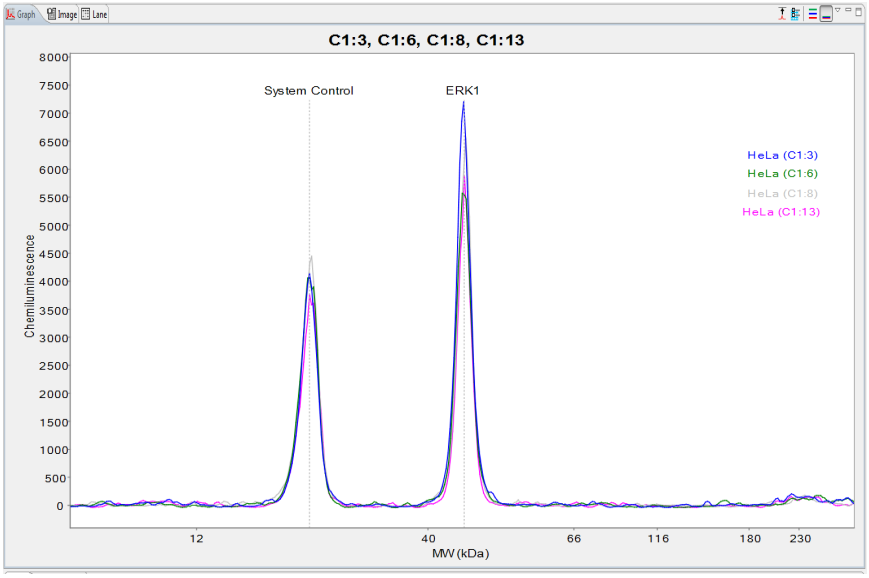
1. Click the **View Selected** icon. You can also select **View** in the main menu and click **Selected**.




2. Select multiple rows in the Experiment pane.
3. Click the **Overlay the Plots** button.




4. The individual electropherograms for each row selected will overlay in the Graph pane.

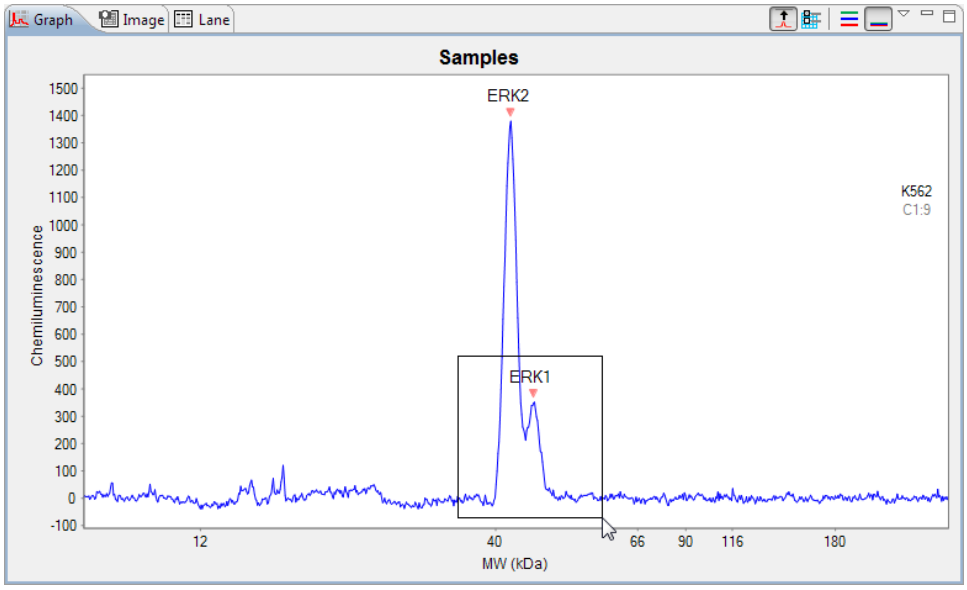



You can customize the colors used for the overlay plot display. For more information see “Selecting Custom Plot Colors for Graph Overlay” on page 342.

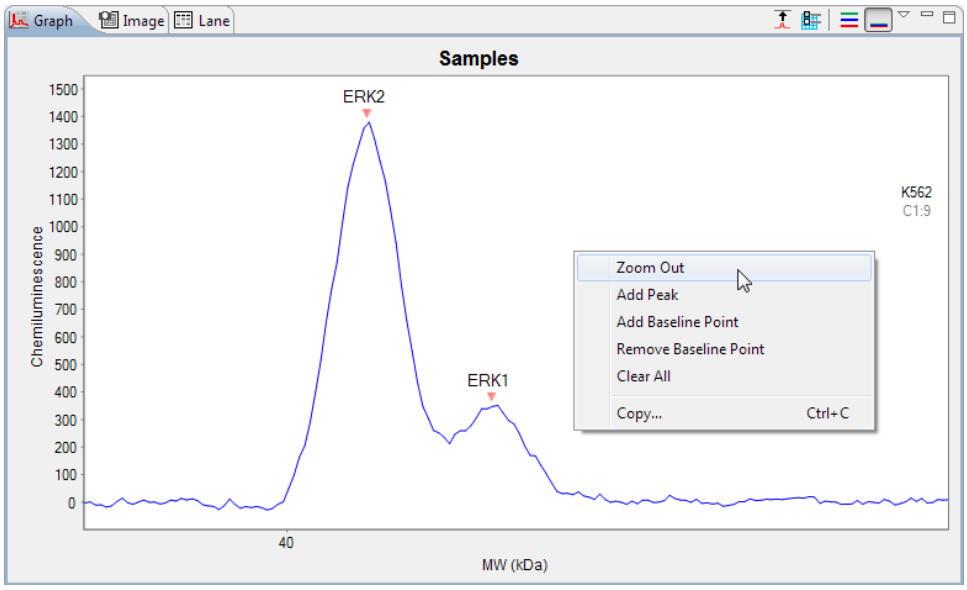
**NOTE:** In Compass for Simple Western version 5.0 and higher, you can no longer change or save Custom Preferences outside of the first instance of the software that’s been opened. When running multiple instances of the software on the same computer, an error message displays if you try to change Custom Preferences in any instance except the first instance opened.

## 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**.

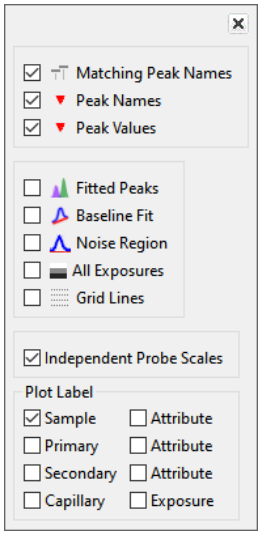


# Graph Options: Customizing the Data Display

**NOTES:**
If you save the run file after updating Graph, Lane, and Table options in the Analysis screen, those selections will override the Default Analysis settings and will automatically display the next time you open the run file. See “Step 8 - Modify Default Analysis View (Optional)” on page 57 for more information.

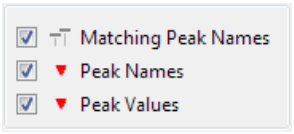
If multiple run files are open, the Graph, Lane, and Table options for the run added last will be applied to the rest of the open runs.

You can customize electropherogram peak labels, plot labels, and display options. To do this, select the **Graph Options** button. An example of the Graph Options for an assay using RePlex is shown below.

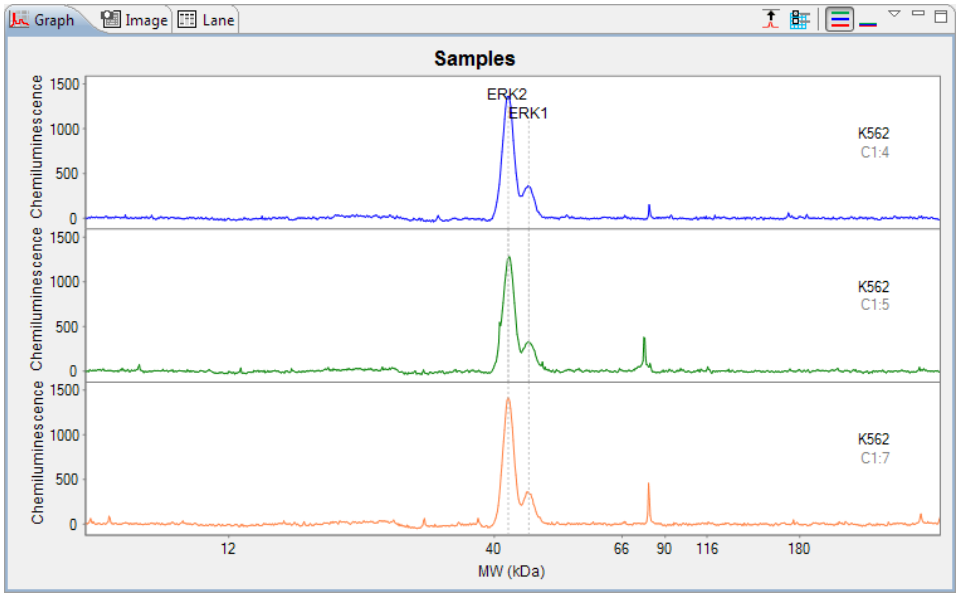



## Peak Labels

You can customize the labels used to identify peaks in the electropherogram with these options:

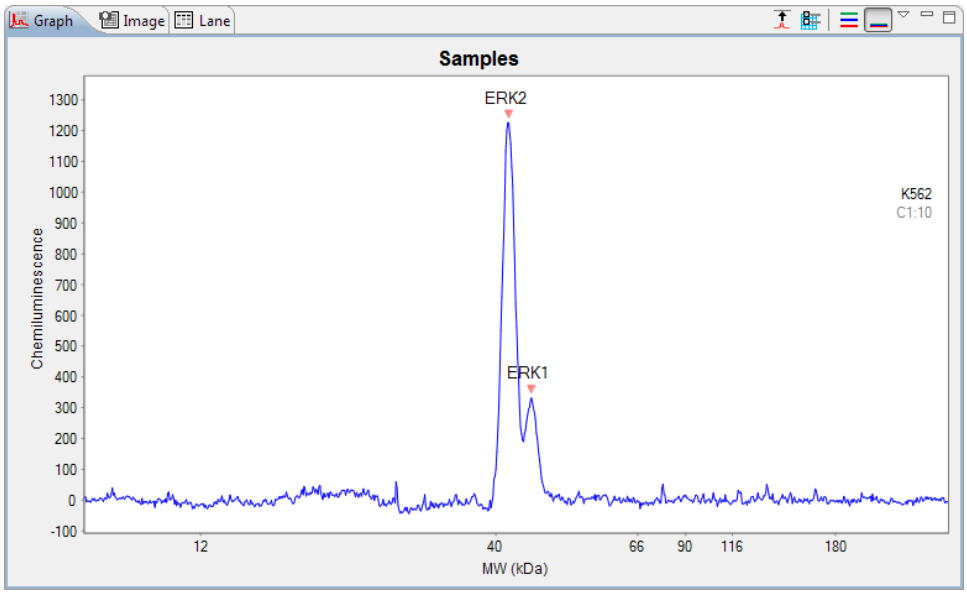


- **Matching Peak Names -** Checking this box will draw vertical lines through each named peak. Using this option with Stack the Plots or Overlay the Plots features is useful for visual comparison of named peaks across multiple capillaries.




- **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.

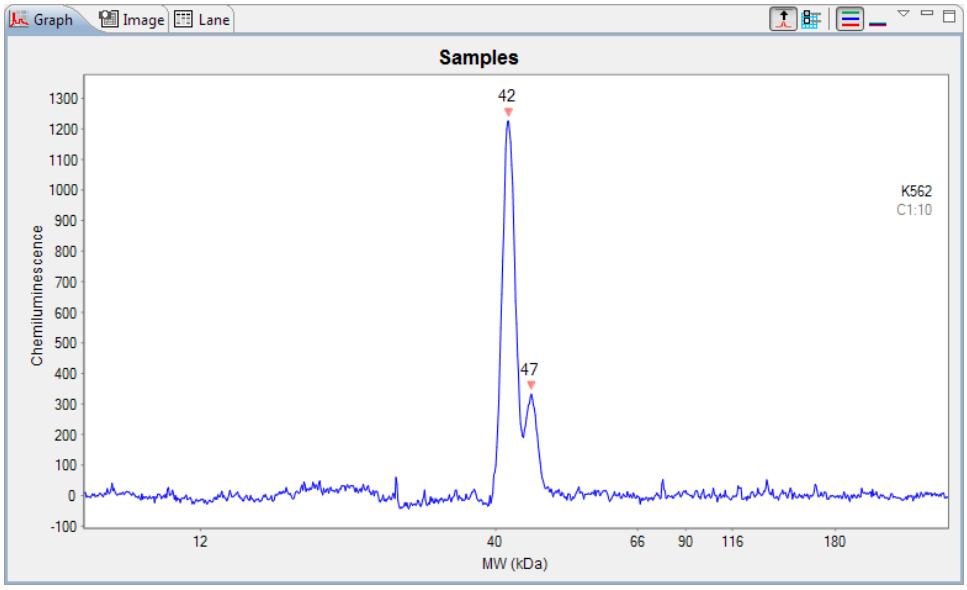


- **Peak Values** - Checking this box will display the molecular weight labels on all peaks in the electropherogram.

**NOTES:**
When viewing standards data, this option is called Peak Positions. Labels displayed are peak positions rather than molecular weight.

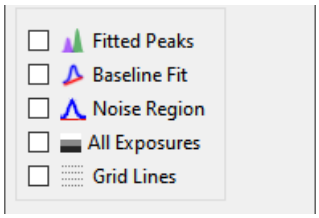
If more than one peak label option is selected, peak name labels will always be used for named peaks.

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.[1,2]



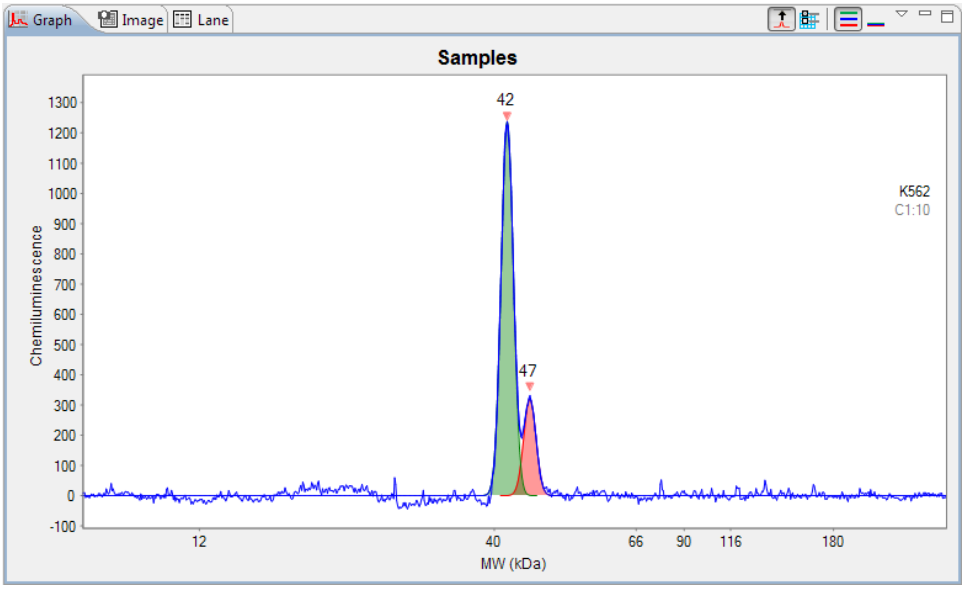

## Baseline, All Exposures, and Grid Options

You can view the calculated baseline fit, peak integration, capillary region used to calculate noise, view all exposures, and show grid lines for sample date with these options. When viewing standards data, only the Grid Lines option is available.

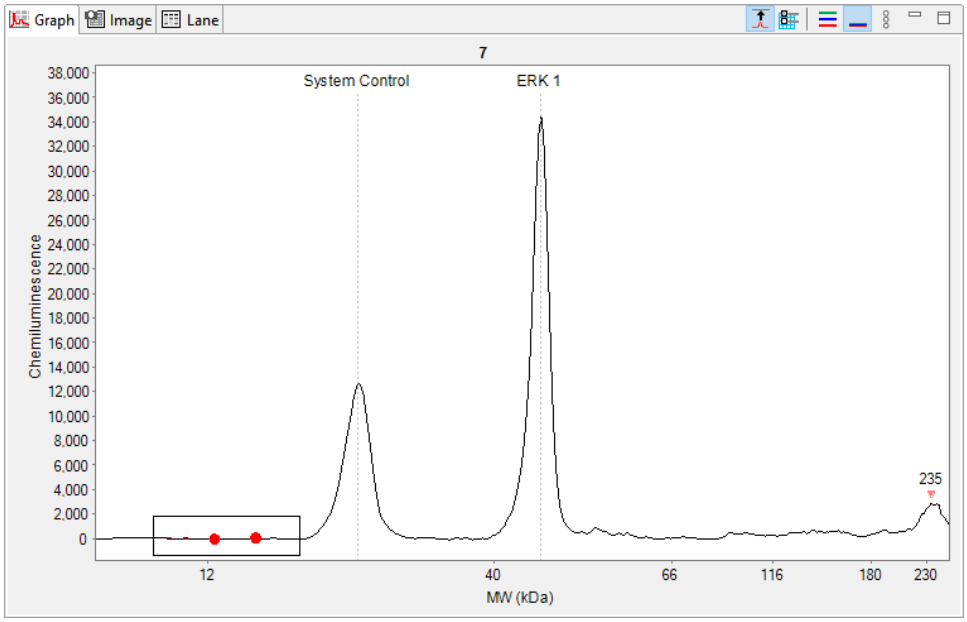


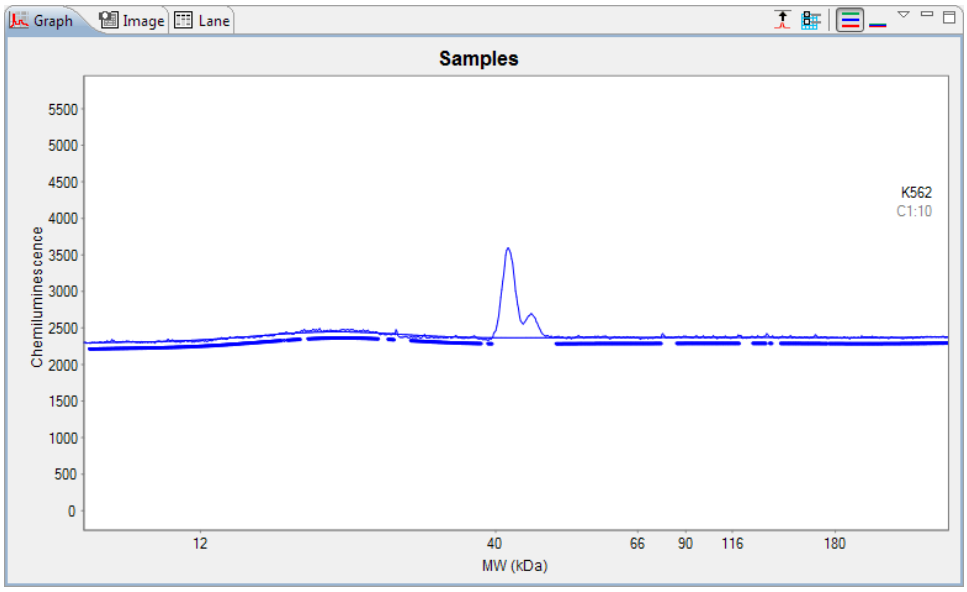

[1] *Electrophoresis. 2021 Aug;42(14-15):1521-1531. doi: 10.1002/elps.202100068.*
[2] *Electrophoresis. 2021 Feb;42(3):206-218. doi: 10.1002/elps.202000199.*

- **Fitted peaks** - Checking this box will display how the peaks were fit by the software.
  - For Immunoassays, the software uses Gaussian fit by default:



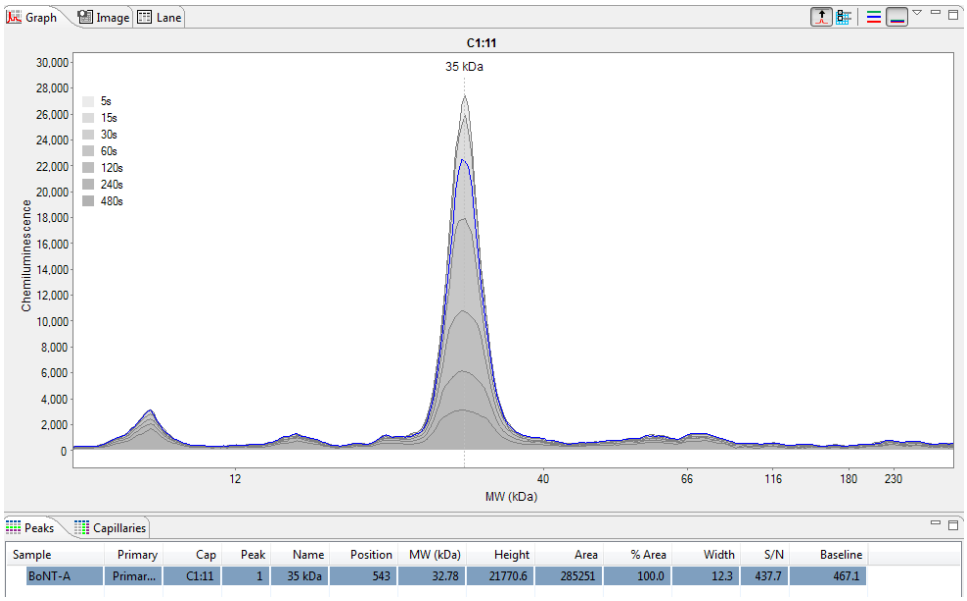

- **Baseline Fit** - Checking this box will display the calculated baseline for the peaks. Depending on your baseline type setting (baseline points or interpolated baseline), baseline points will also display for regions of the electropherogram considered to be at baseline.
- **Noise Region** - Checking this box will overlay the noise region on the electropherogram. The noise region is indicated by the two red dots. For more information on signal to noise methods and calculations, see “Signal to Noise Settings” on page 326.






- **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.

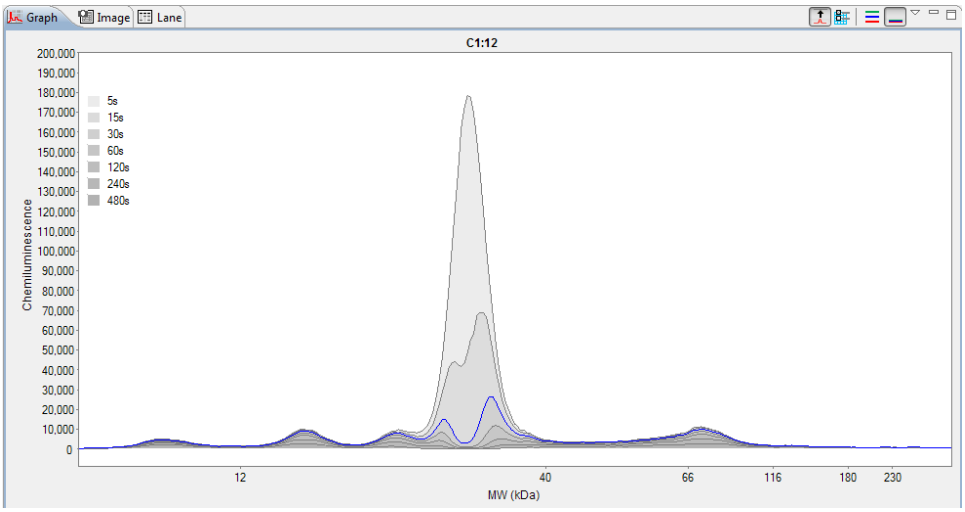
Exposure times are sequential, so longer exposures may have less signal. Since the y-axis in the graph view reports signal/time, the peak heights for the different exposures should be fairly similar. The peak heights and areas will decrease over time with normal HRP enzyme or fluorophore decay, or signal saturation, 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.

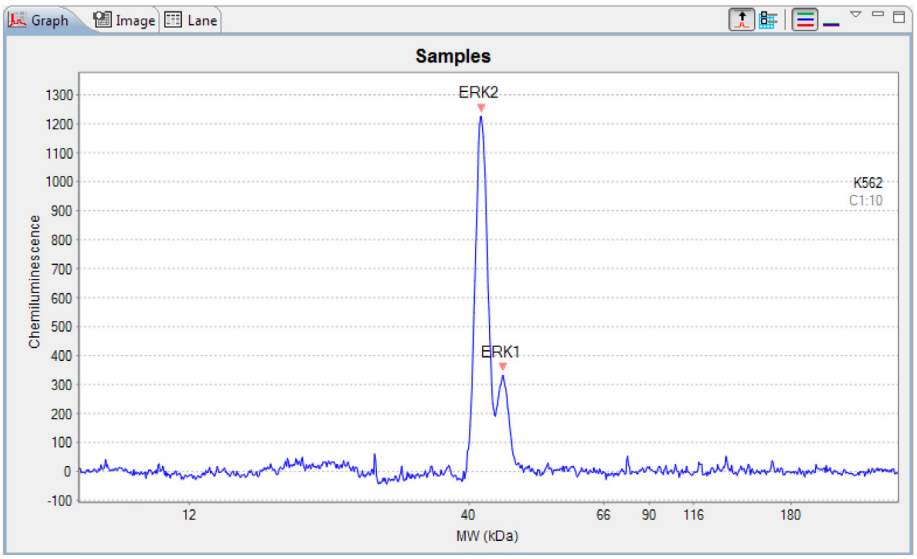
As a rule of thumb, start by looking at the shortest exposure times (typically between 5 to 30 seconds). If the peak heights from each exposure have similar values, then there is likely no signal burnout. If the peak height decreases significantly during those early exposure times, this is an indication of potential signal burn-out. 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.



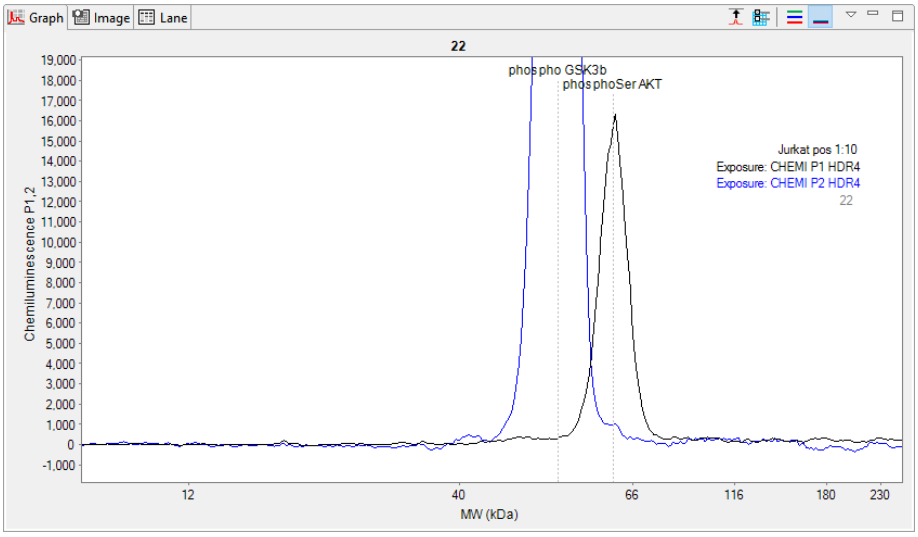
- **Grid Lines** - Checking this box will display grid lines in the graph area.

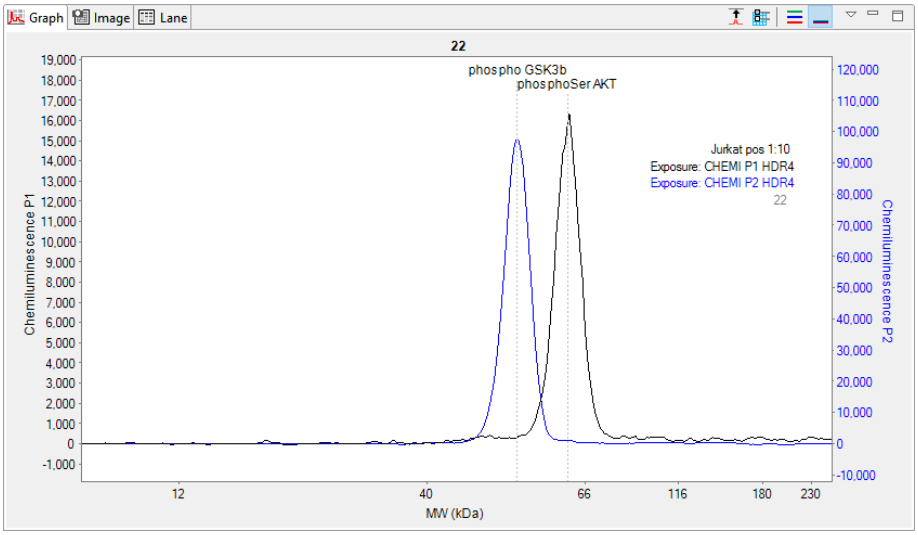



### Independent Probe Scales (RePlex Assays Only)

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).

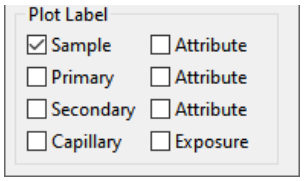
**NOTE:** When more than one channel is used for Probe 1, all data for a channel is displayed on the same y-axis when viewing three or more channels.






## Plot Labels

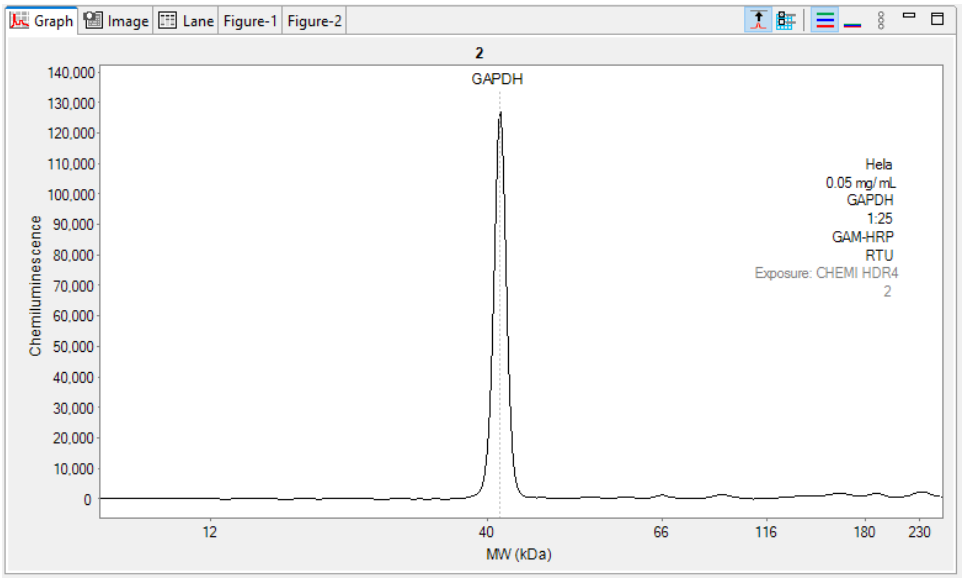
You can customize the plot labels displayed on the electropherogram with these options.




Plot labels are shown on the right side of the Graph pane.

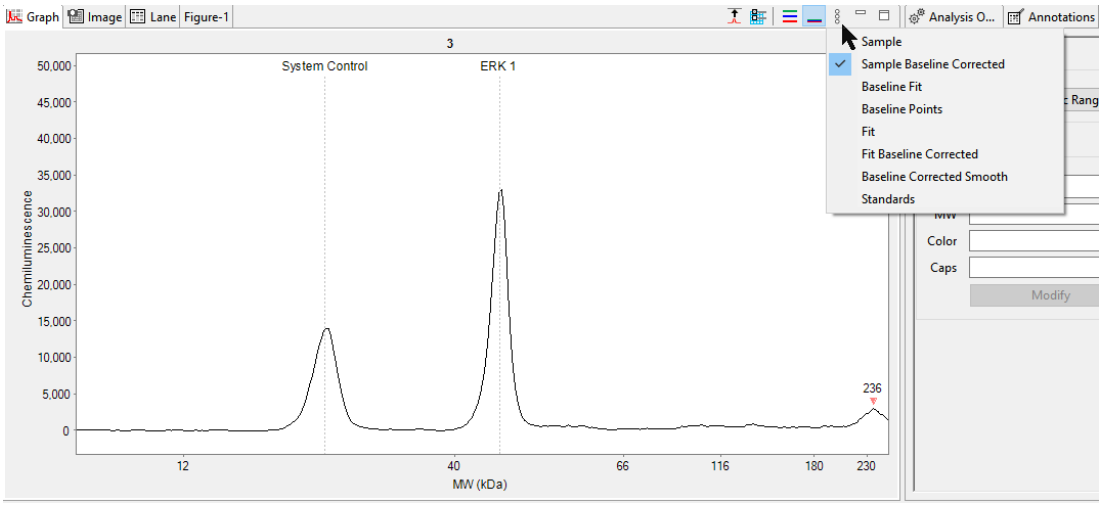
- **Sample** - Checking this box will display the sample name. If sample names were entered in the assay template (Assay screen), those names will display here. Otherwise, Sample (default name) will display.
- **Primary** - Checking this box will display the primary antibody name. If primary antibody names were entered in the assay template (Assay screen), those names will display here. Otherwise, Primary (default name) will display.
- **Secondary** - Checking this box will display the 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.
- **Capillary** - Checking this box will display the capillary number (Jess/Abby) or the capillary and cartridge number (Leo). For example, C1:3 indicates cartridge 1, capillary 3.
- **Attributes** - Checking this box will display attribute text. If attribute information was entered for samples, primary antibodies, or secondary 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

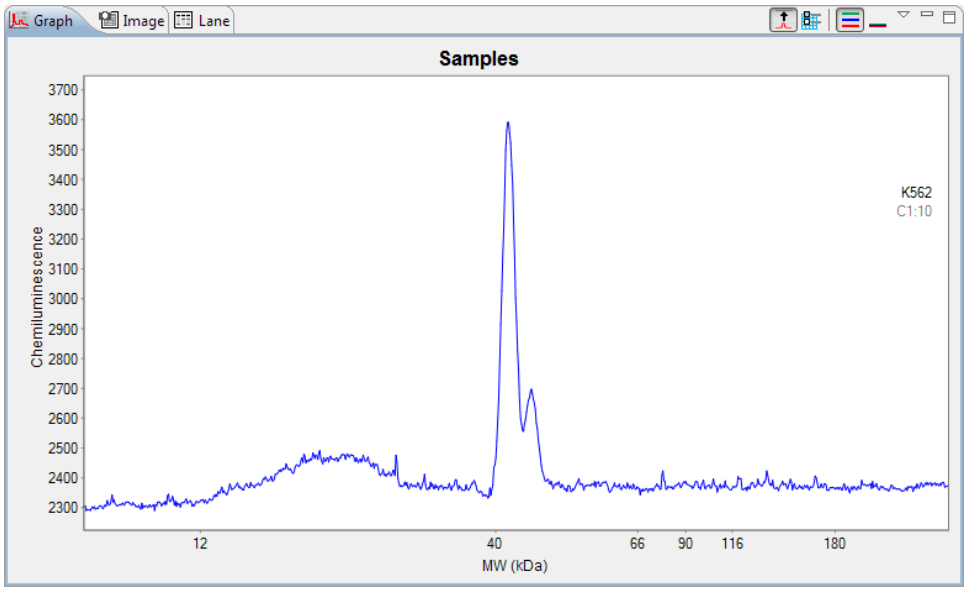
The graph view menu provides you with multiple options for changing what type of electropherogram data is displayed. To access the view menu, click the stacked dot icon in the Graph pane toolbar:



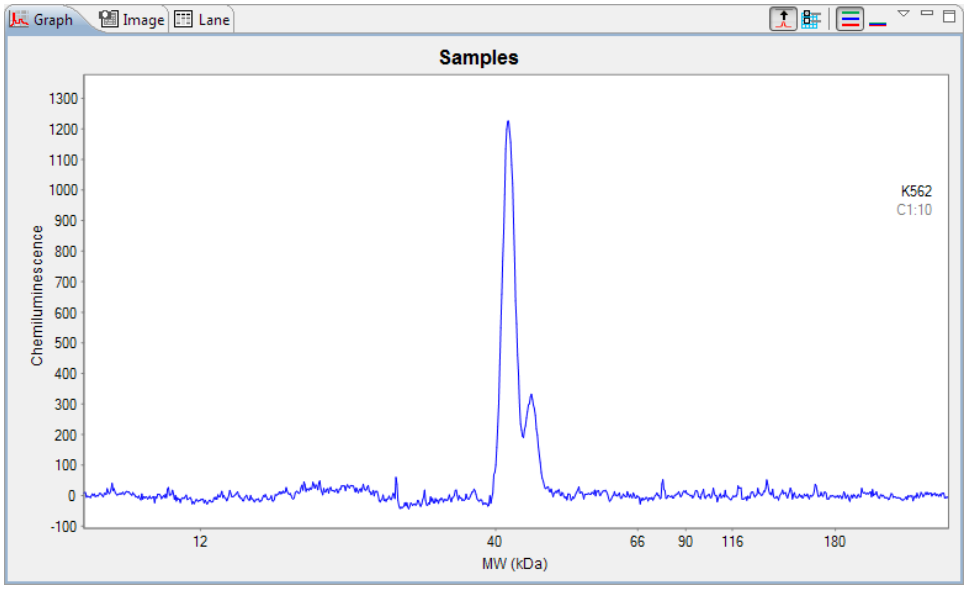
A check mark next to the menu option indicates what is currently selected. Multiple options can be selected at a time.

**NOTE:** Unless otherwise noted, graph view menu options are available for sample data only.

- **Sample** - Clicking this option will display raw, uncorrected sample data.

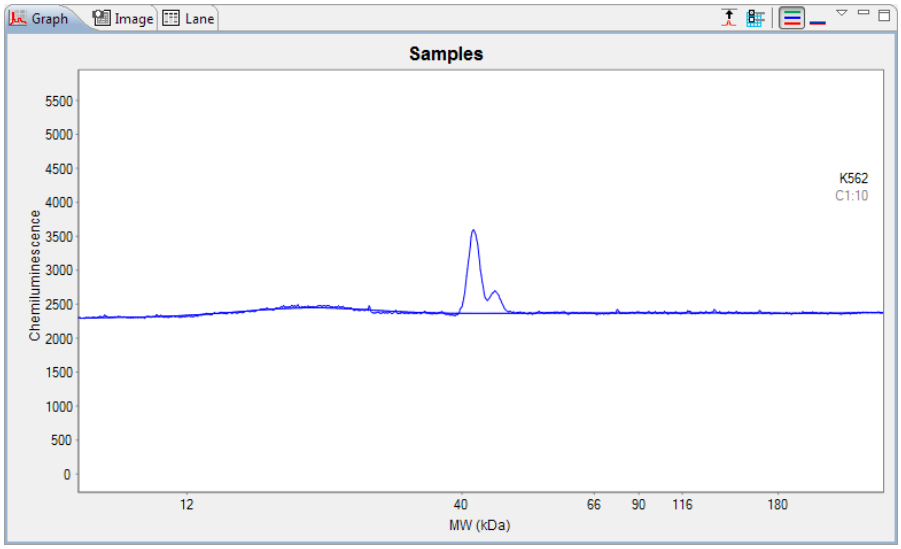



- **Sample Baseline Corrected** - Clicking this option will display sample data with the baseline subtracted (zeroed). This is the default view.



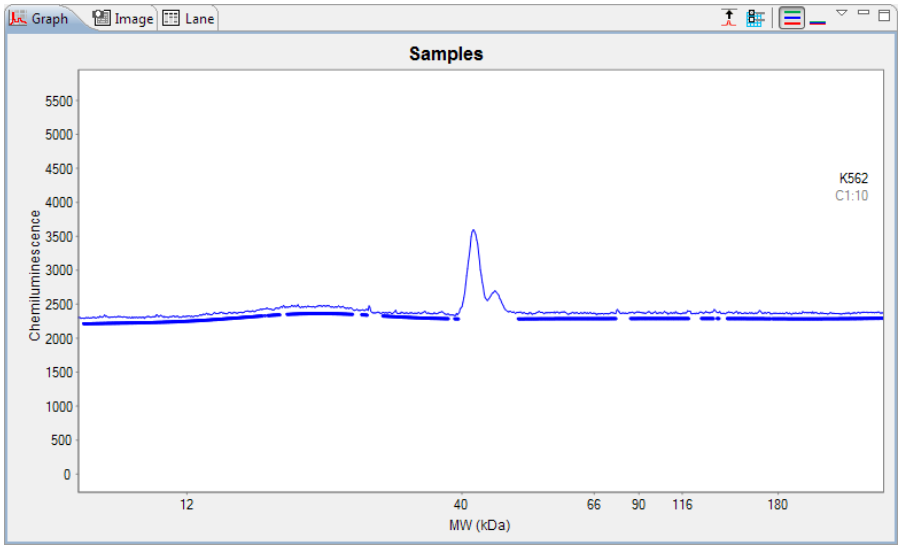
- **Baseline Fit** - Clicking this option will display the calculated baseline for the raw sample data. In the example that follows, both Baseline fit and Sample are selected.

**NOTE:** This option is selected automatically when Baseline Fit is selected in Graph Options.

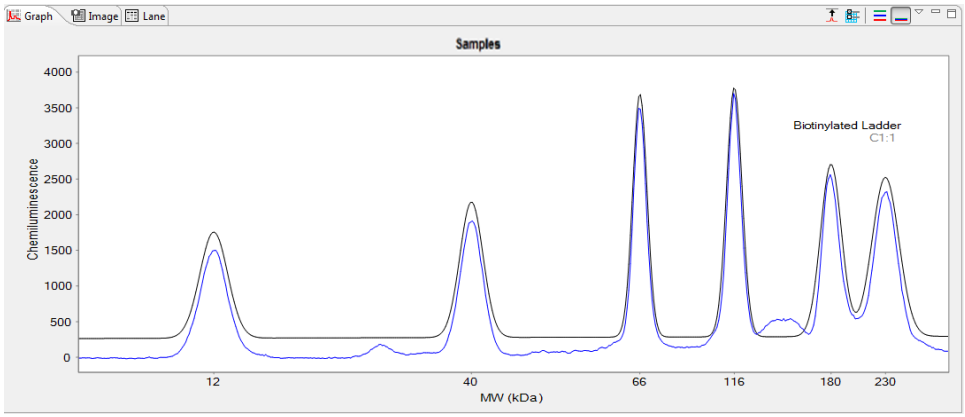



- **Baseline Points** - Clicking this option will display regions of the electropherogram considered to be at baseline. In the example that follows, both Baseline Points and Sample are selected.

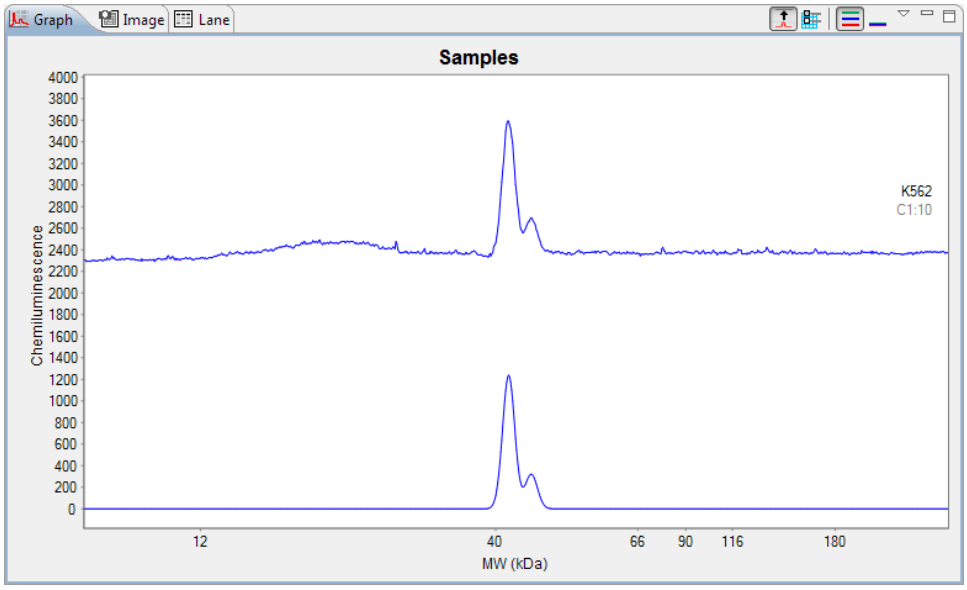
**NOTE:** This option is selected automatically when Baseline Fit is selected in Graph Options.



- **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.

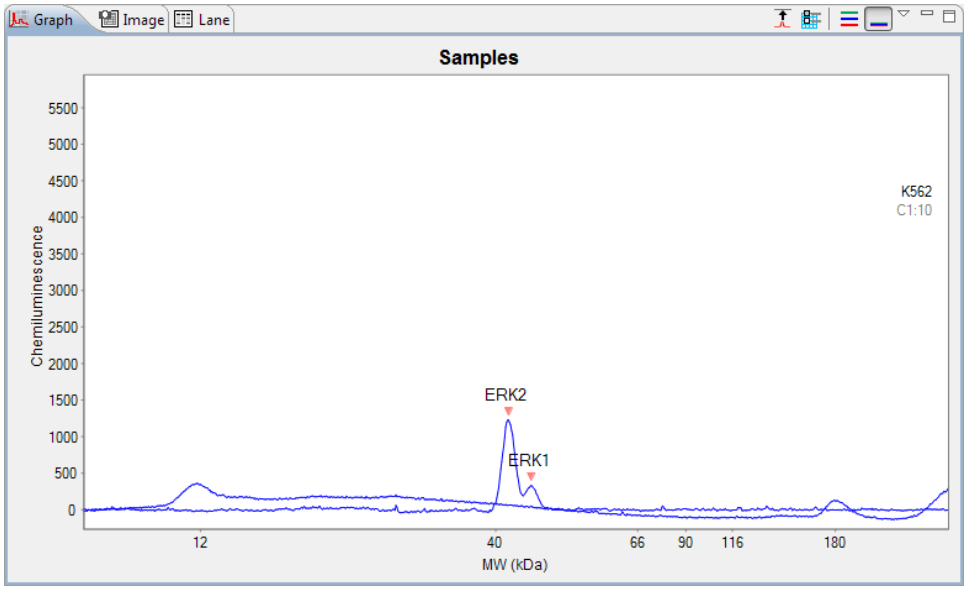



- **Fit Baseline Corrected** - Clicking this option will display the fitted peaks as calculated by the software for the sample baseline corrected data. In the example that follows, both Fit Baseline Corrected and Sample are selected, the fit plot is on the bottom.




**NOTE:** When viewing standards data, this option is called Standards Fluorescence.

- **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.




## Adjusting the Baseline

### Spline Baseline

**NOTES:**
Baseline Type selection is only available when Dropped Lines and Dropped Lines (manual) is used for the Area Calculation.

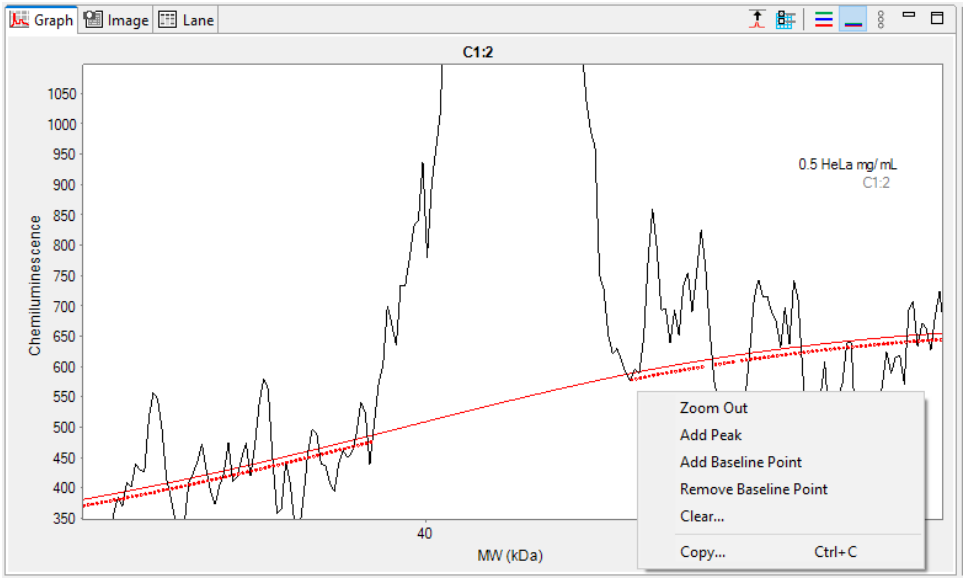
Spine baseline is the default setting for Baseline Type. It cannot be changed if Gaussian Fit is selected in the Peak Fit settings.

Points in the baseline can be added or removed as needed.

1. If you haven't already, name your peaks of interest. Refer to "Changing Peak Names for Sample Data" on page 216 for more information.
2. Select **Edit** > **Analysis** and click **Peak Fit** in the options list.
3. Choose **Spline** as the Baseline Type if it isn't already selected.

Then, to view the baseline:

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**.



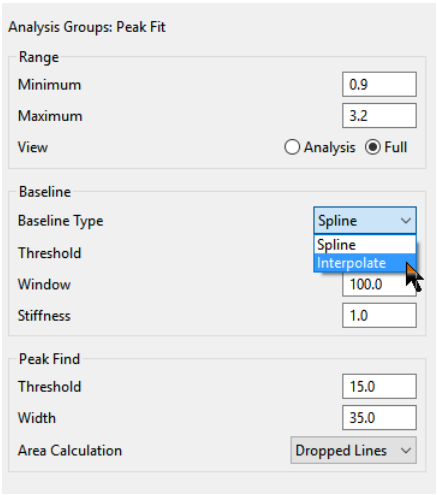

**NOTE:** To clear the manual addition or removal of baseline points and go back to the original view of the data, right-click in the electropherogram and click **Clear**.

## Interpolated Baseline

Compass for Simple Western lets you interpolate baselines from the base of each peak for named peaks that use Dropped Lines (manual) integration.

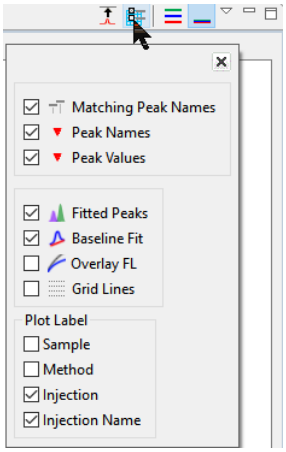
1. If you haven't already, name your peaks of interest.
2. Select **Edit** > **Analysis** and click **Peak Fit** in the options list.

3. Choose **Interpolate** as the Baseline Type if it isn't already selected.




4. Click **OK**.

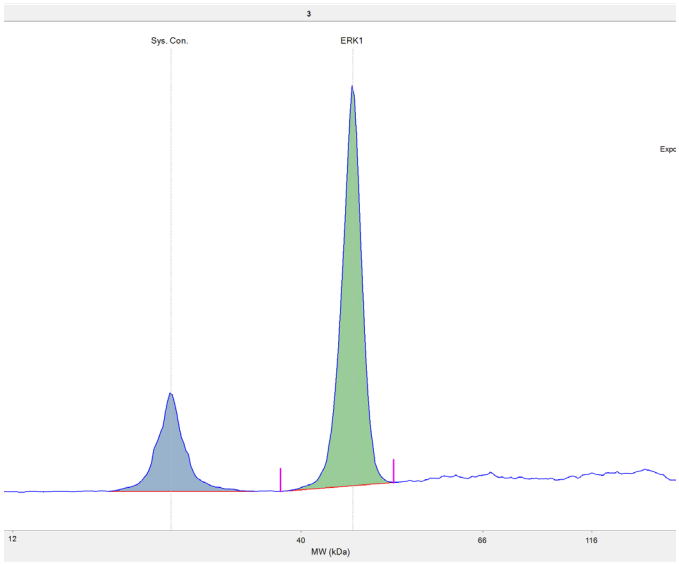
5. In the Analysis window Graph pane, click **Graph Options** and select **Fitted Peaks** and **Baseline Fit**.



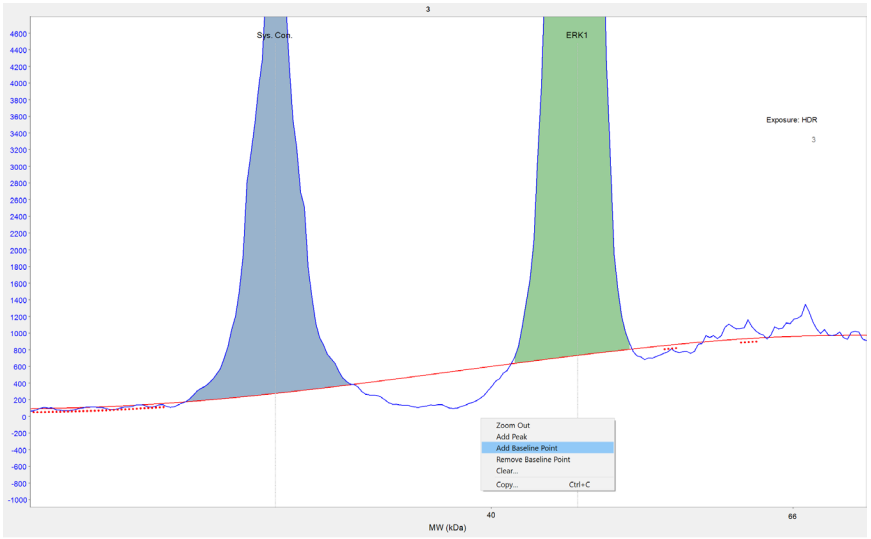

6. Select a capillary in the Experiment pane.

7. Hover the mouse over the peak you want to adjust the baseline on. Two magenta bars will display that show the start and end points of the peak's integration.

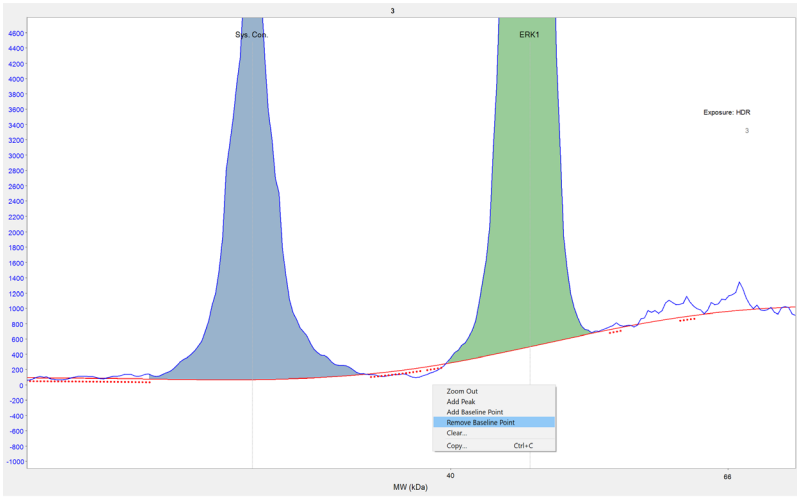
8. To change the baseline, hover the mouse over one of the magenta bars. Then click the mouse and drag the bar to move it. The cursor changes to indicate which direction you can move the integration point.




9. Baseline points can also be added between two adjacent peaks for a better fit. Hover the mouse over the region where you want to add the baseline point, then right-click and select **Add Baseline Point**.

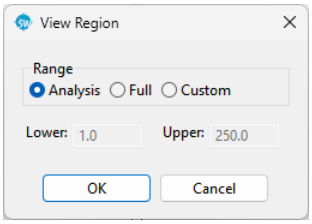


10. To remove the baseline point, hover the mouse over the area that you want to remove the baseline point, then right-click and select **Remove Baseline Point**.

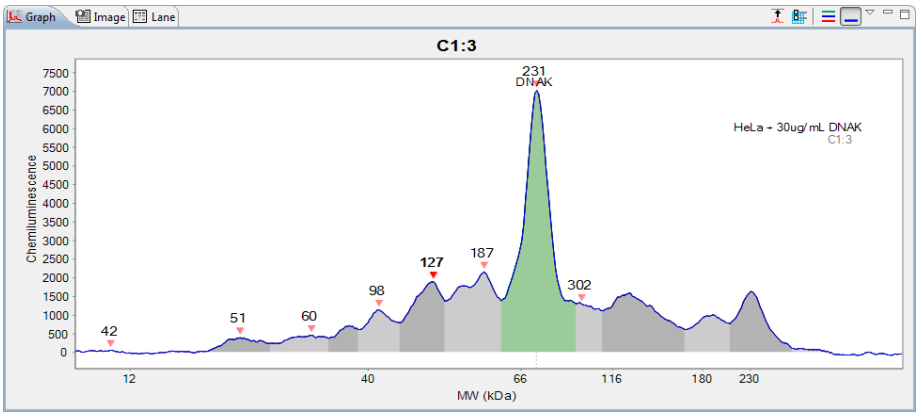



## Selecting the X-Axis Molecular Weight Range

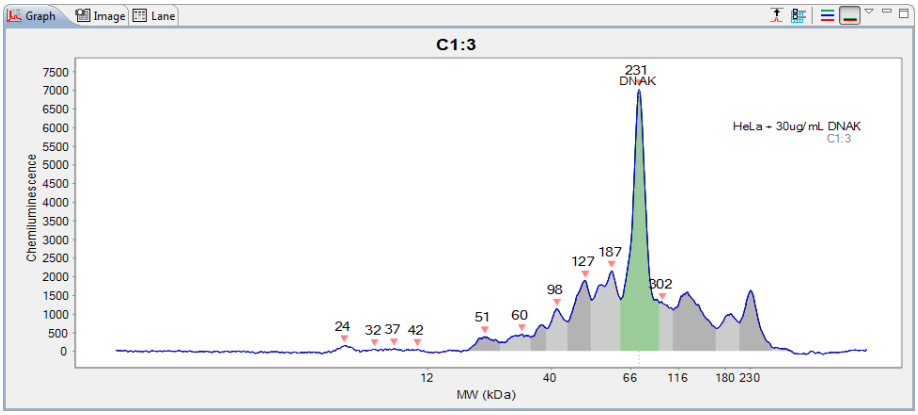
The molecular weight range used for the x-axis can be changed. To do this, select **View** in the main menu and click **View Region**. The following pop-up window will display:



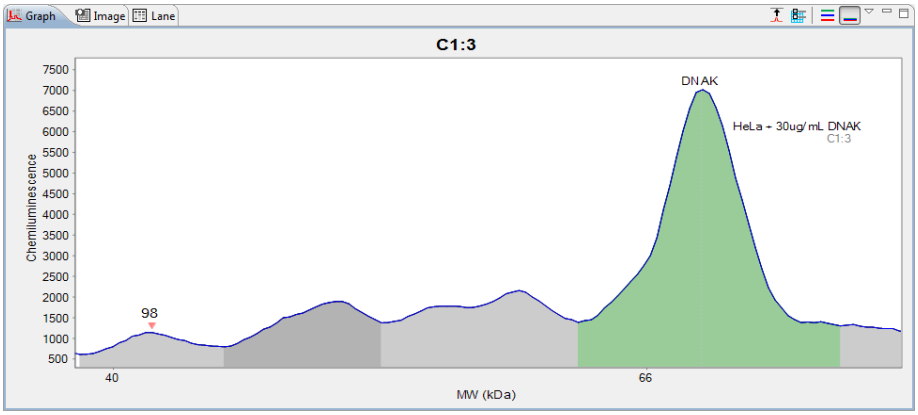

- Selecting **Analysis** will match the range of the run data that was selected in the Peak Fit Analysis Settings in both the electropherogram and virtual blot view.



- Selecting **Full** will display the range of the run data for the entire capillary in both the electropherogram and virtual blot view.




- 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.




**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 316.

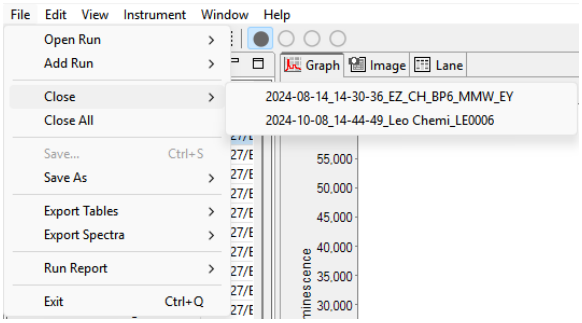
# Closing Run Files

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 run file**

  a. Select **File** from the main menu and then click **Close** to open the submenu.

b. Select the run you want to close.




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

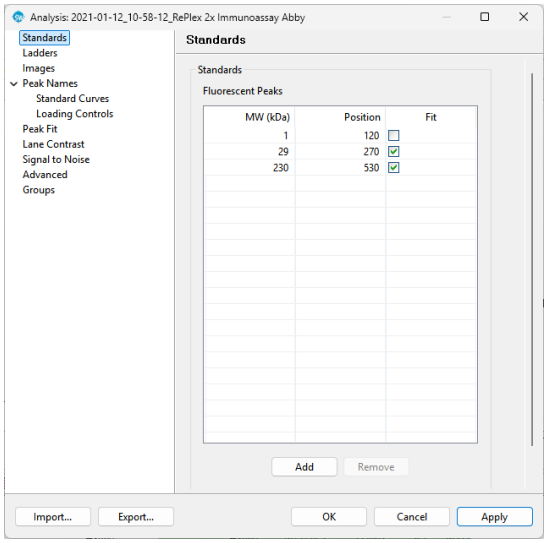
# Analysis Settings Overview

Compass for Simple Western has a variety of analysis features and settings that you can modify as needed to enhance run data.

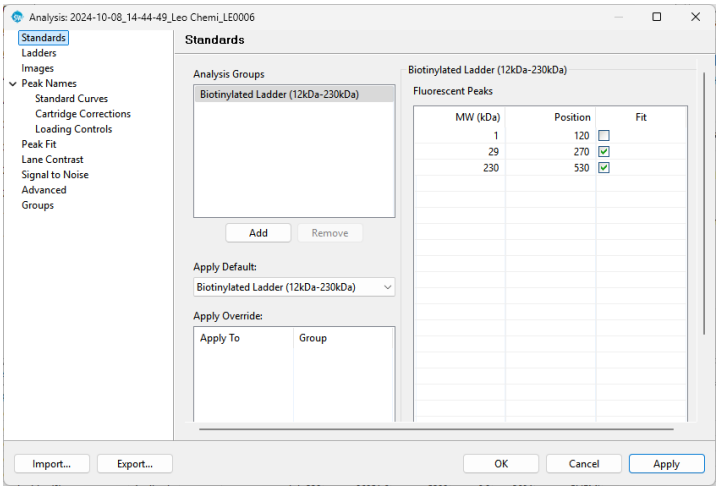
To access the analysis settings, select **Edit** in the main menu and click **Analysis**. If more than one run file is open, select the run file you want to view analysis settings for from the list:

The following screen will display:

**JESS/ABBY**




**LEO**



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 peaks.
- **Ladders** - Lets you customize the molecular weight Compass for Simple Western uses to identify molecular weight ladder peaks, as well as change the capillary, cartridge (Leo), and channel (Jess only) used.
- **Images** - Lets you view the exposures taken during the run and view data for different exposures in the Analysis screen.
- **Normalization** - Lets you change the reference capillary and cartridge (Leo) used for normalization calculations and define the region for normalization area. This setting will only appear if a Total Protein or Protein Normalization assay was run.
- **Peak Names** - Lets you enter custom naming settings for sample proteins associated with specific blocking reagents, primary antibodies, secondary antibodies, or attributes and have Compass for Simple Western automatically label the peaks in the run data.
  - **Standard Curves** – Lets you define a standard curve to quantitate the concentration of a target protein.
  - **Cartridge Correction (Leo only)** – Lets you define how to normalize data across cartridges.
  - **Loading Controls** – Lets you define a loading control to normalize data between capillaries, between runs, and between instruments.
- **Peak Fit** - Lets you customize peak fit and baseline settings for sample data.
- **Lane Contrast** - Lets you enter custom contrast settings for detection channels in Lane View.
- **Signal to Noise** - Lets you change the method used to calculate signal to noise in sample data.
- **Advanced** - Lets you customize analysis settings for samples, standards and image data.
- **Groups** - Lets you customize how to group run data when the Grouping function is enabled.

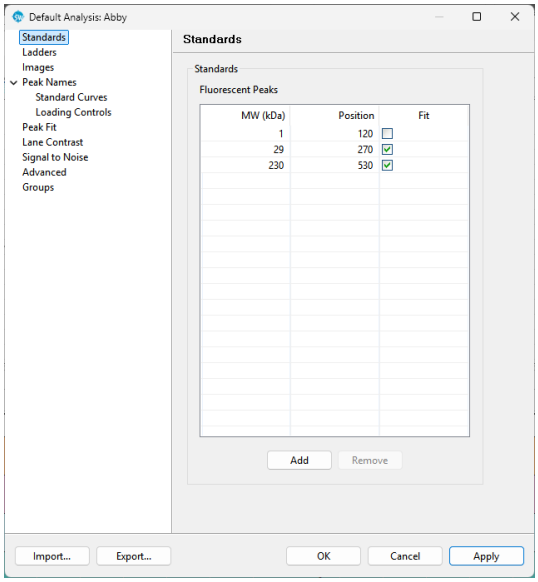
**NOTES:**
Settings can be modified in an assay prior to starting a run, or in a run file once the run has finished executing. Analysis changes made to an executing run will not be saved to the final run file.

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 337.

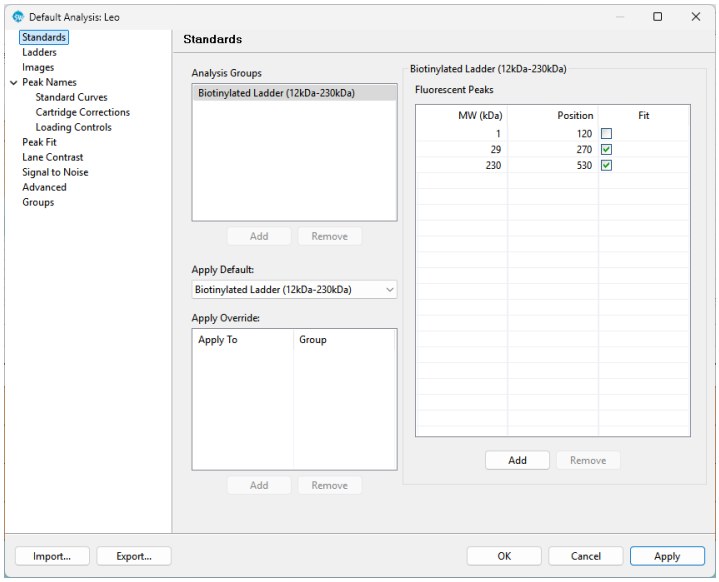
# Standards Settings

The standards analysis settings page lets you view and change the position for fluorescent standards. 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 size range is shown in the following example:

**JESS/ABBY**




**LEO**

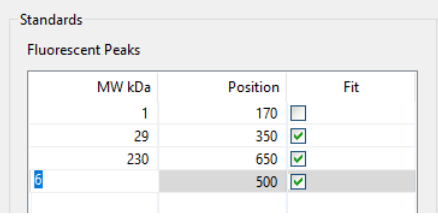



- Click **Import** to import an analysis settings file. This is explained in more detail in “Importing Analysis Settings” on page 337.
- Click **Export** to export the current analysis settings file. This is explained in more detail in “Exporting Analysis Settings” on page 337.
- Click **Apply** to see effect of changes before saving.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

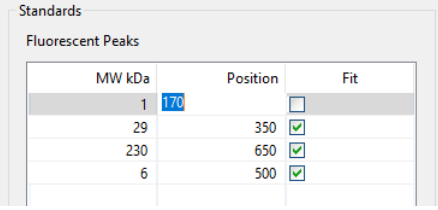
**NOTE:** We recommend using the Compass for Simple Western default values for standards analysis settings. These settings are included in the default Standards group.

## 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 Fluorescent Peaks table. 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.

## Standards Analysis Settings Groups (Leo only)

Standards settings are saved as a group, and multiple settings groups can be created. Specific group settings can then be applied to individual cartridges in the run data.

# Ladders Settings

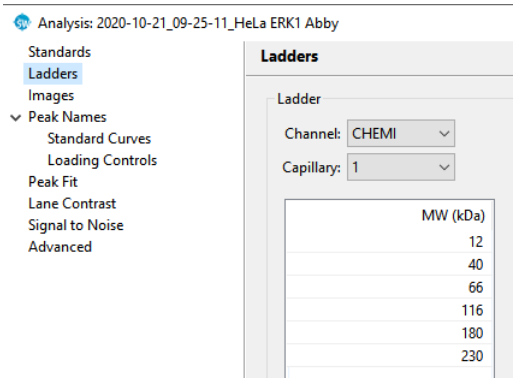
The ladders analysis settings page lets you view and change the molecular weight for the ladder and change the capillary, probe (RePlex assays), channel (Jess only), or cartridge (Leo only) used for it. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Ladders** in the options list.

For RePlex Assays, select the ladder in Probe 1 or Probe 2, depending on the assay setup.

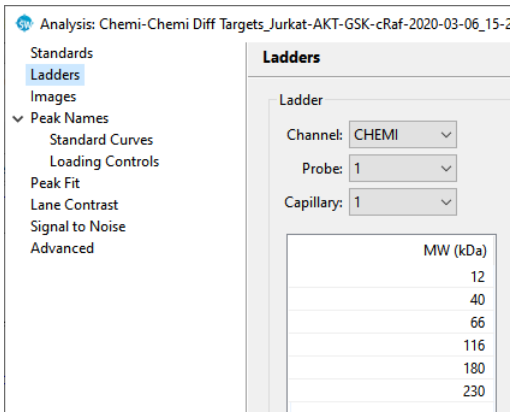
**NOTE:** Probe selection is not available with RePlex Assays that include Total Protein.

The default ladder settings for the 12-230 kDa size range is shown in the following example for a Chemiluminescence Immunoassay on Jess/Abby (top) and a RePlex Assay (middle) on Jess/Abby, and a Chemiluminescence Immunoassay (bottom) on Leo.

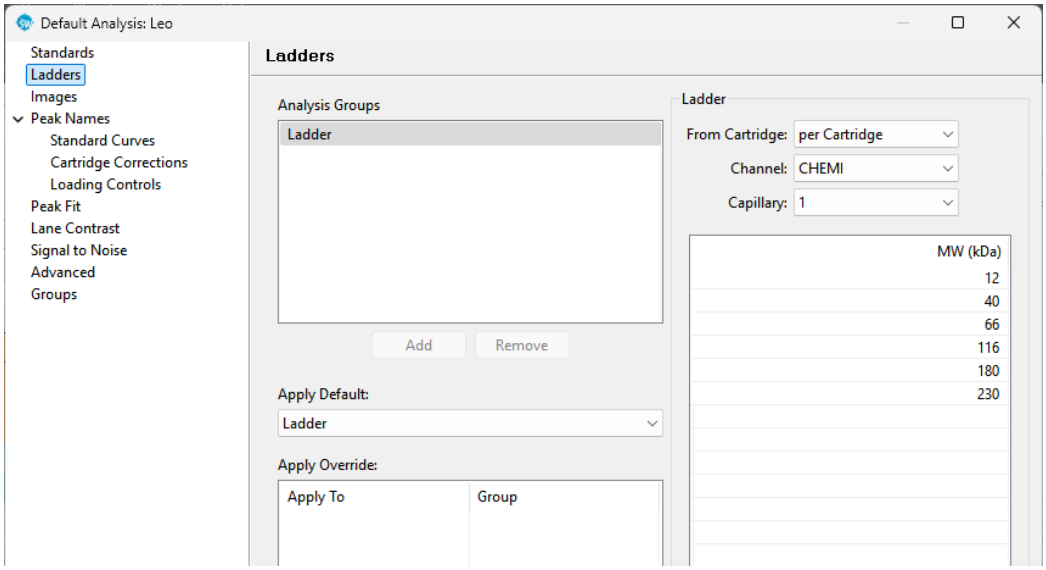
### CHEMILUMINESCENCE IMMUNOASSAY ON JESS/ABBY




### REPLEX ASSAY ON JESS/ABBY



#### CHEMILUMINESCENCE IMMUNOASSAY ON LEO




**NOTE:** We recommend using the Compass for Simple Western default values for ladders analysis settings. These settings are included in the default Ladders group.

### Changing the Capillary Used for the Ladder

Known ladders are used to calculate the molecular weights of unknown sample proteins. 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.

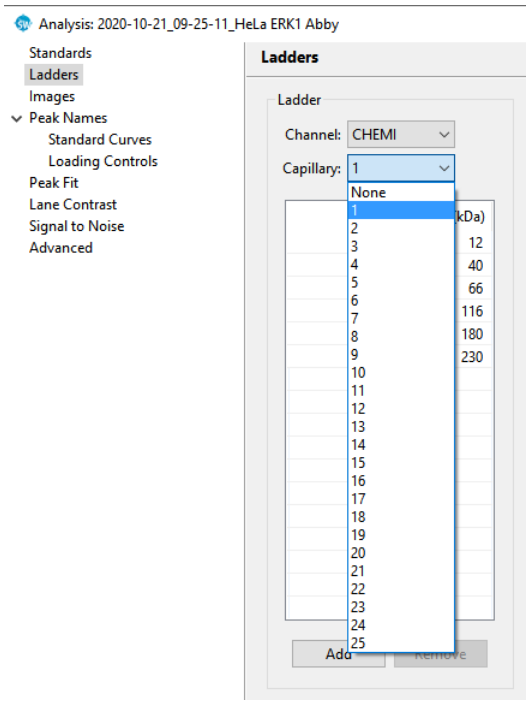
**NOTES:**
When the ladder capillary is set to None, fluorescent standards information is used to calculate sample protein molecular weight instead of the ladder.

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.[1,2]

[1] *Electrophoresis. 2021 Aug;42(14-15):1521-1531. doi: 10.1002/elps.202100068.*
[2] *Electrophoresis. 2021 Feb;42(3):206-218. doi: 10.1002/elps.202000199.*

**To change the ladder capillary on Jess/Abby:**

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.

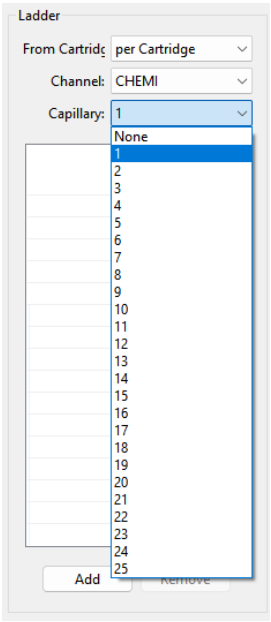



3. Click **Apply** and then **OK** to save changes and exit.

**To change the ladder capillary on Leo with one ladder setting:**

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 From Cartridge and select **per Cartridge** so each cartridge will have the same reference ladder capillary settings.

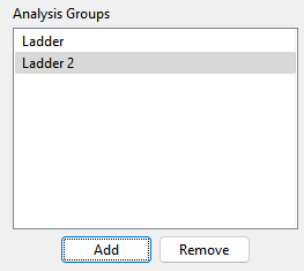
3. Click the arrow in the drop-down list next to Capillary, then click a capillary number or None from the list.




4. Click **Apply** and then **OK** to save changes and exit.

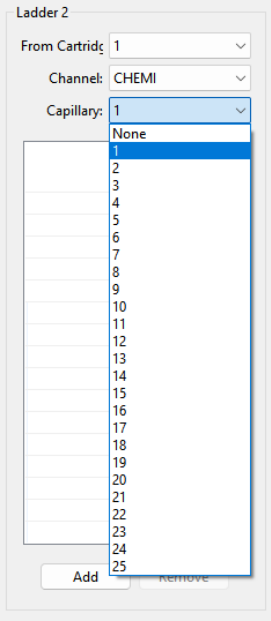
**To change the ladder capillary on Leo with multiple ladder settings:**

1. Select **Edit** in the main menu and click **Analysis**, then click **Ladders** in the options list.
2. Click **Add** under the Analysis Group box. A new group will be created. Optional: click on the new group and enter a new name.

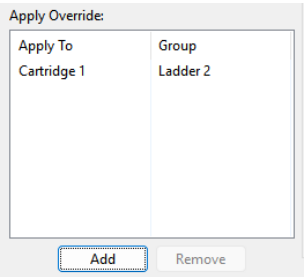



3. Click the arrow in the drop-down list next to From Cartridge and select a cartridge.

4. Click the arrow in the drop-down list next to Capillary, then click a capillary number or None from the list.




5. Click the arrow in the drop-down list in the Apply Default menu to define the default ladder group.
6. Apply a ladder analysis group to specific run data using the Apply Override box. Click **Add** under the override box. The override data set will be created.

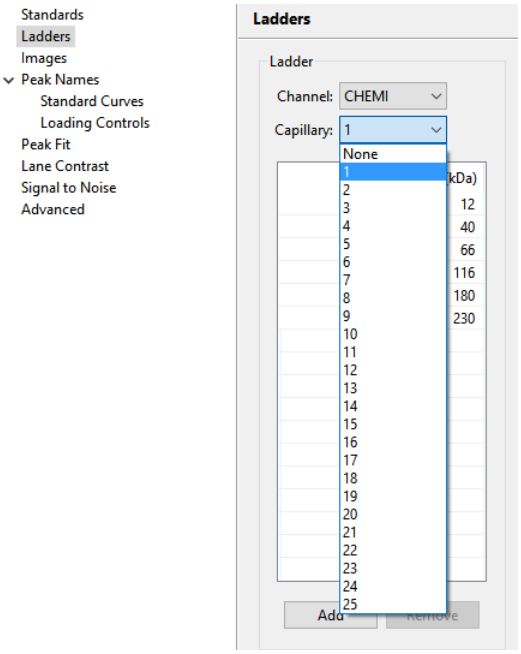



7. Click the cell in the **Apply To** column, then click the down arrow. Select the cartridges to apply the cartridge analysis group to.
8. Click **Apply** and then **OK** to save changes and exit.

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.

## Creating a New Ladder

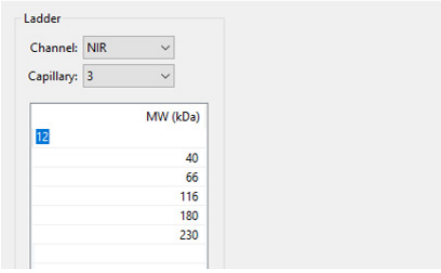
1. Select **Edit** in the main menu and click **Analysis**, then click **Ladders** in the options list.
2. Click the arrow in the drop-down list next to Capillary, then click a capillary number. Capillary 1 is typically used for the ladder.




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.

**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.[1,2]

3. Click in the first cell in the **MW** column in the ladder table. Enter the molecular weight (in kDa) for the ladder standard.




[1] *Electrophoresis. 2021 Aug;42(14-15):1521-1531. doi: 10.1002/elps.202100068.*
[2] *Electrophoresis. 2021 Feb;42(3):206-218. doi: 10.1002/elps.202000199.*

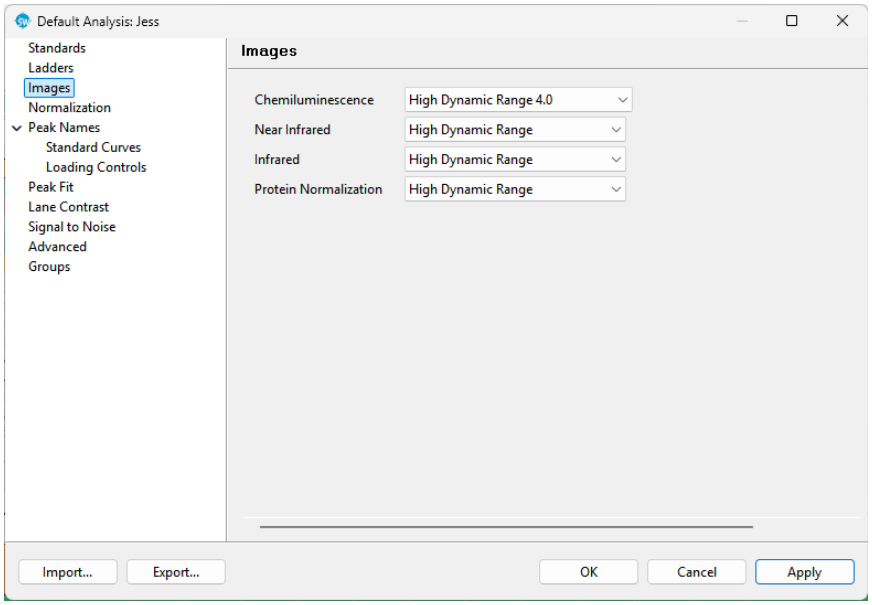
4. Repeat the steps above for the remaining ladder MW values in the table.
5. Click **Apply** and then click **OK** to save changes.

# 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 Jess, Abby, and Leo chemiluminescent 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.

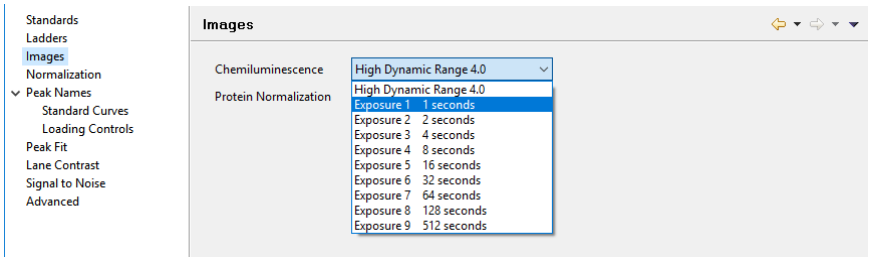
- Click **Import** to import an analysis settings file. This is explained in more detail in "Importing Analysis Settings" on page 337.

- Click **Export** to export the current analysis settings file. This is explained in more detail in “Exporting Analysis Settings” on page 337.
- Click **Apply** to see effect of changes before saving.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

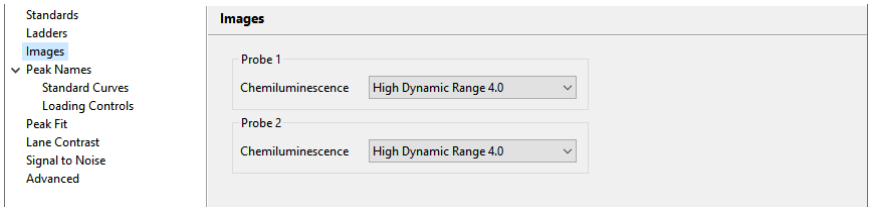
## Exposure Settings

The exposure used for the sample data displayed in the Analysis screen is shown below for a Chemiluminescence Immunoassay with Protein Normalization (top), a 2 Chemiluminescence Immunoassay with RePlex (second), a Leo Chemiluminescence Immunoassay (third), and a Stellar Assay on Jess (bottom):

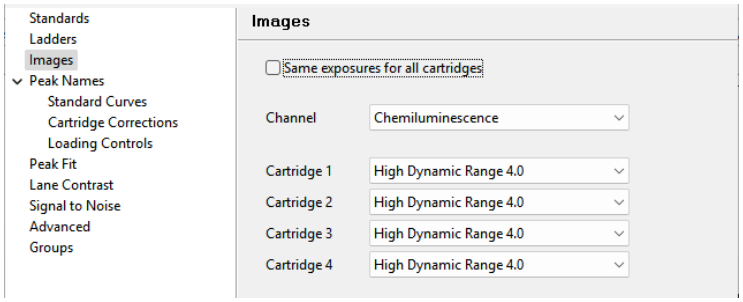
#### CHEMILUMINESCENCE IMMUNOASSAY WITH TOTAL PROTEIN NORMALIZATION USING PROTEIN NORMALIZATION CHANNEL



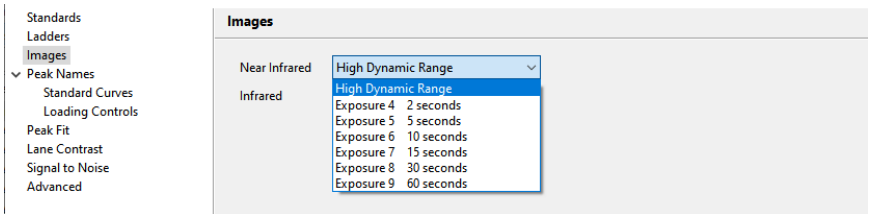

#### CHEMILUMINESCENCE IMMUNOASSAY + CHEMILUMINESCENCE IMMUNOASSAY USING REPLEX




#### CHEMILUMINESCENCE IMMUNOASSAY ON LEO



**STELLAR FLUORESCENCE NIR/IR IMMUNOASSAY**




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).

- **High Dynamic Range** (Chemiluminescence only) - 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 rate at which burnout occurs.
- **Exposure #** - Sample data displayed in the Analysis screen is for this specific exposure only.

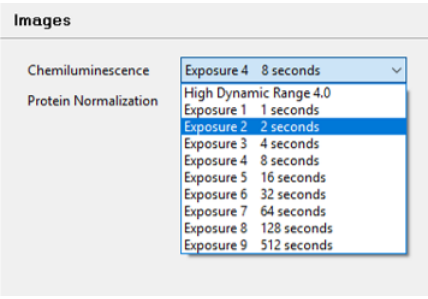
  To see the number of exposures and exposure times used for the run data, click the arrow in the drop-down.

**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 to 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

The Normalization settings in the Analysis window lets you view and change the reference capillary used for total 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 (Jess Only) or when enabling the Total Protein feature for RePlex and Stellar 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, RePlex with Total Protein, and Stellar with Total Protein, the default Reference Capillary is set to 2.

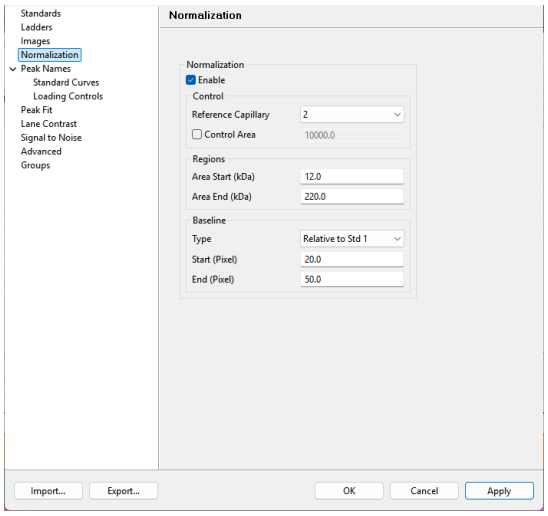
For Abby runs that include RePlex with Total Protein, the default Reference Capillary is set to 2.

For Leo runs that include RePlex with Total Protein, the default Reference Capillary is set to 2 for each cartridge.

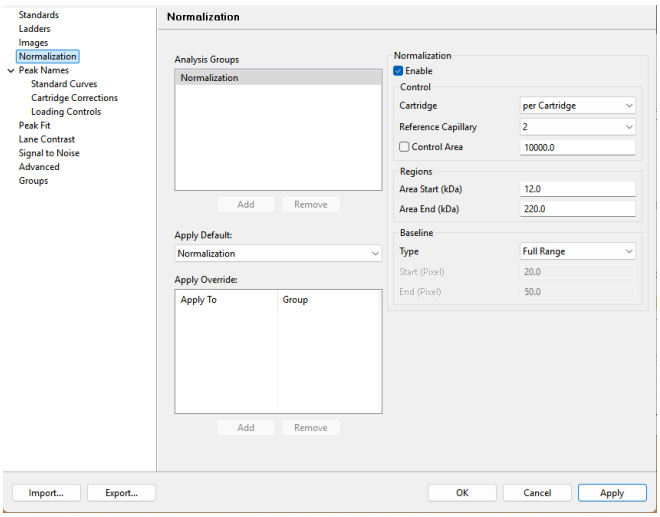
The Enable checkbox is selected by default and must stay selected to normalize immunoassay targets.

Only one type of normalization is allowed per analysis. Cartridge Correction and Loading Controls cannot be used if Normalization is enabled.

**JESS/ABBY**




**LEO**




- Click **Import** to import an analysis settings file. This is explained in more detail in “Importing Analysis Settings” on page 337.
- Click **Export** to export the current analysis settings file. This is explained in more detail in “Exporting Analysis Settings” on page 337.
- Click **Apply** to see effect of changes before saving.

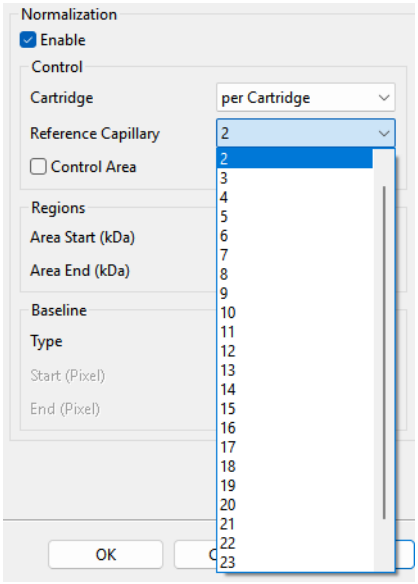
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

**NOTE:** If you deselect the Enable checkbox in the Normalization settings, only total protein values will display, immunoassay target data won't be normalized.

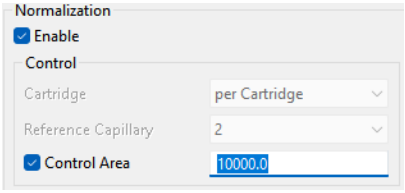
**To use the Protein Normalization fluorescence signal or RePlex/Stellar Total Protein chemiluminescent signal from a reference capillary for protein normalization:** Click the Reference Capillary drop-down menu and select the capillary you'd like to use as the normalization control to normalize all other capillaries against.

For Leo RePlex runs with Total Protein chemiluminescent signal, you can choose to normalize each cartridge's data to a reference capillary within each cartridge by clicking the Cartridge drop-down menu and selecting **per Cartridge**. To normalize all cartridges to a single reference capillary within the run, click the Cartridge drop-down menu and select the cartridge number.

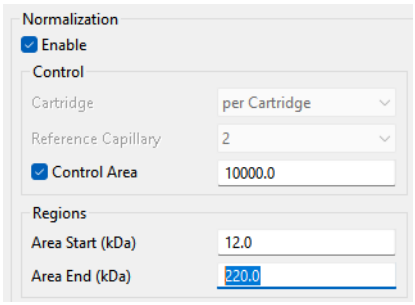
We recommend choosing the capillary that had the highest sample concentration. The default reference capillary is 2.



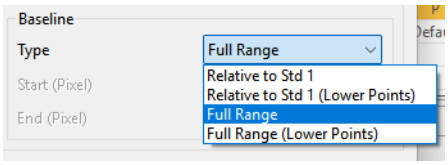

**To manually assign a reference signal for normalization:** Select the **Control Area** box and enter a signal value. This setting is helpful when you want to use the average signal from more than one capillary for normalization.



**To change the sizing region that the normalization total area is determined:** Enter new kDa values in the **Area Start** and **Area End** boxes.




**To change the baseline type for normalization:** Select the Type in the drop-down menu.




**To change the region of points from which the baseline is being determined:** Enter new pixel values in the **Start (Pixel)** and **End (Pixel)**. This only applies to the Relative to Std 1 and Relative to Std 1 (Lower Points) baseline types.

- **Relative to Std 1** - Uses the region defined by Start (Pixel) and End (Pixel) as it relates to the pixel position of the left-most fluorescent standard (position 0).
- **Relative to Std 1 (Lower Points)** - Uses the region defined by Start (Pixel) and End (Pixel) as it relates to the pixel position of the left-most fluorescent standard (position 0). Within this region, greater weighting is given to pixel positions exhibiting lower signal.
- **Full Range** - Uses the region defined by the entire length of the capillary.
- **Full Range (Lower Points)** - Uses the region defined by the entire length of the capillary. Within this region, greater weighting is given to pixel positions exhibiting lower signal.

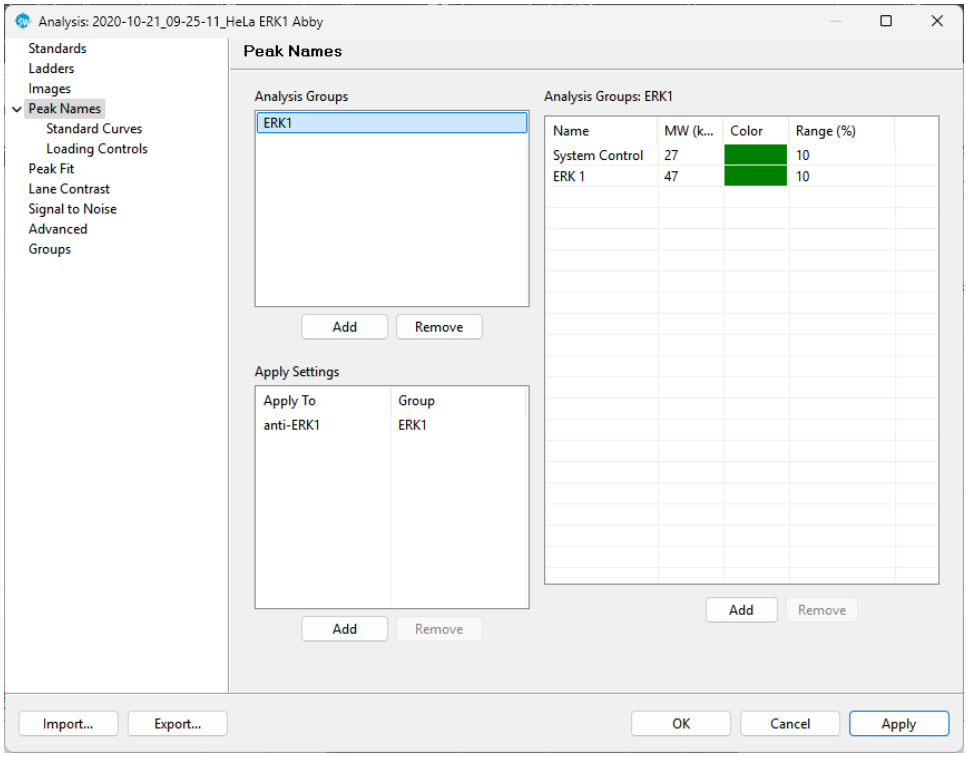
Click **Apply** and then click **OK** to save changes and exit. Updated sample data using the new normalization settings will display in the Analysis screen.

# Peak 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, secondary antibody names, attributes, probes (RePlex assays only), and cartridges (Leo only). To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.

**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.

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 337.



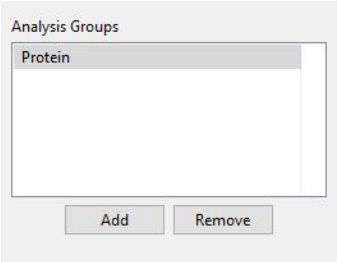

- Click **Import** to import an analysis settings file. This is explained in more detail in “Importing Analysis Settings” on page 337.
- Click **Export** to export the current analysis settings file. This is explained in more detail in “Exporting Analysis Settings” on page 337.
- Click **Apply** to see effect of changes before saving.

- 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, primary antibody names, secondary antibody names, attributes, probes (RePlex assays only), and cartridges (Leo only) in the run data.

Peak name groups are displayed in the Analysis Groups box:

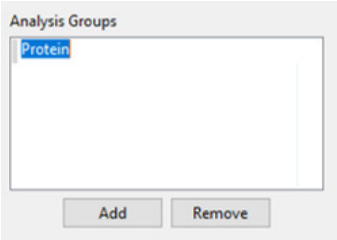



The Peak Names group shown is a Compass for Simple Western template group. You can make changes to this group and create new groups.

To view settings for a group, click on the group name in the Analysis Groups box.

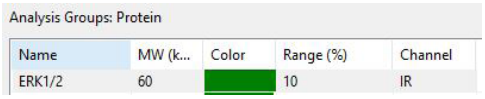
## Creating a Peak Names Group

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 Groups box.

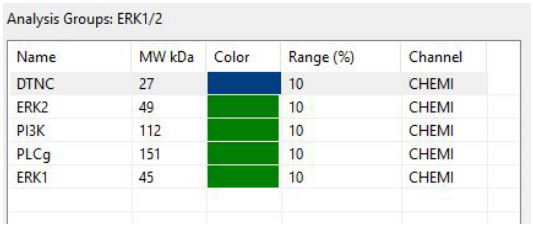



3. Enter a new name for the group.
4. Click in the first cell in the Name column in the Analysis Groups peak table.

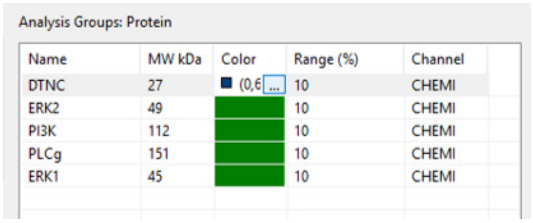
5. Enter a sample protein name associated with the primary antibody used in the run.



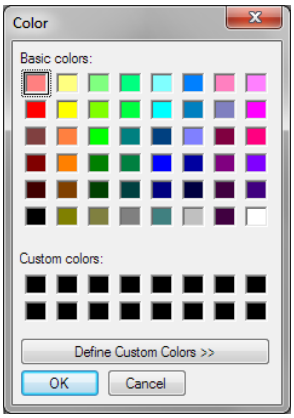

6. Click in the first cell in the MW column.
7. Enter the molecular weight (in kDa) for the sample protein.




8. Click in the first cell in the **Color** column, then click the button.

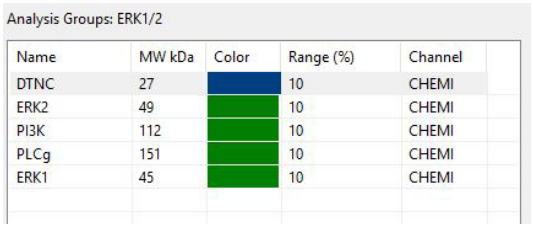



The color selection box will display:

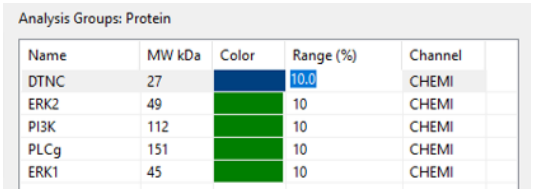



The color selected will be used to identify the sample protein peak in the Peaks and Capillaries table in the Analysis screen.

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.



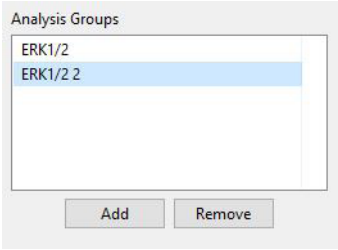

11. Enter a range window for the MW entered. Compass for Simple Western will automatically name peaks found within this percent of the molecular weight entered in the table. For example, if the molecular weight entered is 40 kDa and a 10% range is used, all peaks between 36 and 44 kDa will be identified 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.[1,2]

12. **For assays with more than one detection channel:** Click in the Channel cell and select a channel from the drop-down menu.

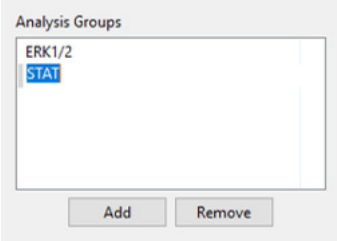
## Adding Peak Names Groups

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:




[1] *Electrophoresis. 2021 Aug;42(14-15):1521-1531. doi: 10.1002/elps.202100068.*
[2] *Electrophoresis. 2021 Feb;42(3):206-218. doi: 10.1002/elps.202000199.*

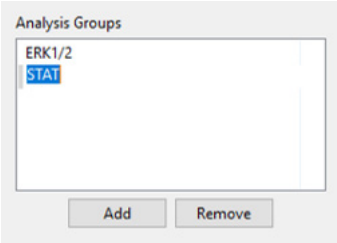
3. Click on the new group and enter a new name.




4. Enter information in the Analysis Groups peak table as described in "Creating a Peak Names Group" on page 294.
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 New Peak Fit Group" on page 319.
4. Click **Apply** and then click **OK** to save changes.

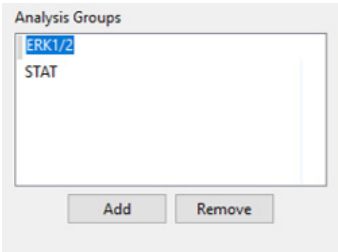
## Deleting a Peak Names Group

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.

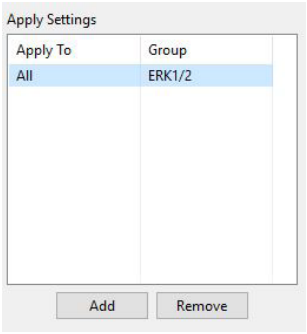
## Applying Peak Names Groups to Run Data

1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Names** in the options list.

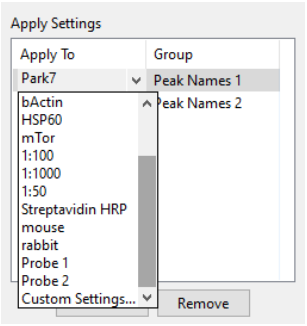
2. Click on the group in the Analysis Groups box you want to apply to specific run data.

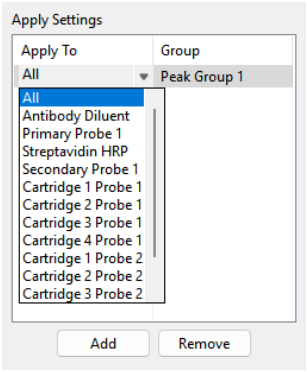



3. Application of peak names groups to specific run data is done in the Apply Settings box. Click **Add** under the Apply Settings box. The settings will be applied to the data.




4. Click the cell in the **Apply To** column, then click the down arrow.

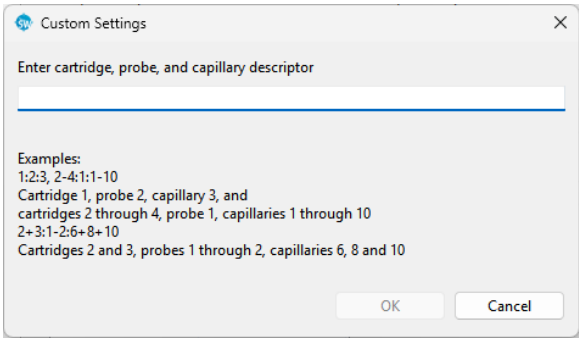


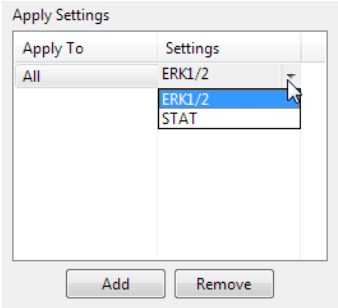

5. Select an option from the drop-down list. This will apply the settings group selected to specific run data as follows:

   - **All** - When this option is selected, peak names group settings will be applied to all capillaries in the run file.
   - **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 and Secondary 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.
- **Probe (RePlex Assays only)** - Select a probe to apply group settings to all capillaries within that probe.
- **Cartridge (Leo only)** - Applies group settings to a specific cartridge.
- **Custom settings** - Lets you choose specific cartridges and 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 for a specific probe and/or cartridge depending on the assay type:

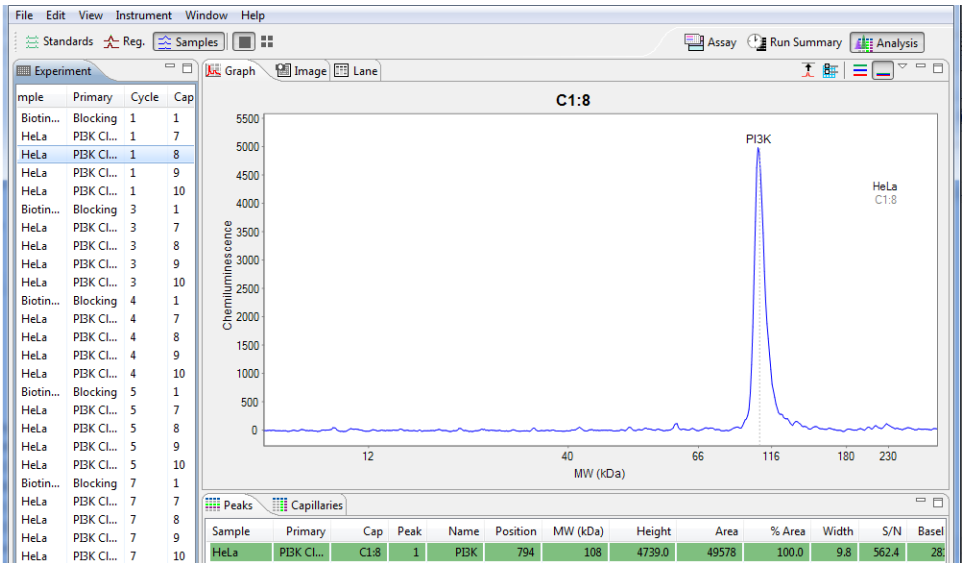



6. If you need to change the peak names group used for a data set, click the cell in the **Settings** column and click the down arrow. Select a group from the drop-down list.



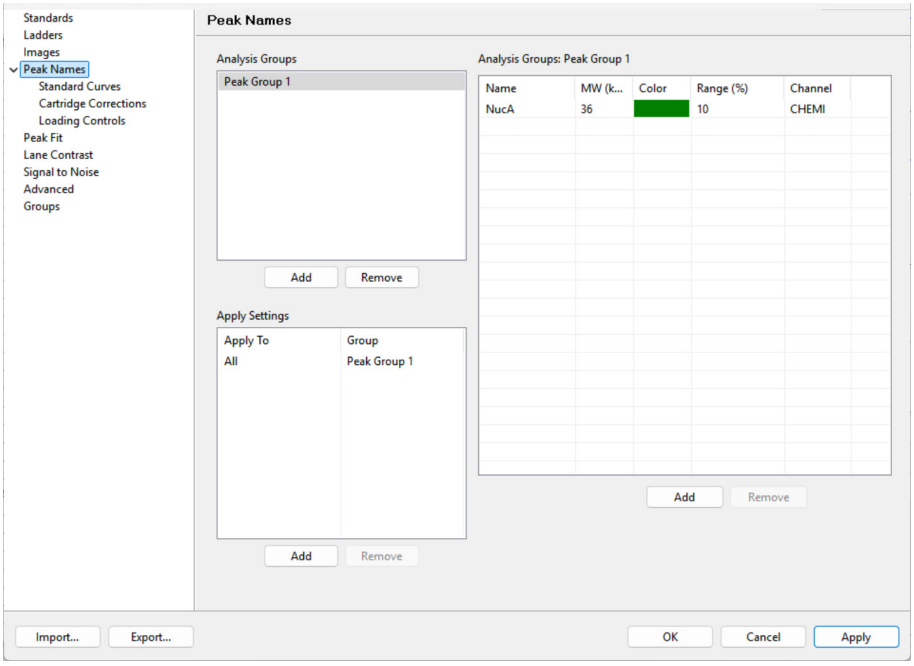

7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.

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

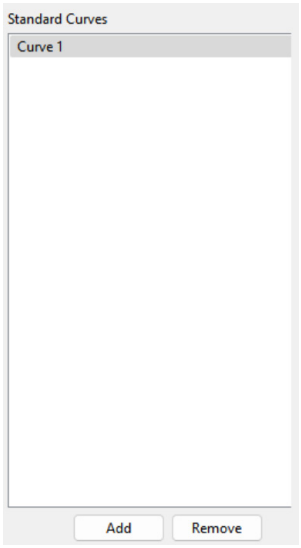
To use a standard curve to quantitate the concentration of a target protein detected, first create peak names groups as described in “Creating a Peak Names Group” on page 294 for your standard curve protein and the target protein. In the example below, NucA is the standard curve protein and the target 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 337.

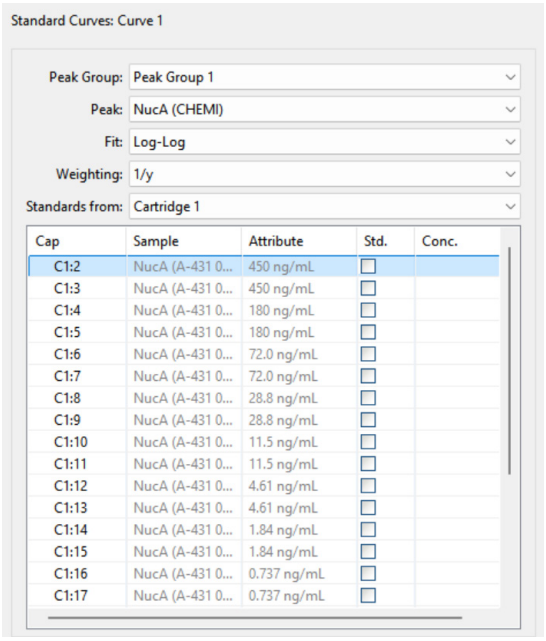
To set up a standard curve:

1. Select **Edit** in the main menu and click **Analysis**.
2. Click **Standard Curves** from the Peak Name submenu in the options list.
3. Click **Add** under Standard Curves. “Curve 1” will appear with default standard curve settings. Double-click on “Curve 1” to edit the name.




4. From the Peak Group drop-down list, select a Peak Group.

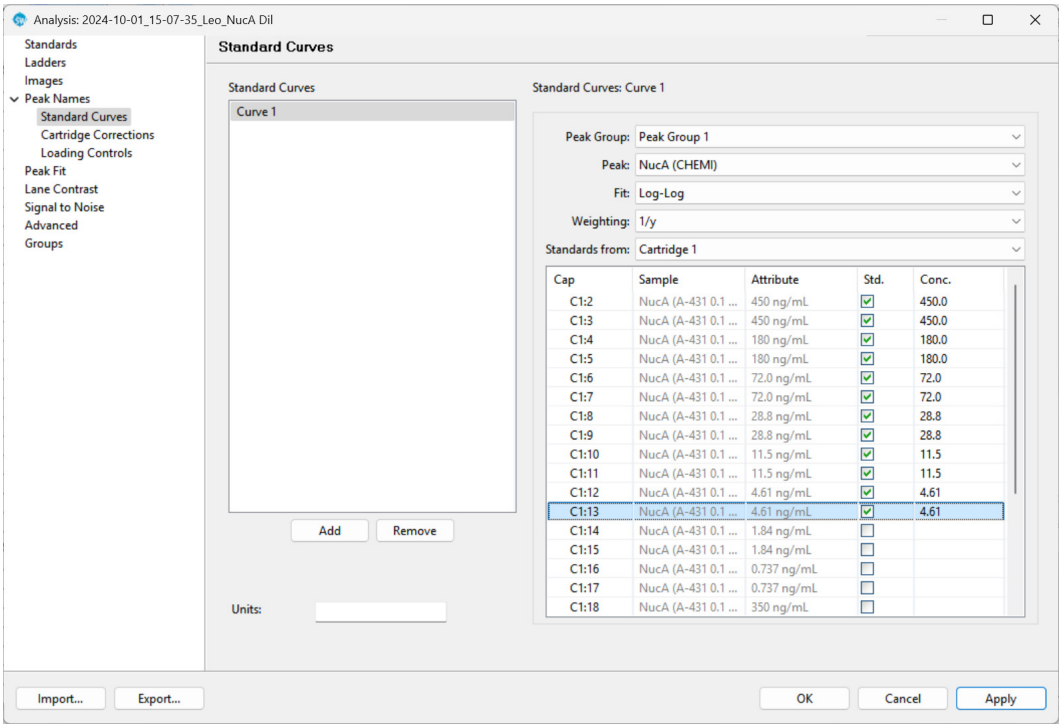
5. From the Peak drop-down list, select the peak name for your standard curve protein.




6. Choose either a **Linear**, **4 Parameter (4PL)**, or **Log-Log** curve fit from the Fit drop-down list.
7. Select a weighting factor from the drop-down list. Weighting factors are used to calculate a weighted least square calibration curve, which can be used to determine the %-error for each experimental value. Refer to “Standard Curve Fits and Weighting Factors” on page 305 for more information.
8. In the Std column, click the checkboxes for the capillaries with standard curve samples.

9. In the Conc. column, enter the sample concentrations. In the following, twelve concentrations were entered.

**NOTE:** If concentrations are entered in the Assay template, the Conc. column will automatically be filled with the Sample name or Sample attribute values in the template. If no number is available in the Sample name or Sample attribute, 1.0 will be entered by default.



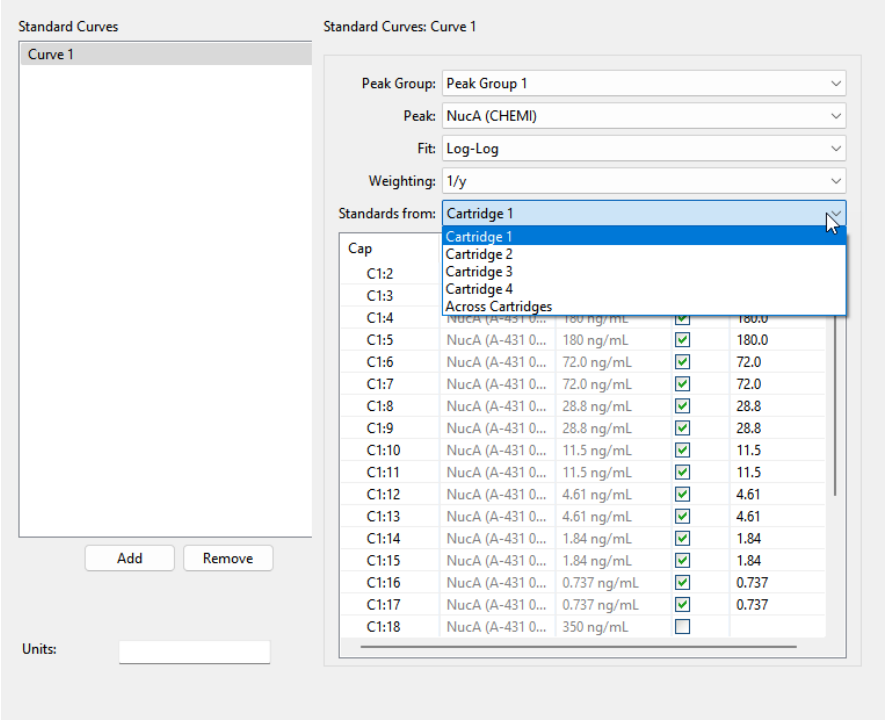

To remove a concentration, uncheck the box in the Std. column.

10. Enter the concentration units in the Units box (for example, pg/µL)
11. Click **OK** to save changes.

When running standard curves, capillaries with standard curve samples can be selected from a single cartridge or across multiple cartridges (Leo only).

1. Set up a standard curve as described in “Standard Curve Settings” on page 300.

2. In the Standards from drop-down list, select the cartridge to use for the standard curve or **Across Cartridges**.




**NOTE:** We recommend enabling Cartridge Corrections when a standard curve is performed on Leo, especially if standard curve samples are all run on a single cartridge and will be used to calculate concentrations on multiple cartridges. See “Cartridge Corrections (Leo only)” on page 311 for more information.

# Standard Curve Fits and Weighting Factors

Compass for Simple Western provides three different types of standard curve fits: Linear, 4 Parameter Logistic (4PL), and Log-Log. The equations applied are:

The linear curve is calculated using:

**y = mX + c**

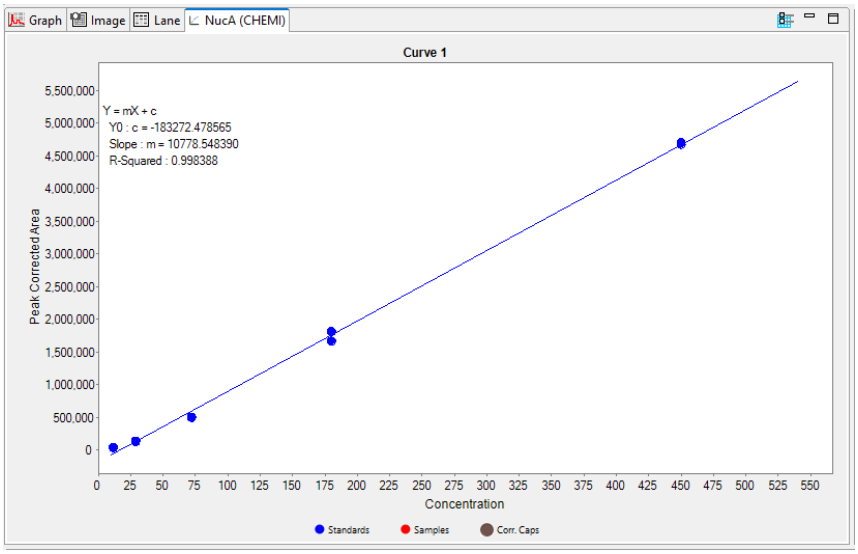
where:

**y** = The peak area value

**X** = The concentration value

**m** = The slope of the fitted line

**c** = The intercept of the y-axis




The weighting factors associated with linear fits are calculated using:

For 1/y weighting factor: w=1/max(abs(y), ε)

For 1/y2 weighting factor: w=1/max(y2, ε)

where:

ε = 10.

The 4PL curve is calculated using:

$$y = Max + \frac{Min - Max}{1 + \left[\frac{x}{EC50}\right]^{HillSlope}}$$

where:

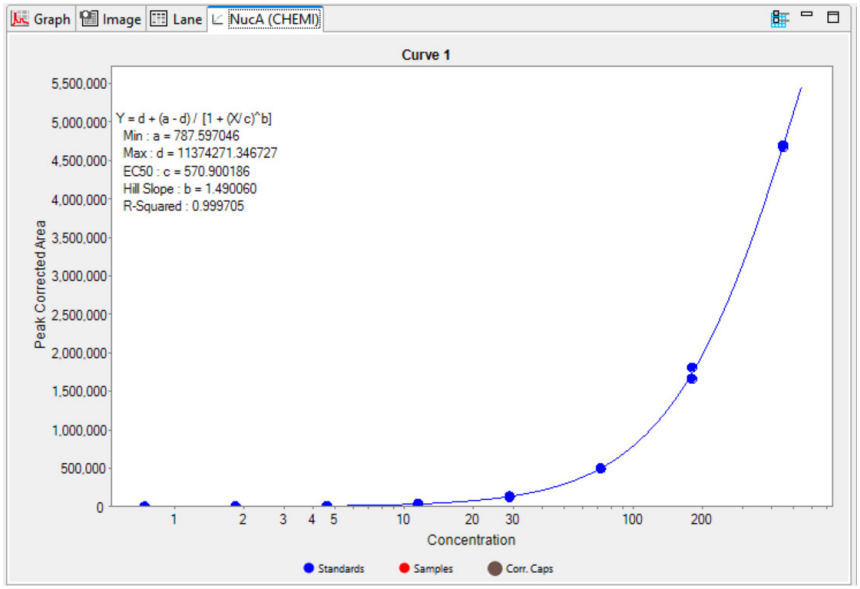
**y** = The peak area value

**Min** = The minimum peak area value that can be obtained

**Max** = The maximum peak area value that can be obtained

**EC50** = The concentration at the midpoint of the curve

**HillSlope** = The slope at the midpoint of the curve




The weighting factors associated with 4PL fit are calculated using:

For 1/y weighting factor: w=1/max(abs(y), ε)

For 1/y2 weighting factor: w=1/max(y2, ε)

where:

ε = 10.

The Log-Log curve is calculated using:

**Log(Y)=m*Log(X)+c**

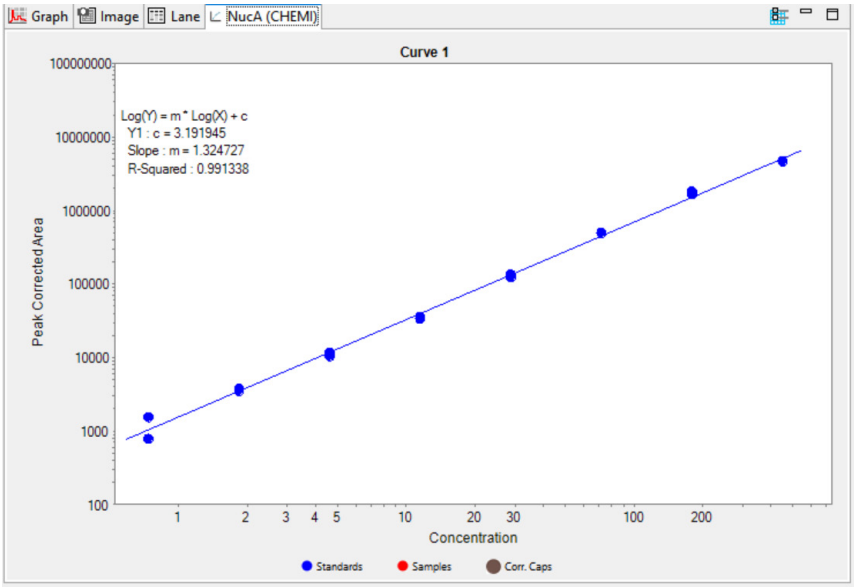
where:

**Y** = The peak area value

**X** = The concentration value

**m** = The slope of the fitted line

**c** = The intercept of the y-axis




The weighting factors associated with log-log fit are calculated using:

For 1/y weighting factor: w=1/log(max(abs(Y), ε)

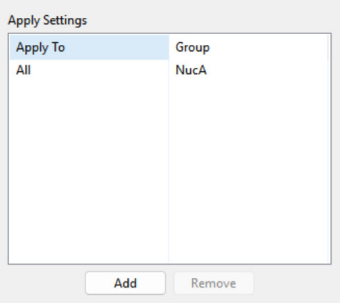
For 1/y2 weighting factor: w=1/log(max(y2, ε)

where:

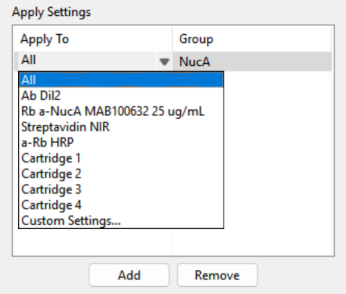
ε = 10.

# Modifying Peak Names Groups Used for Standard Curves Settings

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 Apply Settings box you want to apply to specific run data.




3. Click the cell in the **Apply To** column, then click the down arrow.

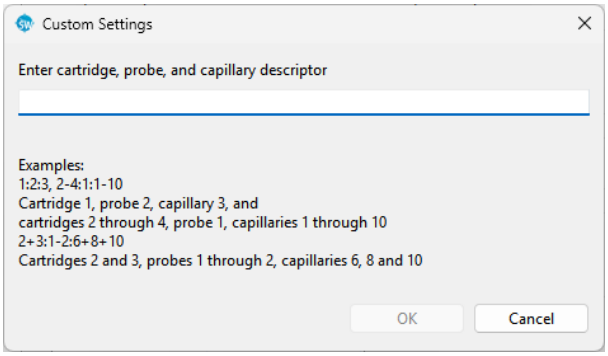



4. Select an option from the drop-down list. This will apply the settings group selected to specific run data as follows:
    a. **All** - When this option is selected, peak names group settings will be applied to all capillaries in the run file.
    b. **Blocking reagent** - When this option is selected, 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. **Primary antibody 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. **Secondary antibody names** - All secondary antibody names entered in the assay template (Assay screen) will display in the drop-down list, otherwise the default name of Secondary will be shown. Select a name to apply group settings to all capillaries that use the secondary antibody name in the run file.
    f. **Secondary antibody attributes** - All 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.

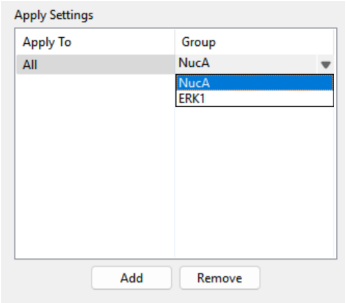
g. **Probe (RePlex Assays only)** - Select a probe to apply group settings to all capillaries within that probe.

h. **Cartridge (Leo only)** - Applies group settings to a specific cartridge.

i. **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 for a specific Probe and/or cartridge:




5. If you need to change the peak names group used for a data set, click the cell in the **Group** column and click the down arrow. Select a group from the drop-down list.

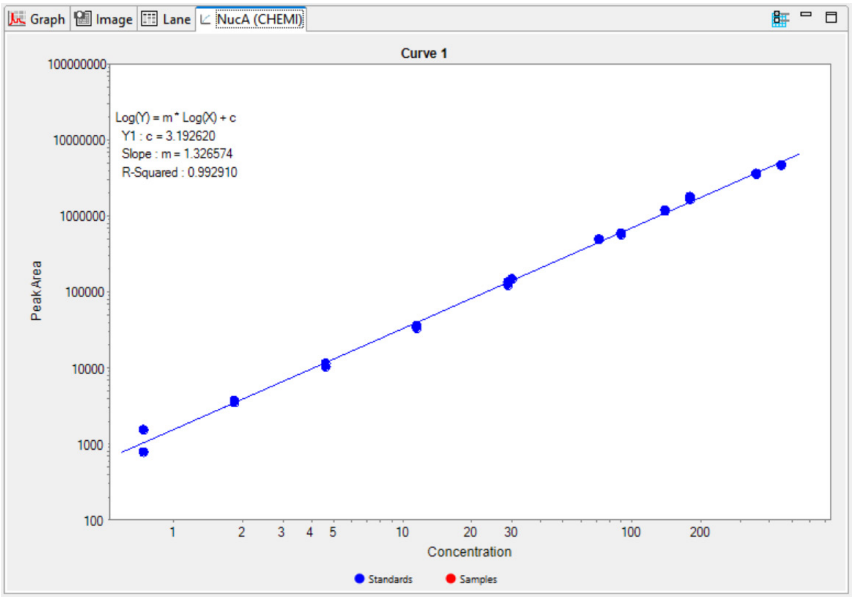



6. Repeat the previous steps to apply other groups to specific run data.

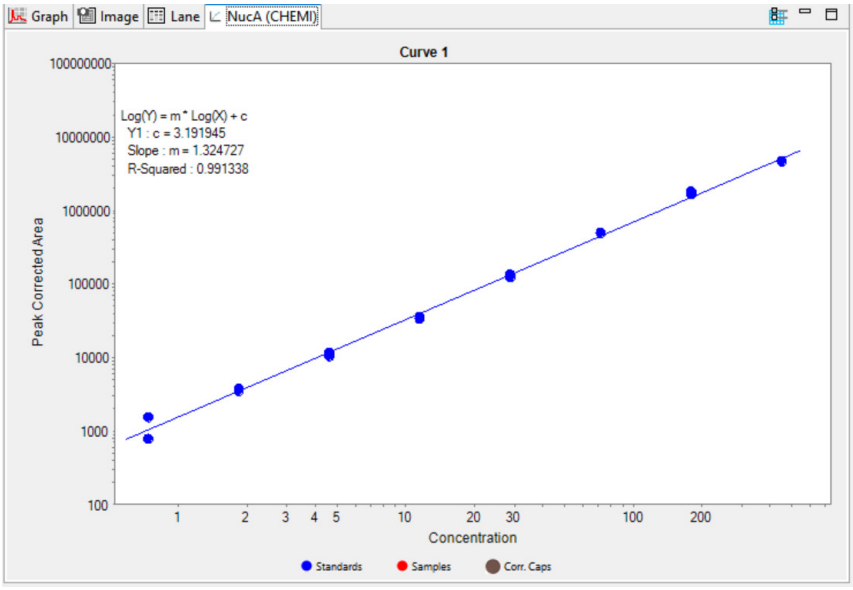
7. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.

8. 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:

#### STANDARD CURVE WITH NO NORMALIZATION (TOTAL PROTEIN OR LOADING CONTROL) OR CARTRIDGE CORRECTION APPLIED

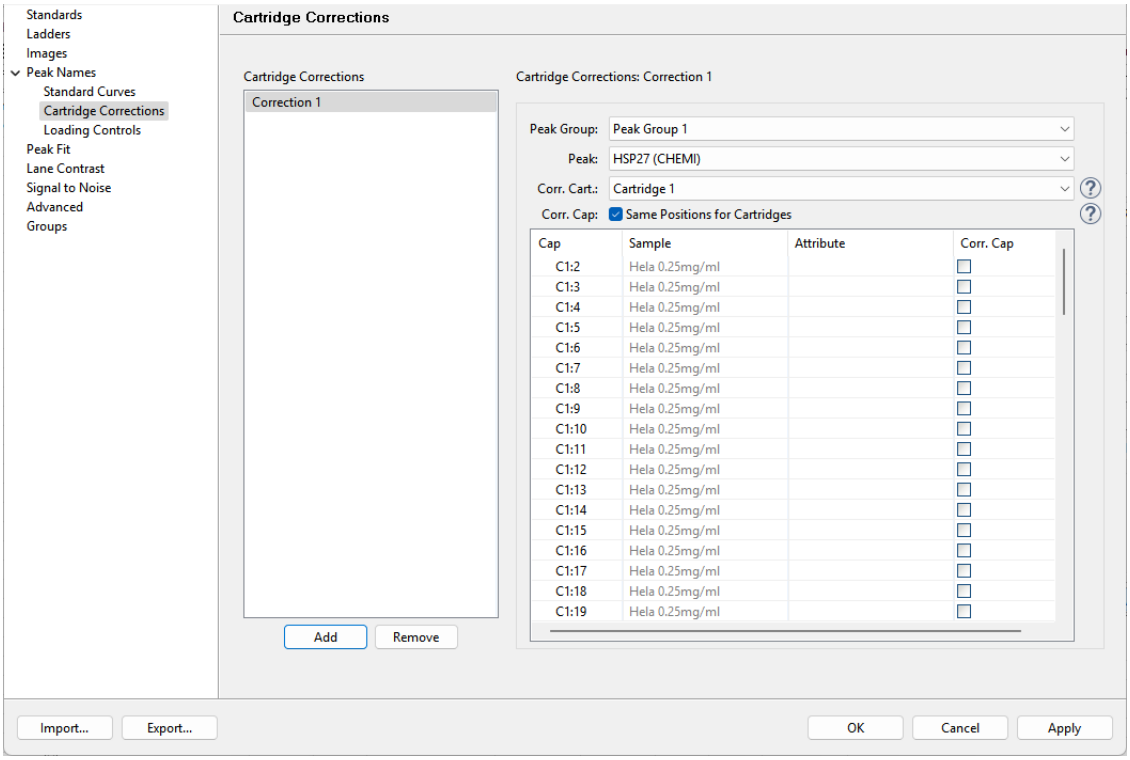



#### STANDARD CURVE WITH NORMALIZATION (TOTAL PROTEIN OR LOADING CONTROL) OR CARTRIDGE CORRECTION APPLIED



# Cartridge Corrections (Leo only)

The Cartridge Corrections settings in the Analysis window lets you view and change the reference cartridge and peak for cartridge correction calculations. Cartridge normalization settings are applied when enabling Cartridge Corrections. Cartridge Corrections is only applied to the named peak chosen under Peak in Cartridge Corrections analysis settings. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Cartridge Corrections** under Peak Names in the options list.




**NOTES:**
You will need to set up a Peak Names Analysis Group to set up Cartridge Corrections. See “Peak Names Analysis Groups” on page 294.

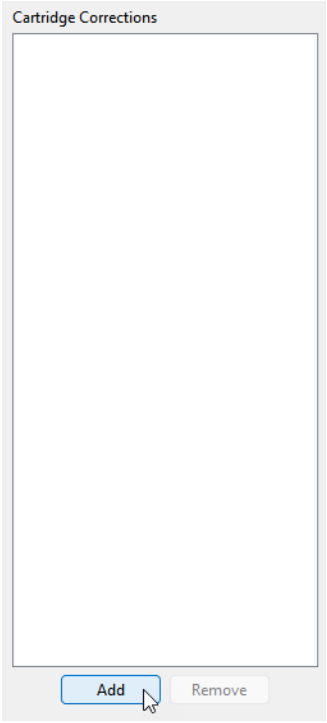
Only one type of normalization is allowed per analysis. Normalization using Total Protein with RePlex assays and Loading Controls cannot be used if Cartridge Corrections is set up.

- Click **Import** to import an analysis settings file. This is explained in more detail in “Importing Analysis Settings” on page 337.
- Click **Export** to export the current analysis settings file. This is explained in more detail in “Exporting Analysis Settings” on page 337.
- Click **Apply** to see effect of changes before saving.

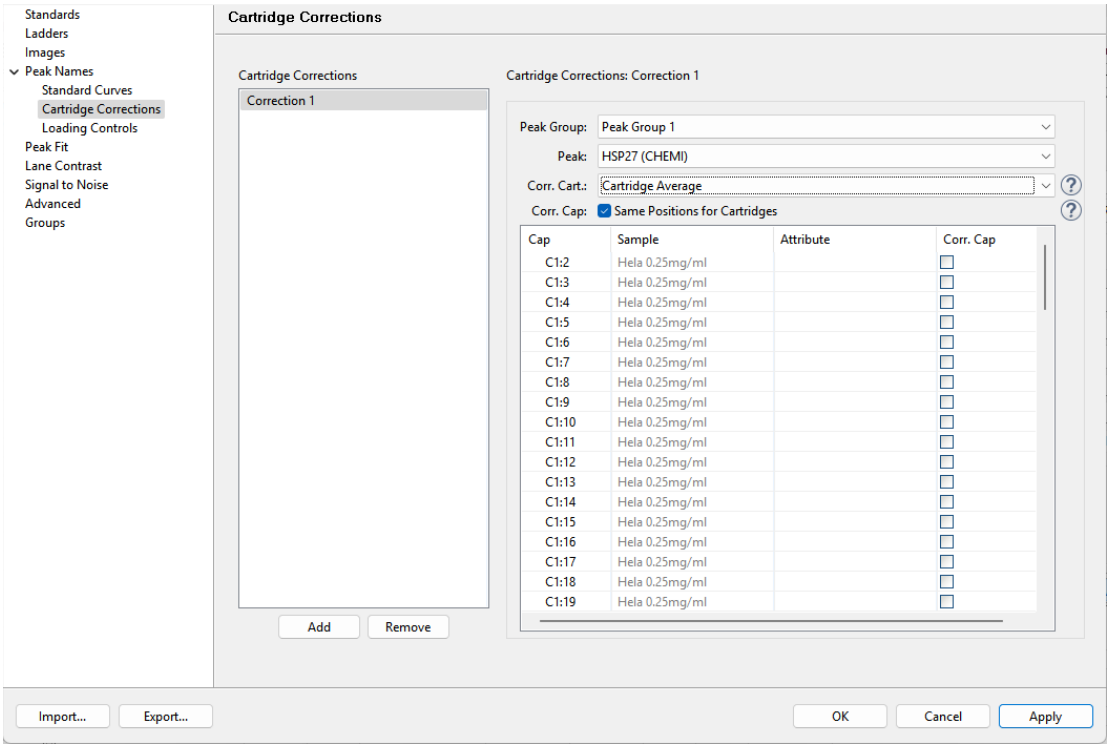
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

To use Cartridge Corrections to normalize data:

1. Select **Edit** in the main menu and click **Analysis**.
2. Click **Peak Names** in the options list and select **Cartridge Corrections**.
3. Click **Add** under Cartridge Corrections.



4. "Correction 1" will appear along with default Cartridge Corrections settings. Optional: Click on the "Correction1" to enter a new name.




5. Adjust the settings as needed.

   - **Peak Group** – Lets you select the Peak Names Analysis Group.
   - **Peak** – Lets you select the named peak to use for the cartridge correction calculations.
   - **Corr. Cart** – Lets you select the reference cartridge. The reference signal can be from a specific cartridge or the average signal from all cartridges.
   - **Corr. Cap** – Selecting the Same Positions for Cartridges checkbox enables the automatic selection of the reference capillary between cartridges to use for cartridge correction calculations. For example, capillary 1 in cartridges 2–4 will automatically be selected when capillary 1 in cartridge 1 is selected. When the box is unchecked, unique capillary numbers can be used as the reference capillary for each cartridge.

6. Click **Apply** and then click **OK** to save changes and exit. Updated sample data using the cartridge correction settings will display in the Analysis screen.

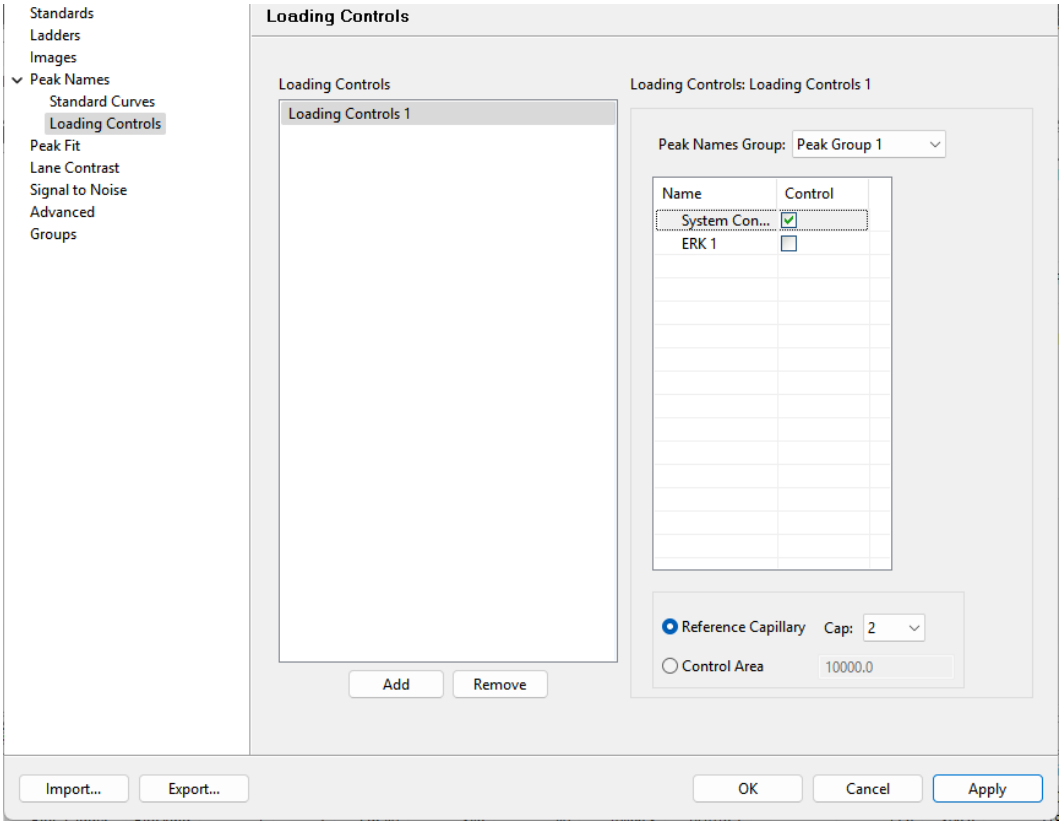
**NOTE:** Normalization when using Cartridge Corrections is only applied to the peak selected in the Cartridge Corrections analysis settings. The normalization will not be applied to other named peaks or peaks identified in the samples.

# Loading Controls Settings

It is possible to use loading control protein to normalize data between capillaries, between runs and between instruments. First create peak names groups as described in “Creating a Peak Names Group” on page 294 for your control protein and the target protein in the same peak analysis group. In the example below, System Control is the control protein and ERK1 is the target protein.

**NOTES:**
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 337.

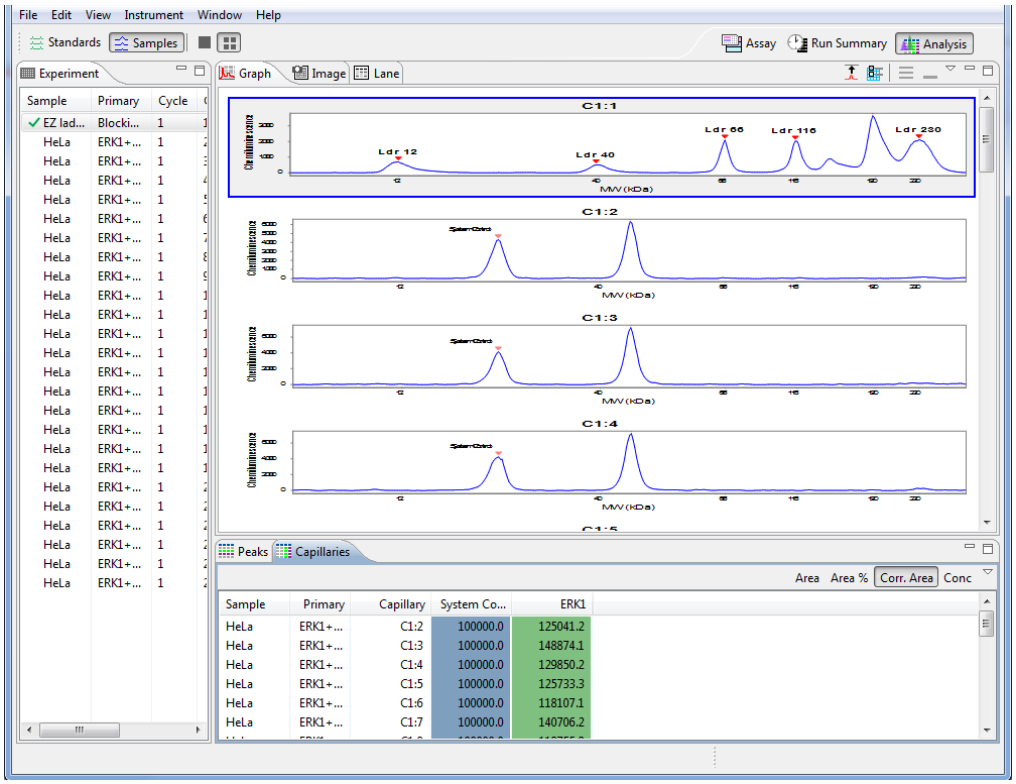
Only one type of normalization is allowed per analysis. Normalization and Cartridge Correction cannot be used if a Loading Control is set up.




To identify the control protein:

1. Select **Edit** in the main menu and click **Analysis**.
2. Click **Peak Names** in the options list and select **Loading Controls**.

3. Select the desired peak name group using the Peak Names Group drop-down menu. All peak names in that group will be listed.
4. Select the checkbox in the Control column next to the peak name to use as the loading control.
5. Select a Reference Capillary from the drop-down menu(s). The peak area of this capillary will be used to normalize the peak area of the named peaks. For RePlex assays, 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. For Leo runs, specify which cartridge to use as the reference.
6. To manually assign the Control Area, click the box and enter the peak area of your control protein.
7. 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:



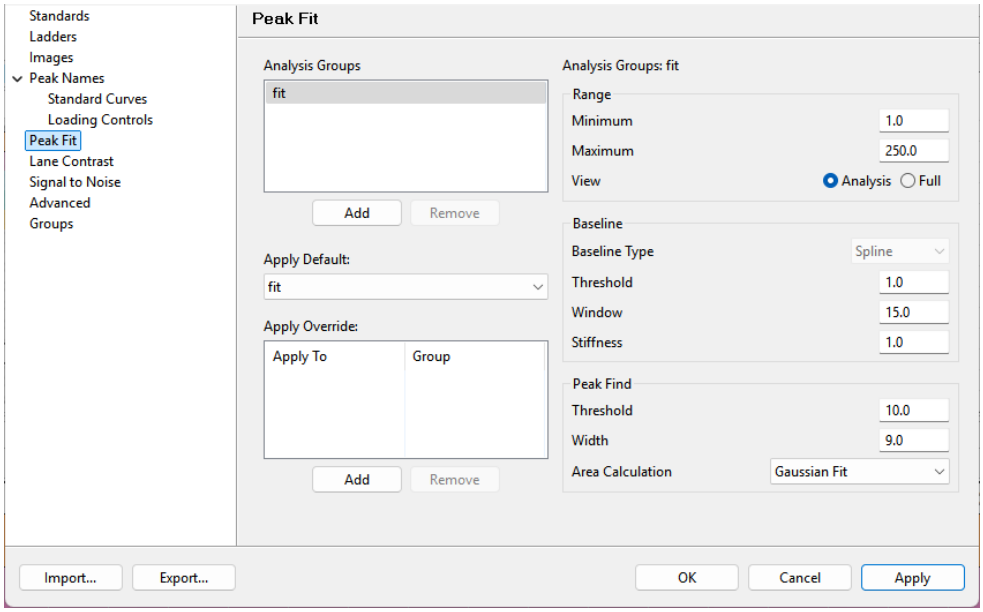
# Peak Fit Analysis Settings

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.

**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 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 337.




- Click **Import** to import an analysis settings file. This is explained in more detail in “Importing Analysis Settings” on page 337.
- Click **Export** to export the current analysis settings file. This is explained in more detail in “Exporting Analysis Settings” on page 337.
- Click **Apply** to see effect of changes before saving.
- 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.
- **View** - Changes the molecular weight range used for the x-axis.
  - **Analysis** matches the range of the run data that was selected in the Range Analysis Settings in both the electropherogram and virtual blot view. This is the default setting.
  - **Full** will display the range of the run data for the entire capillary in both the electropherogram and virtual blot view.

## Baseline Settings

- **Type -** Changes the type of baseline fit used for the run data.

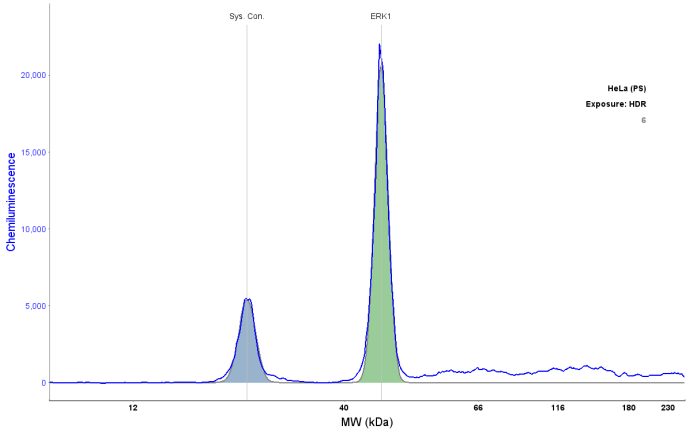
**NOTE:** Baseline Type selection is only available when Dropped Lines and Dropped Lines (manual) is used for the Area Calculation

- **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.

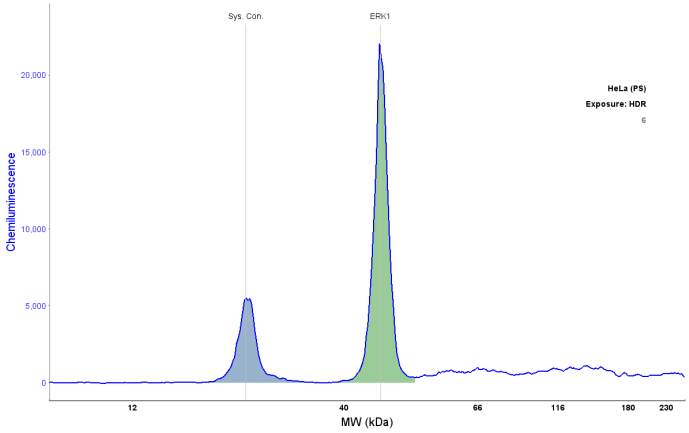
## Peak Find Settings

- **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** - Three fits are provided: Gaussian Fit, Dropped Lines, or Dropped Lines (manual). These settings can be changed before or after the run is finished.
  - For Immunoassays, peak area is calculated using Gaussian distribution by default. Dropped Lines (manual) allows for manually adjusting the start and stop positions of peak boundaries. This type of area calculation is also often called the perpendicular drop method. This is the preferred method when peaks overlap or are close to each other. It 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. Adjustments can then be made to manually set the start and stop positions of the peak boundaries.



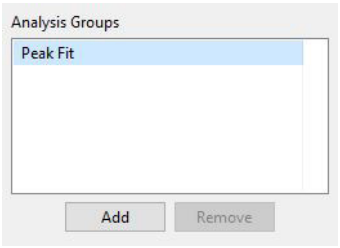

This next view is of the same data using Dropped Lines (manual) instead:




## 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, attributes, probes (RePlex assays only), and cartridges (Leo only) in the run data.

Peak fit groups are displayed in the analysis settings box:

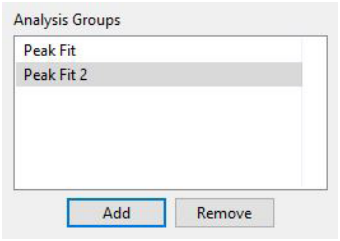



The Peak Fit group shown contains the Compass for Simple Western default analysis settings. You can make changes to this group and create new groups.

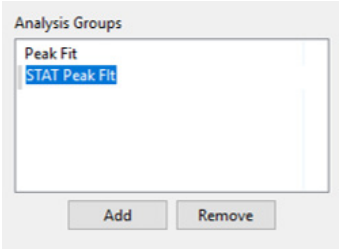
To view settings for a group, click on the group name in the analysis settings box.

## 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 Groups box. A new group will be created:

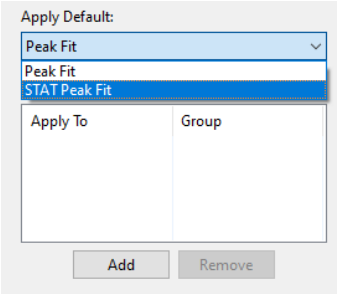



3. Click on the new group and enter a new name.




4. Modify range, baseline or peak find parameters as needed.

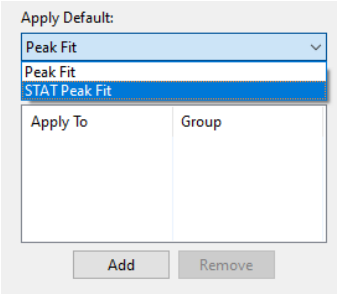
5. To use the new group as the default peak fit settings for the run file data, click the arrow in the drop-down list below Apply Default, then click the new group from the list. Peak fit settings in the new group will then be applied to the run data.




6. Click **Apply** and then click **OK** to save changes.

## Changing the Default Peak Fit Group

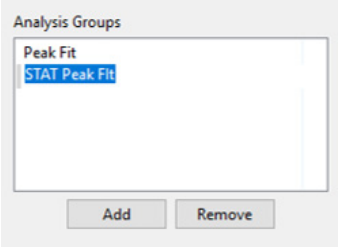
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click the arrow in the drop-down list below Apply Default, then click a new default group from the list.




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

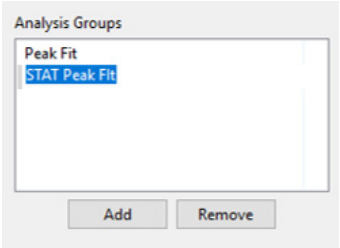
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the Analysis Groups box you want to modify.



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

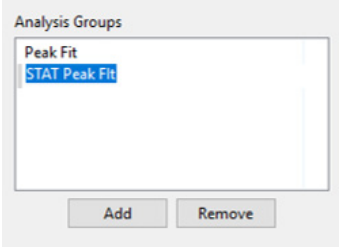
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the Analysis Groups box you want to delete and click **Remove**.



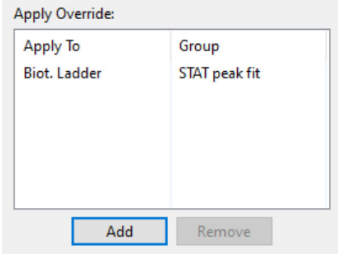

3. Click **OK** to save changes.

## Applying Peak Fit Groups to Specific Run Data

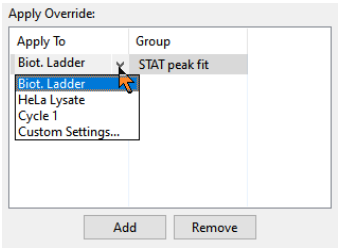
1. Select **Edit** in the main menu and click **Analysis**, then click **Peak Fit** in the options list.
2. Click on the group in the Analysis Groups box you want to apply to specific run data.




3. Application of peak fit groups to specific run data is done in the Apply Override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.

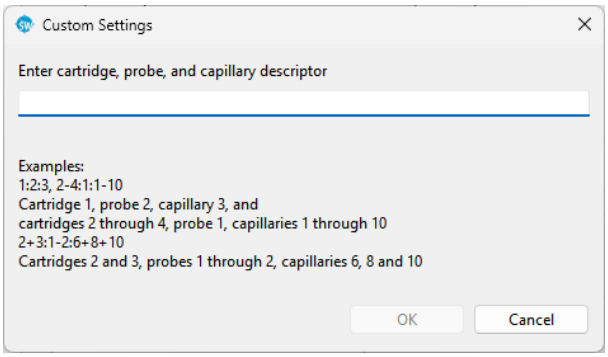


4. Click the cell in the **Apply To** column, then click the down arrow.

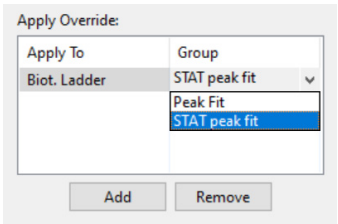



5. Select an option from the drop-down list. This will apply the settings group selected to specific run data as follows:

   - **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.
   - **Probe 1 and 2 (RePlex Assays only)** - Select a probe to apply Peak Fit group settings to all capillaries within that probe.
   - **Cartridge (Leo only)** - Applies group settings to a specific cartridge.
   - **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 for a specific Probe and/or cartridge:




6. If you need to change the peak fit group used for a data set, click the cell in the Group column and click the down arrow. Select a group from the drop-down list.

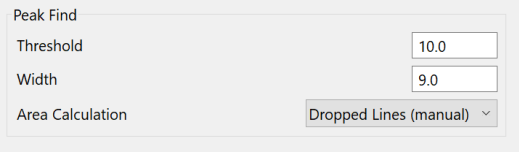


7. Repeat the previous steps to apply other groups to specific run data.
8. To remove a data set, click on its cell in the **Apply To** column, then click **Remove**.
9. Click **Apply** and then click **OK** to save changes.

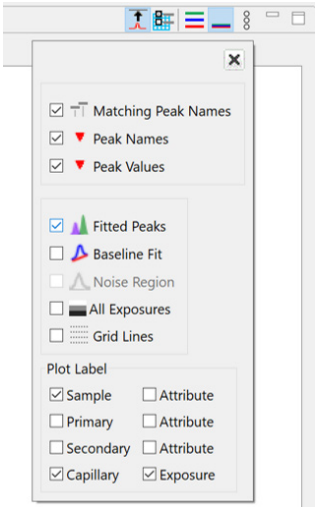
## Manual Peak Integration

Compass for Simple Western lets you manually integrate peaks in individual electropherograms.

1. Select **Edit** in the main menu, click **Analysis**, and select **Peak Fit** in the options list.
2. Select **Dropped Lines (manual)** as the area calculation if it isn't already selected.

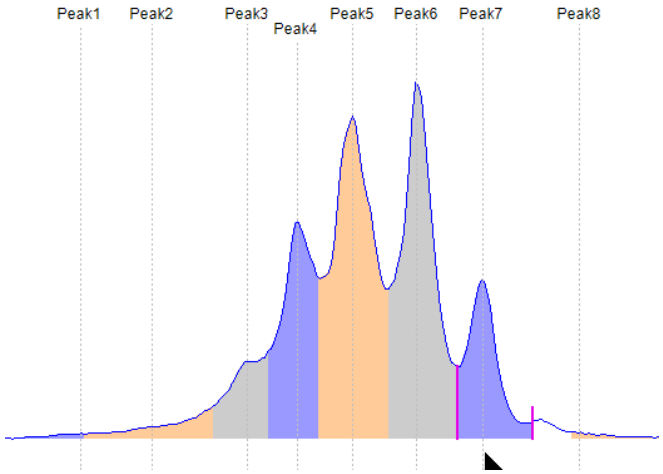



3. Click **OK** to save changes.
4. In the Analysis screen, select **Fitted Peaks** in the Graph Options.



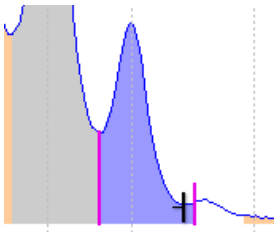

5. Select a capillary in the Experiment pane.

6. Hover the mouse over the peak you want to adjust. Two magenta bars will display that indicate the start and stop points of its integration.

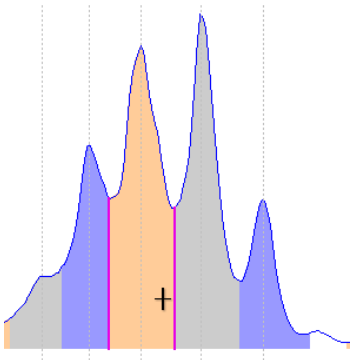



7. To change the integration, hover the mouse over either of the magenta bars. Then click the mouse and drag the bar to move it.
   - If the cursor changes to ⊢ this is the peak start for the peak on the right.

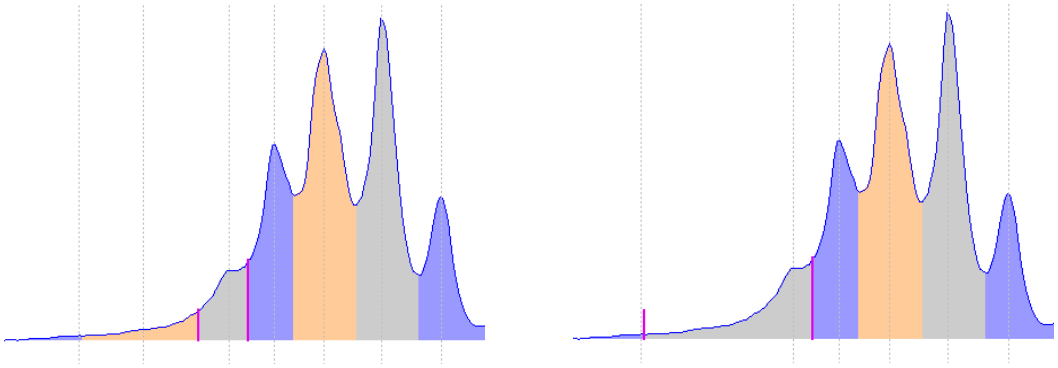
   - If the cursor changes to ⊣ this is the peak end for the peak on the left.



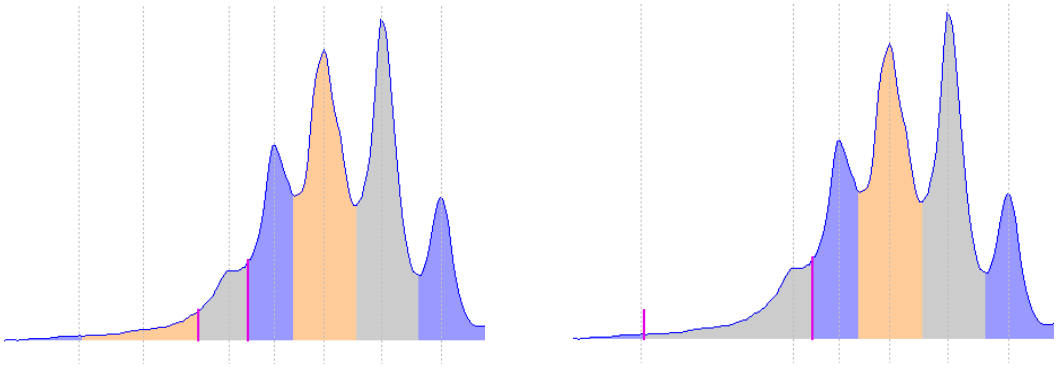
- If the cursor changes to ┼ this is a joint boundary for the peaks on the left and right.



In the example below, we moved the start and end points of the peak to include more area under the peak:



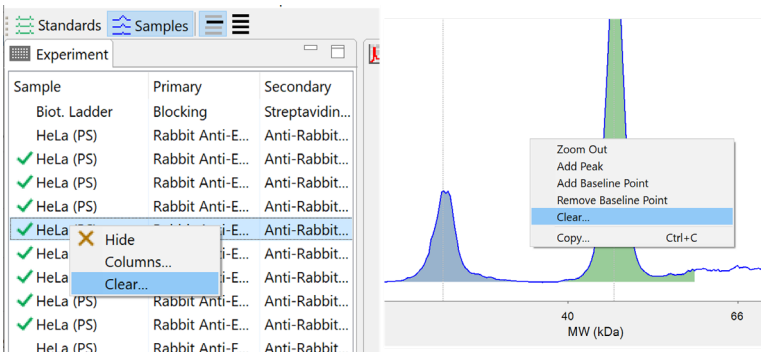
You can also combine peaks by dragging the magenta lines left or right:



You can also separate areas between peaks. Whenever you have a ┼ cursor between two peaks that aren't baseline resolved, move the mouse slightly to the right or left until you get the ┣ or ┫ cursor.

Click and hold the mouse. A black drop line will display indicating where the current peak start or end point is. While holding the mouse, drag the cursor to the right or left to move the start or end point to where you want it, then release the mouse.

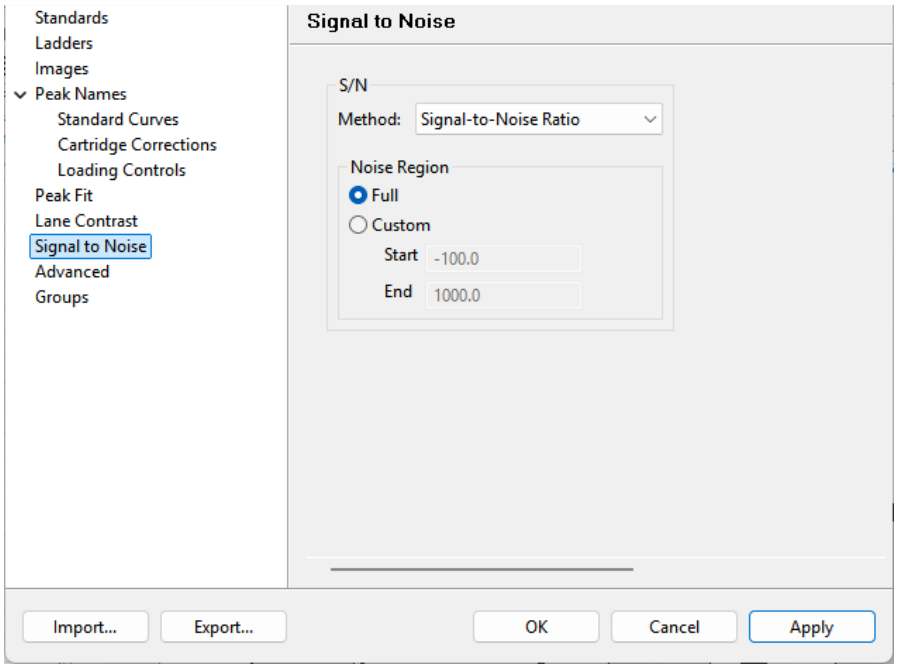
8. To remove the manual integration settings, right-click on the graph or on the sample in the Experiment pane and select **Clear**.




# Signal to Noise Settings

The signal to noise settings page lets you view and change the method used for the signal to noise calculation in the sample data. To access these settings, select **Edit** in the main menu and click **Analysis**, then click **Signal to Noise** 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 is explained in more detail in “Importing Analysis Settings” on page 337.
- Click **Export** to export the current analysis settings file. This is explained in more detail in “Exporting Analysis Settings” on page 337.
- Click **Apply** to see effect of changes before saving.
- Click **OK** to save changes and exit.
- Click **Cancel** to exit without saving changes.

## S/N Compass v5.0

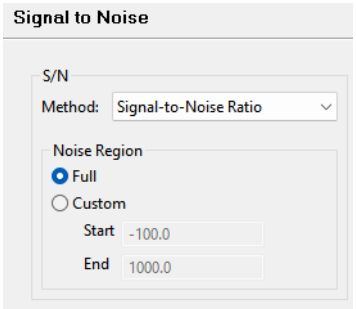
S/N Compass v5.0 is a method that calculates peak score based on a peak finding algorithm to provide a relative ratio comparing all peaks within the electropherogram in Compass. S/N Compass v5.0 was referred to as ‘Peak Score (v1-v5)’ in Compass for Simple Western versions 6.0. With some run data, Peak Score can result in negative signal to noise values. If this happens, using the Signal-to-Noise Ratio instead will correct this.

## Signal-to-Noise Ratio

The calculation used for the Signal-to-Noise Ratio follows USP/NF (United States Pharmacopeia/National Formulary) regulations with the difference on how noise is determined. This is the default selection for run data generated using Jess and Abby assays in Compass for Simple Western version 6.0 and higher and Leo assays in Compass for Simple Western version 7.0 and higher.

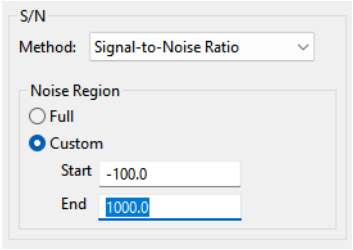
When this option is selected, you can set the following parameters to select the region of data used to identify the best noise sampling:

- **Full** - Selects the entire data range of the run data within the capillary along the x axis (in kDa) for the noise region. The default range used will be the size range for the run data: 2-40, 12-230, or 66-440 kDa. This is the default setting for the Noise Region and is recommended for most use cases.

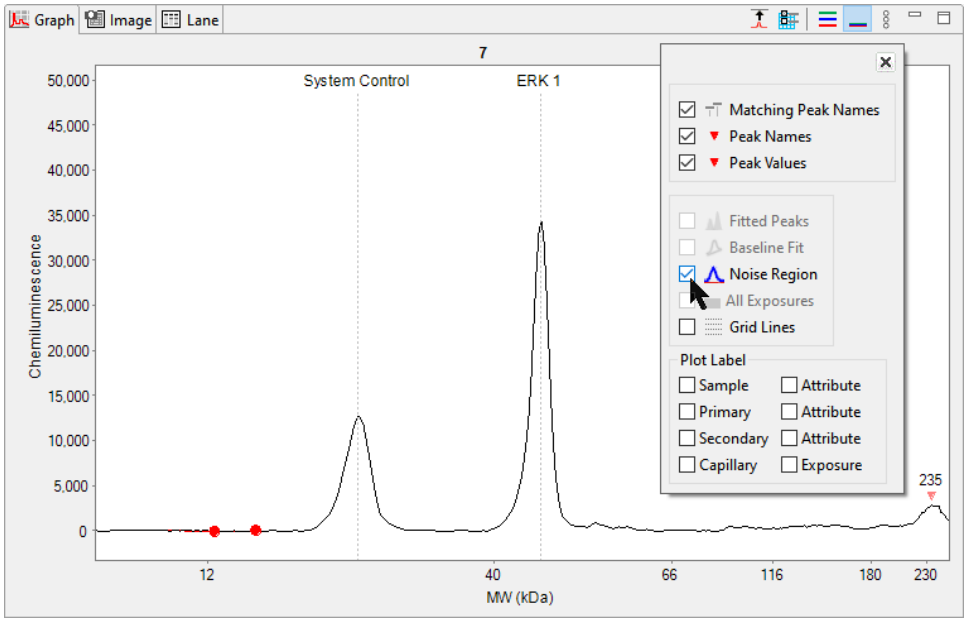



- **Custom** - Lets you set the data range along the x axis (in kDa) to narrow the search region used for the signal to noise calculation. To use this option, enter a **Start** and **End** value in kDa.

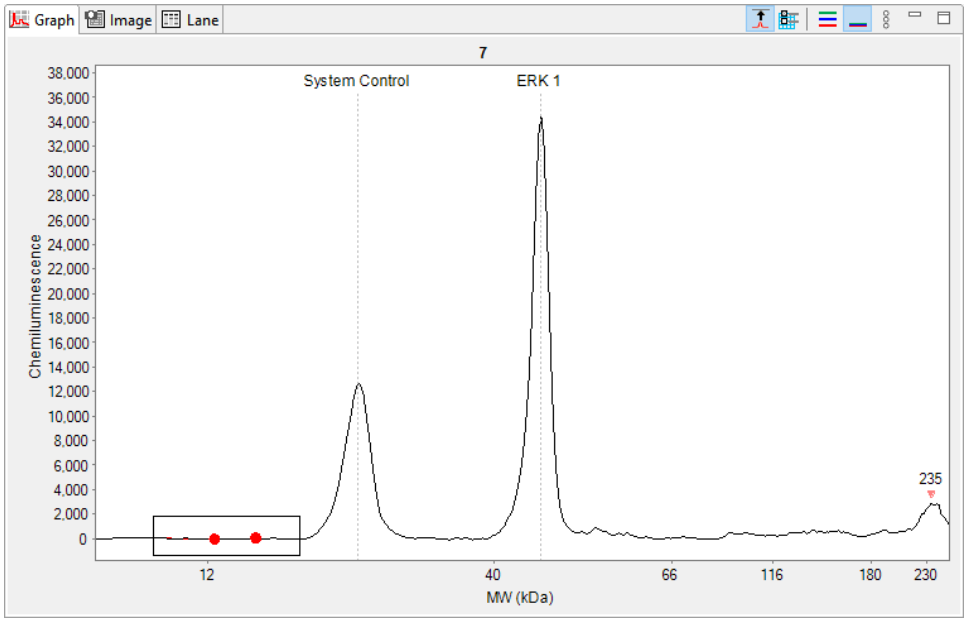
**NOTE:** There are no custom range recommendations from ProteinSimple. The start and end value entry depends on the user application and noise variation in the run data.



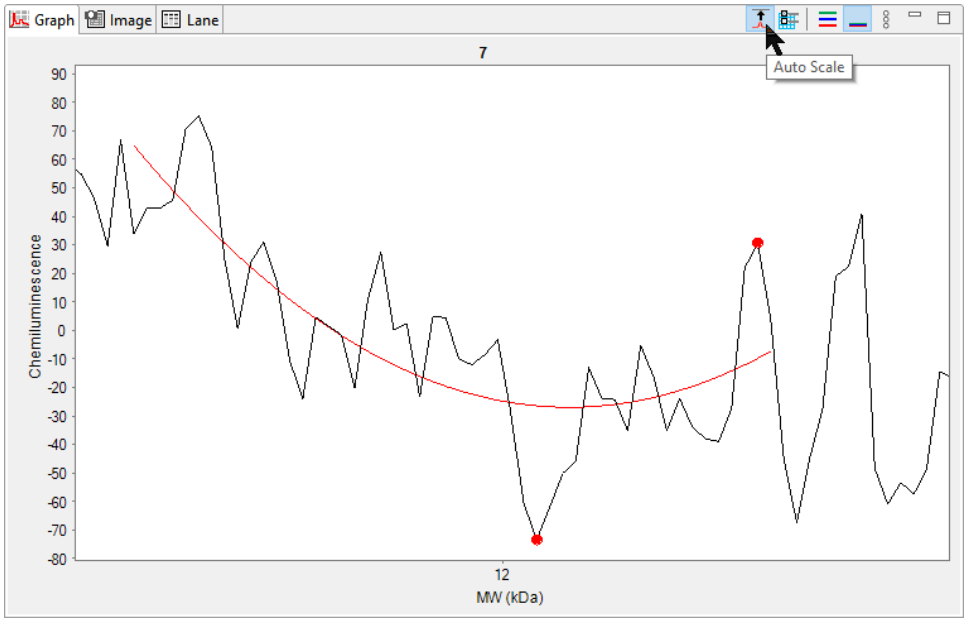

When Signal-to-Noise Ratio is selected, the software can overlay the noise region on the electropherogram in the Graph pane. To display the overlay, select **Noise Region** from the Graph Options menu:



The noise region is indicated by the two red dots on the electropherogram. To zoom in on the noise region: select that area with your mouse:



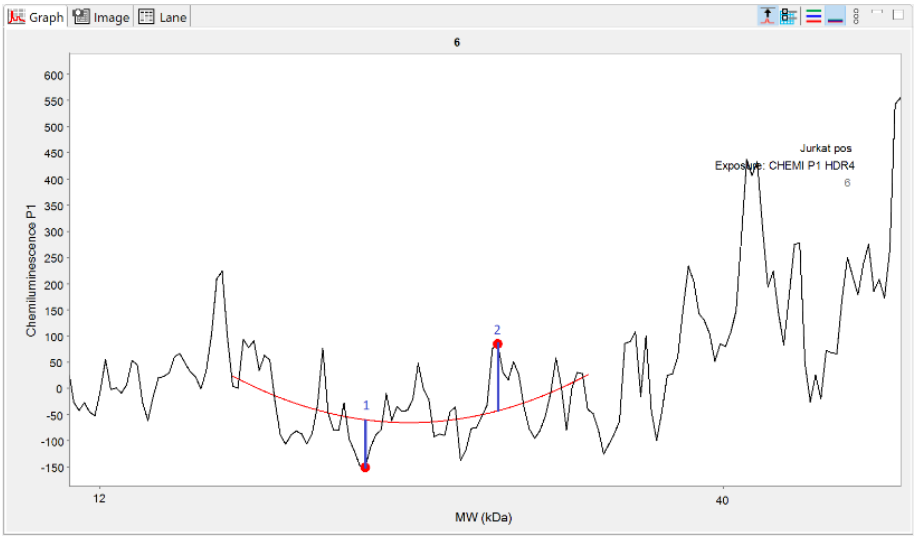

Next, select **Auto Scale**. This makes it easier to see the noise fit region, as well as the points used in the overall noise calculations:




The red line represents the quietest noise fit region within the bounded area. The two points are the greatest difference in noise between the real and fit data within the quietest region.

To find the noise fit region, Compass for Simple Western utilizes a rolling window over the bounded area based on the Start and End values. Beginning at the Start value, the software fits a curve using polynomial regression to a window of real data and calculates the maximum difference between the real data and the fit data above and below the curve. It then searches for the quietest region via multiple searches within the bounded area and chooses the region with the minimum noise.

Noise is found by taking the distance between the actual data points and the fit curve and adding those values. In the following data, those values have been marked (1, 2), and the distance between the real point and the fit curve is shown as the straight blue line:




The noise for this noise fit window is the Length of Blue line 1 + Length of Blue Line 2. This process continues until the window reaches the End value.

**h = Length of Blue line 1 + Length of Blue line 2**

This is the highest noise within the determined region of minimum noise. The signal to noise ratio given the peak height is then calculated using:

**2.0 * Peak Height/h**

This calculation is done for each peak, and is represented as the S/N value in the Peaks table:

Peaks | Capillaries

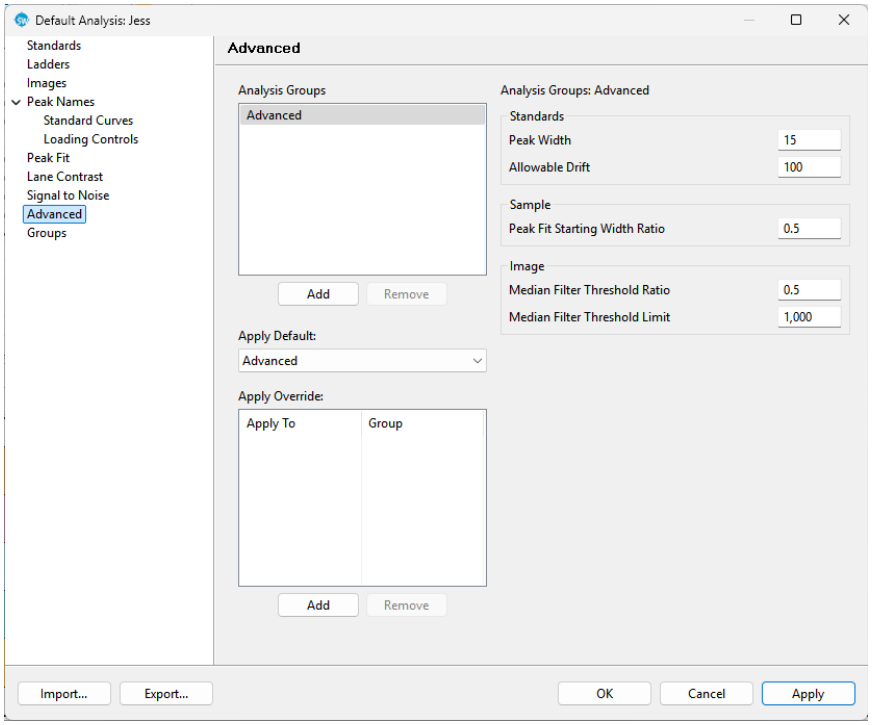
| Sample | Primary | Cap | Peak | Name | Position | MW (kDa) | Height | Area | % Area | Width | S/N | Baseline |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HeLa | Anti-R... | 7 | 1 | Syste... | 286 | 27 | 12274.1 | 163252.7 | 35.5 | 12.5 | 280.8 | 474.0 |
| HeLa | Anti-R... | 7 | 2 | ERK 1 | 361 | 47 | 33062.9 | 296673.6 | 64.5 | 8.4 | 756.4 | 660.9 |
| HeLa | Anti-R... | 7 | 3 | | 522 | 235 | 2637.3 | 38650.2 | | 13.8 | 60.3 | 726.0 |

**NOTE:** Per USP standards, 'h' is derived from the noise region around the peak of interest and is used in the S/N ratio calculation for that individual peak. Compass for Simple Western calculates 'h' in the quietest noise fit region in the boundary area (not around the individual peak of interest). This value is used to calculate the S/N ratio across the capillary for all peaks, where the only value that changes for individual peaks is the Peak Height.

# 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 is explained in more detail in “Importing Analysis Settings” on page 337.
- Click **Export** to export the current analysis settings file. This is explained in more detail in “Exporting Analysis Settings” on page 337.
- Click **Apply** to see effect of changes before saving.
- 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 1000.

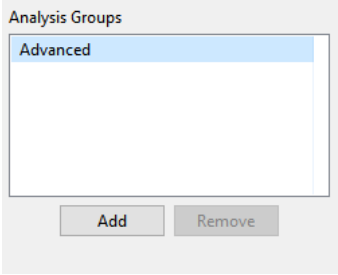
## Advanced Analysis Settings Groups

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 337.

Analysis groups are displayed in the analysis settings box:

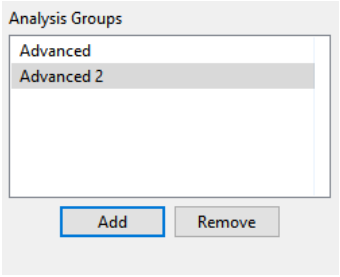


The Advanced group shown contains the Compass for Simple Western default analysis settings. You can make changes to this group and create new groups.

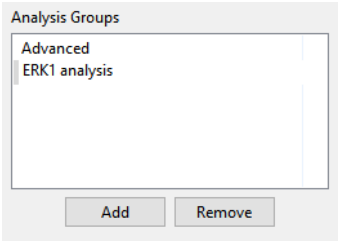
To view settings for a group, click on the group name in the analysis settings box.

## Creating a New Analysis Group

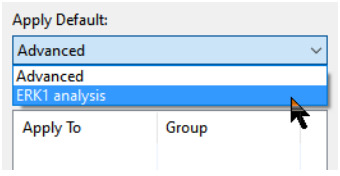
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click **Add** under the Analysis Groups box. A new group will be created:




3. Click on the new group and enter a new name.



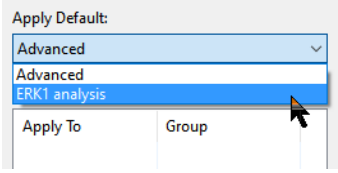

4. Modify standards, sample or image parameters as needed.
5. To use the new group as the default analysis settings for the run file data, click the arrow in the drop-down list below Apply Default, then click the new group from the list. Analysis settings in the new group will then be applied to the run data.




6. Click **OK** to save changes.

## Changing the Default Analysis Group

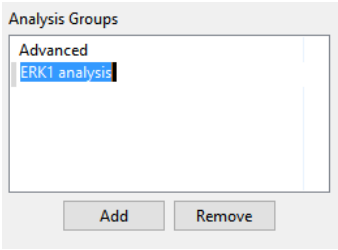
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click the arrow in the drop-down list below Apply Default, then click a new default group from the list.




3. Click **OK** to save changes. Analysis settings in the group selected will be applied to the run data.

## Modifying an Analysis Group

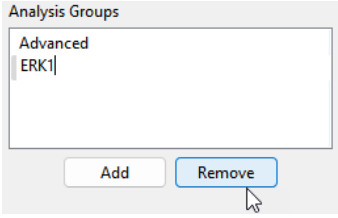
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click on the group in the Analysis Groups box you want to modify.




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

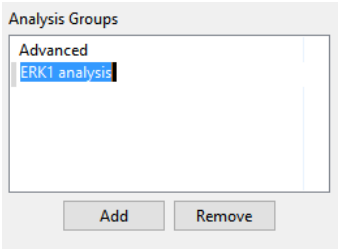
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click on the group in the Analysis Groups box you want to delete and click **Remove**.



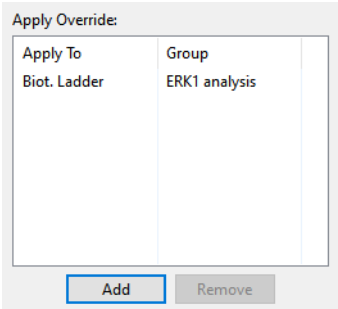

3. Click **OK** to save changes.

# Applying Analysis Groups to Specific Run Data

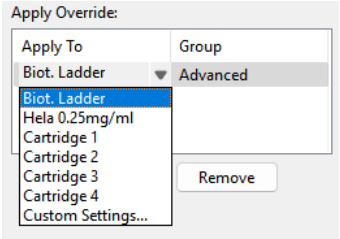
1. Select **Edit** in the main menu and click **Analysis**, then click **Advanced** in the options list.
2. Click on the group in the Analysis Groups box you want to apply to specific run data.




3. Application of analysis groups to specific run data is done in the Apply Override box. Click **Add** under the override box. A default override data set will be created from sample information found in the run file.



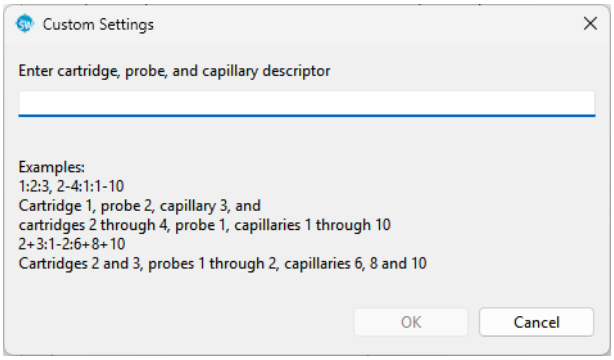

4. Click the cell in the **Apply To** column, then click the down arrow.



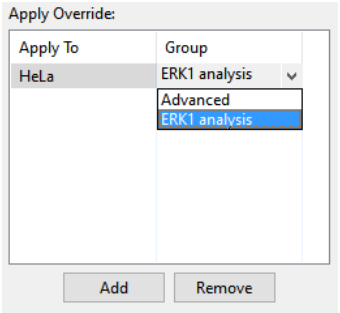

5. Select an option from the drop-down list. This will apply the settings group selected to specific run data as follows:

   - **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.
   - **Probe 1 and 2 (RePlex Assays only)** - Applies group settings to all capillaries within that probe.

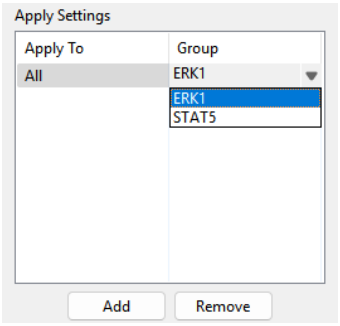
- **Cartridge (Leo only)** - Applies group settings to a specific cartridge.
- **Custom settings** - Lets you choose specific cartridges and capillaries to apply the group settings to. When this option is selected, the following pop-up box appears to let you enter a specific capillary number or range of capillaries for a specific Probe and/or cartridge:




6. If you need to change the analysis group used for a data set, click the cell in the Group 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.




10. 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.

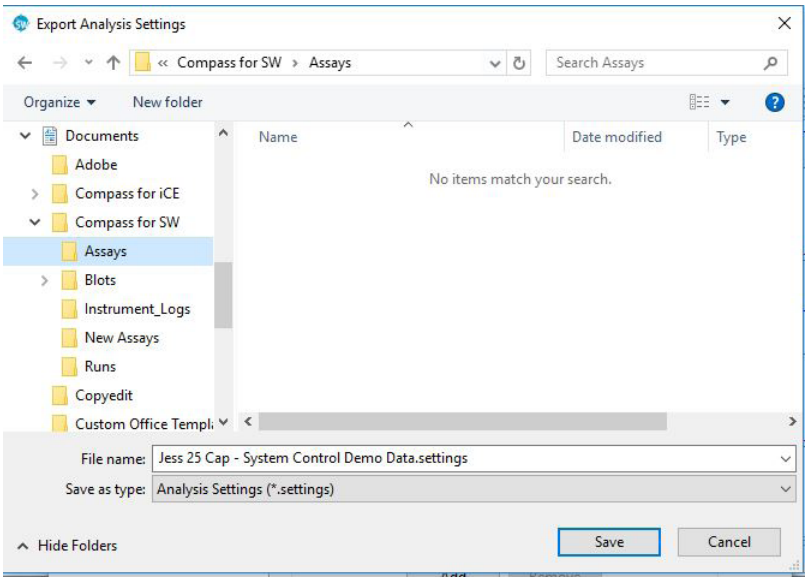
1. Open the run file or assay you want to import analysis settings to.
2. Select **Edit** in the main menu and click **Default Analysis** (Assay screen) or **Analysis** (Analysis screen).
3. Click **Import** on any page.
4. Select a settings file (*.settings) and click **OK**. The imported settings will 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 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 8:

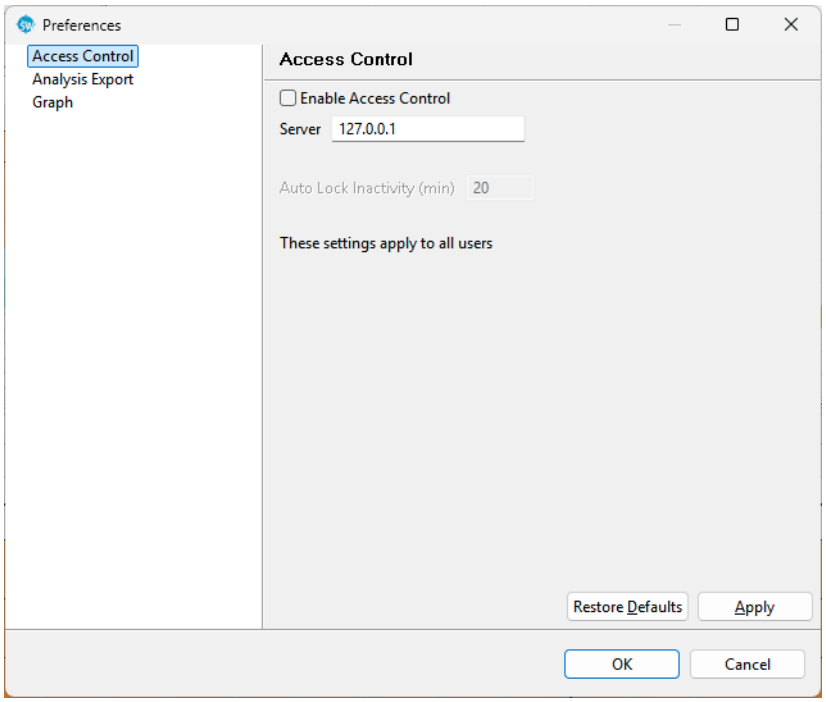
# Setting Your Preferences

## Chapter Overview

- Custom Preference Options
- Setting Data Export Options
- Selecting Custom Plot Colors for Graph Overlay

# Custom Preference Options

You can set and save custom preferences for access control, data export, and plot colors in the Graph pane. To access these settings, select **Edit** in the main menu and click **Preferences**.



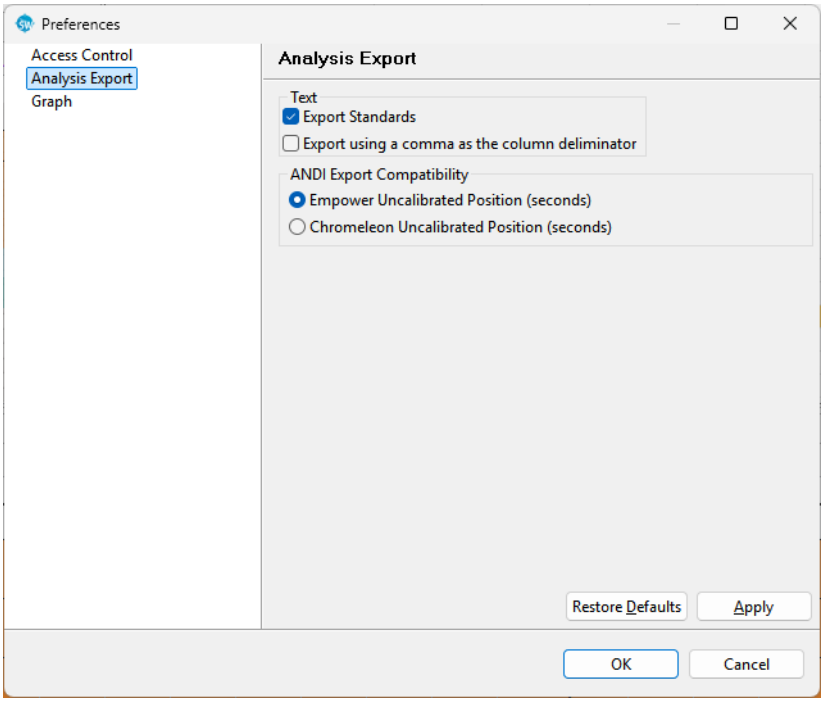

**NOTE:** In Compass for Simple Western version 5.0 and higher, you can no longer change or save Custom Preferences outside of the first instance of the software that's been opened. When running multiple instances of the software on the same computer, an error message displays if you try to change Custom 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:

- **Access Control** - This feature can be used to help satisfy the 21CFR Part 11 data security requirements. See "Enabling Access Control" on page 356 for more information.
- **Analysis Export** - Lets you customize data export options.
- **Graph** - Lets you customize electropherogram color displays in the Graph pane.

# Setting Data Export Options

Select **Edit** in the main menu and click **Preferences**, then choose **Analysis Export** in the options list.

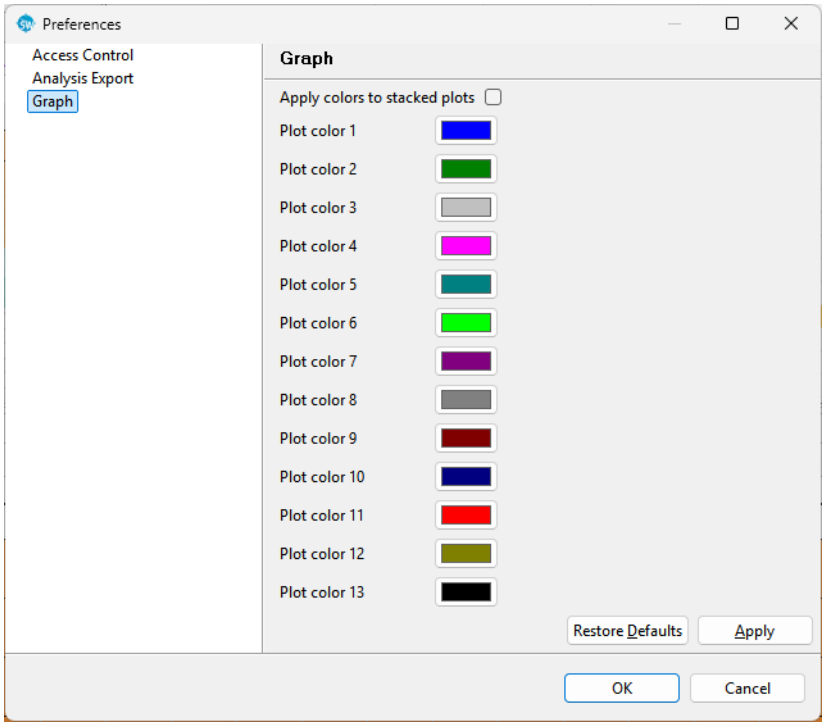



- **Export Standards** - Selecting this option includes data for the standards in each sample when run data is exported. When this option is deselected, only sample data will be exported. This option is selected by default.
- **Export using a comma as the column delimiter** - Selecting this option exports 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™ Software (Waters™) 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 Chromatography Data System (CDS) Software (Thermo Scientific™) 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 choose **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.
  - When this option is deselected, plots will use Compass for Simple Western default colors.

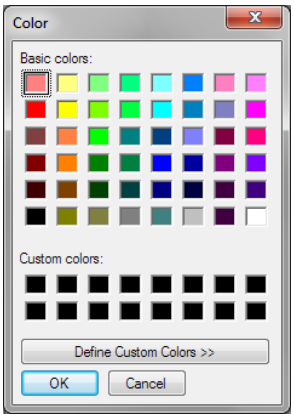
**NOTE:** If Apply colors to stack plots is not checked, the colors shown in the preferences screen will be applied only to overlaid electropherograms when the Overlay the Plot option is selected in the Graph pane.

- 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 choose **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.

Chapter 9:

# Compass Access Control and 21 CFR Part 11 Compliance

## Chapter Overview

- Overview
- Compass Authorization Server for Simple Western
- Enabling Access Control
- Changing the Software Inactivity Auto Lock
- Logging In to Compass for Simple Western
- Saving Changes
- Signing Files
- Exporting Uncontrolled Files
- Instrument Command Log
- Run File History
- Troubleshooting Problems and Suggested Solutions

# Overview

The Compass Access Control feature can be used to help support the 21CFR Part 11 data security requirements when using Simple Western instruments. When Access Control is enabled and the Compass Authorization Server for Simple Western has been installed:

- 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.

# Compass Authorization Server for Simple Western

The Compass Authorization Server (CAS) for Simple Western 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 for Simple Western 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 for Simple Western, click **Edit**, then **Preferences** and **Access Control** and enter the server IP address using format X.X.X.X.

## Server Administration

**NOTES:**
Always use the default port setting of 8443, this should not be changed.

If the server is installed on the same computer as Compass for Simple Western (e.g., the local machine), enter localhost:8443 instead of the IP address. Contact your local IT Administrator to assist with installing the Compass Authorization Server for Simple Western in your preferred format.

The Authorization Server for Simple Western is configured through a web interface at the IP address of the server on port 8443. To access the Server home page, open any browser and type the IP address on port 8443 in a X.X.X.X:8443 or https://X.X.X.X:8443 format. Use localhost:8443 instead of the IP address if the Server is installed on the local machine.

**NOTE:** If you have upgraded your system from a previous version of Compass Authorization Service, the localhost login page will have changed from http://localhost:8000/admin/login/?next=/ to https://localhost:8443/admin/login/?next=/

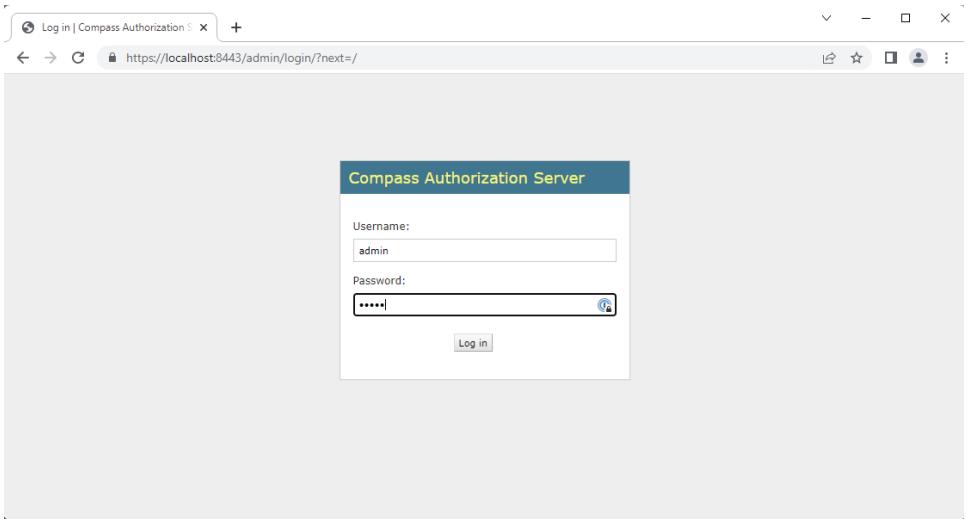
The default server administrator is:

- User: admin
- Password: admin

**NOTE:** The user account “admin” is not an active Compass for Simple Western login account. In order to log into Compass for Simple Western in the 21 CFR Part 11 compliant mode, at least one user account must be created in the Authorization Server for Simple Western application.

After installing the Compass Authorization Server for Simple Western, the administrator user name and password can be changed.

**NOTE:** Sites are responsible for maintaining their own admin and user account credentials. Record these and keep them safe. ProteinSimple can’t provide access once the admin account has been edited.

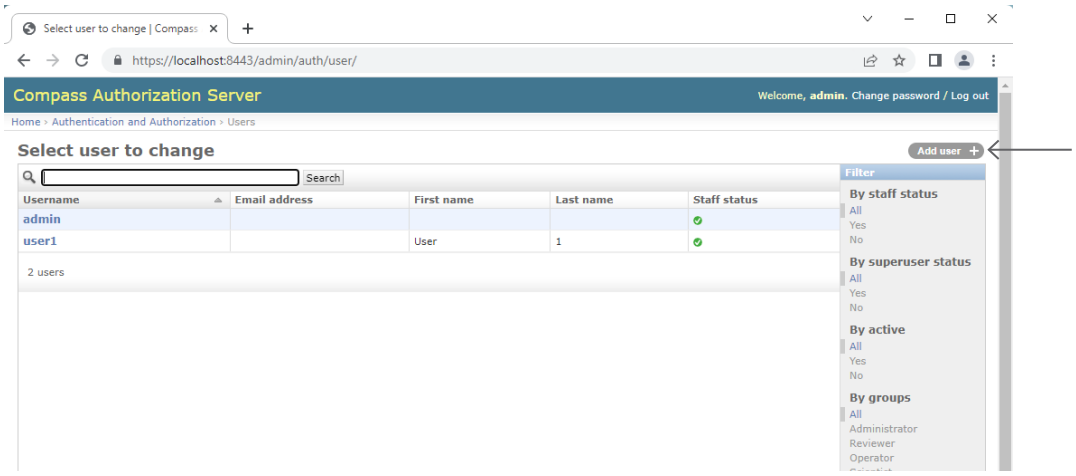



## Adding Non-admin Users

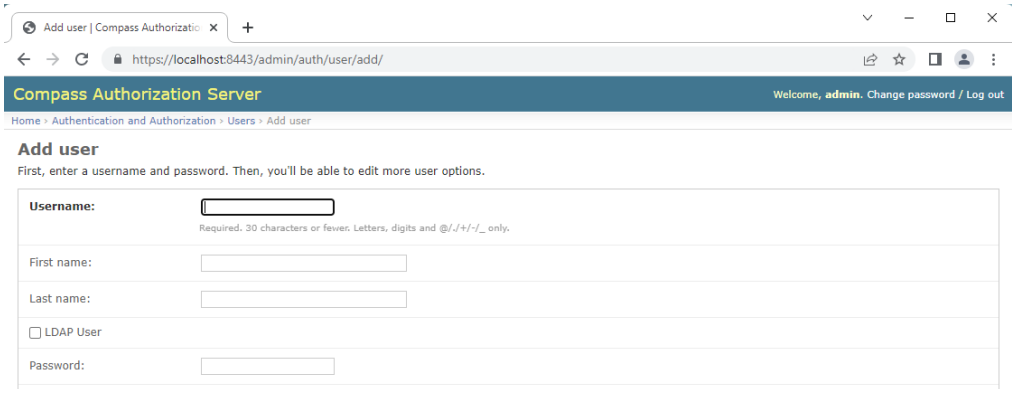
Add a user to the server to allow that user to log in to Compass for Simple Western. To do this:

1. Select **Users** from the Site Administration home page:

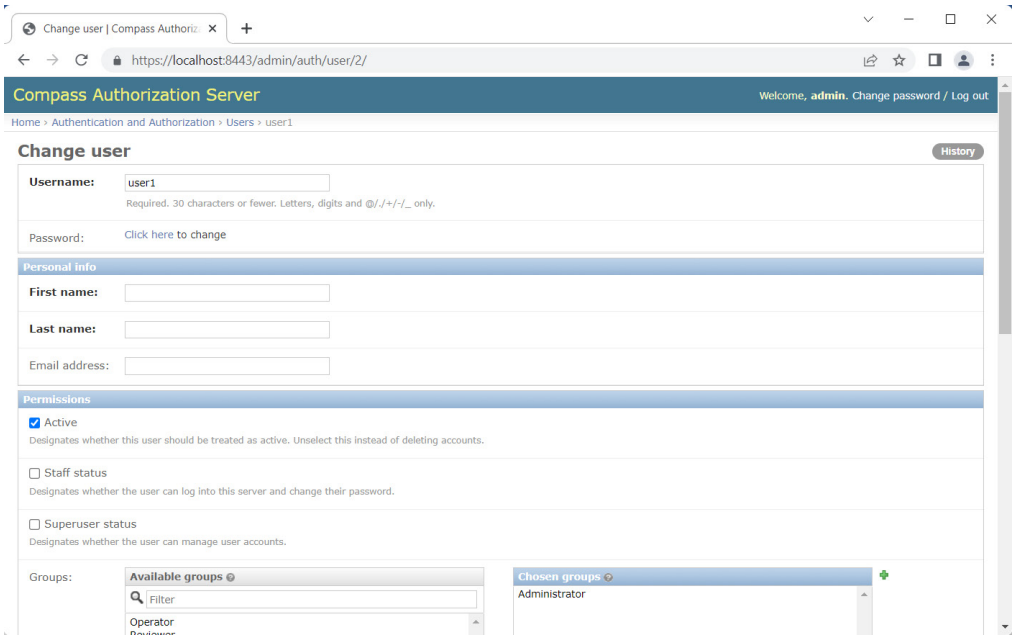

2. From the Users page, select **Add User**:




3. Fill in the fields to create a new user:



After adding a new user more information can be added:



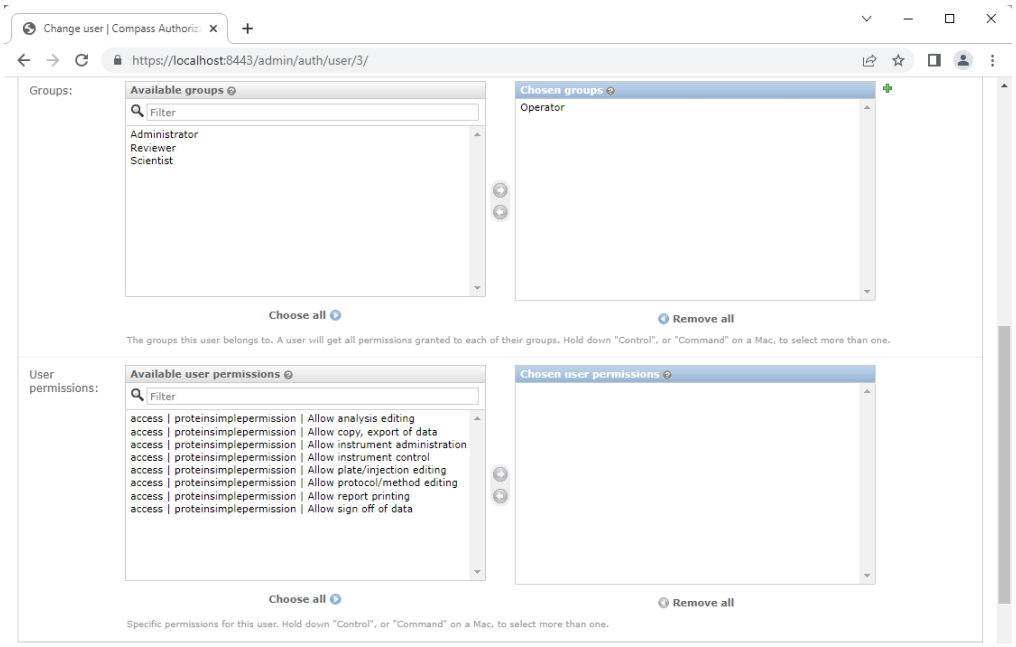

**NOTES:**
Users are blocked after the number of login failures defined in the Password policy setting. See “Password Policy Settings” on page 355 for more information.

Non-admin users will not be able to disable Access Control when the CAS connection is lost.

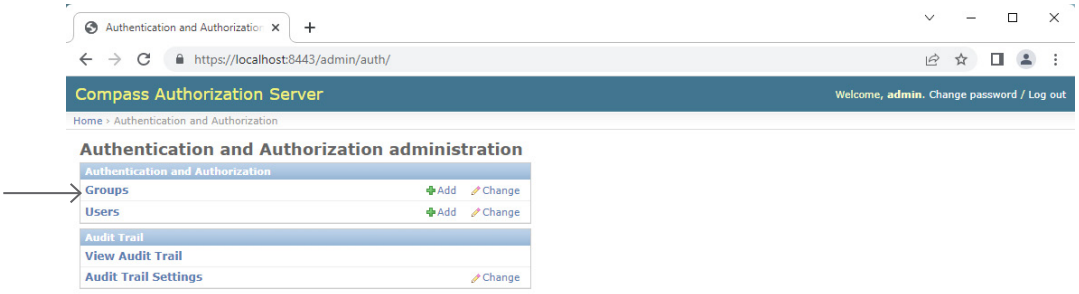
## Permissions

All users can log in to Compass for Simple Western, but the commands available within Compass for Simple Western 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 for Simple Western to use the new permissions.

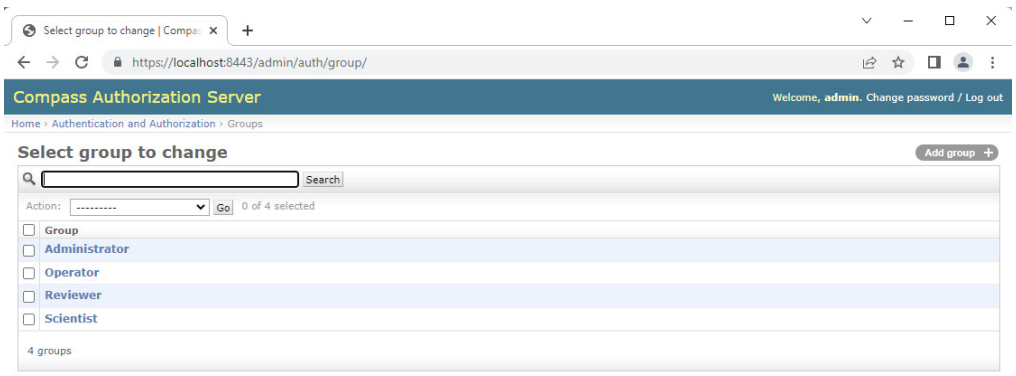
Users can belong to groups that have multiple permissions such as Operator or Scientist:



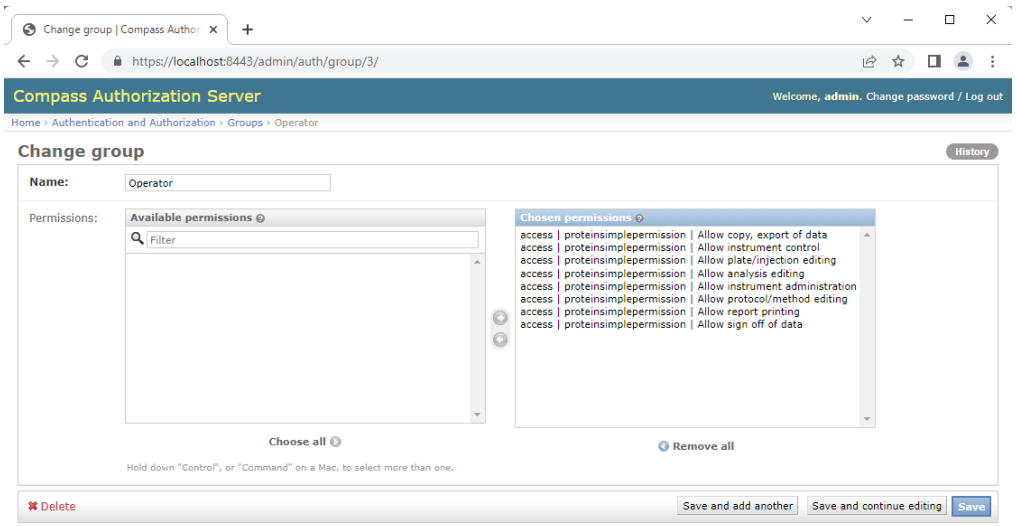

Use the Groups page to change the permissions in a group or create new groups:




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.




Here are the current user and group permissions:

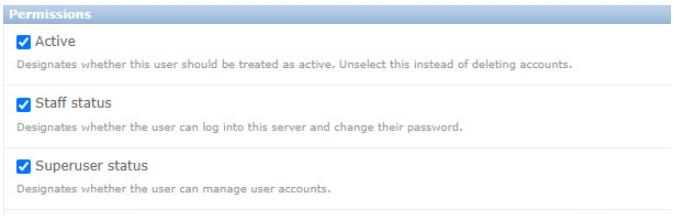
- **Allow analysis editing** - lets users change analysis settings
- **Allow copy, export of data** - lets users copy and export data
- **Allow instrument control** - gives users permission to connect to an instrument
- **Allow plate editing** - lets users update the plate layout and edit sample info
- **Allow protocol/method editing** - gives users permission to edit protocol parameters/methods. Assay setup is allowed for users with this permission
- **Allow report printing** - lets users print the run report
- **Allow sign off of data** - gives users permission to sign off on changes using e-signatures
- **Allow instrument administration** - lets users update the instrument embedded software, delete run data from instruments, update the Compass for Simple Western software and turn Access Control on or off

## Adding Admin Users

To create a user with administrator permissions:

1. Follow the steps described in “Adding Non-admin Users” on page 346 to create the admin user.

2. Under permissions, select **Staff status** and **Superuser status**:




3. Assign the admin user to a group.

**NOTES:**
Selecting Superuser status enables server permissions only. Admin users must also be assigned to a group in order to have Compass for Simple Western permissions.

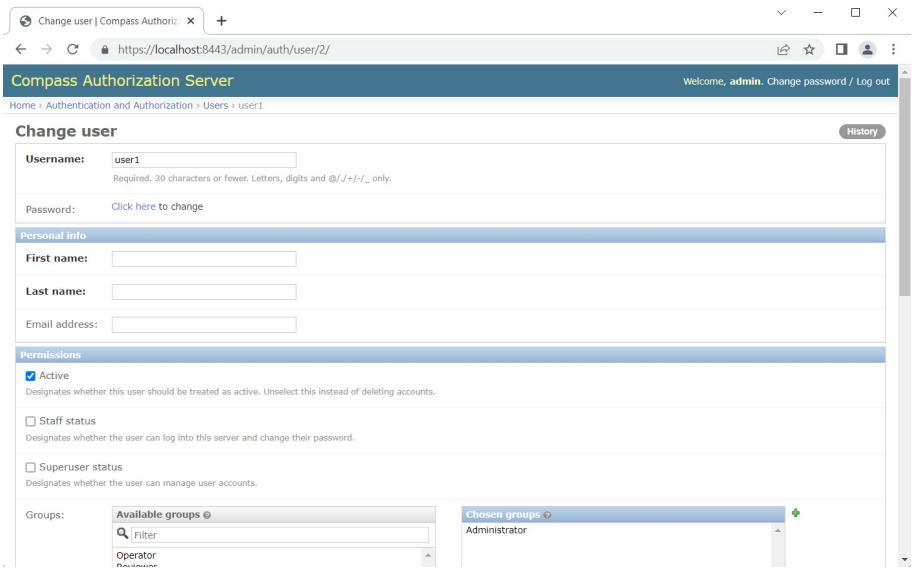
Only Admins will be able to disable Access Control when the CAS connection is lost.

## Resetting User Passwords

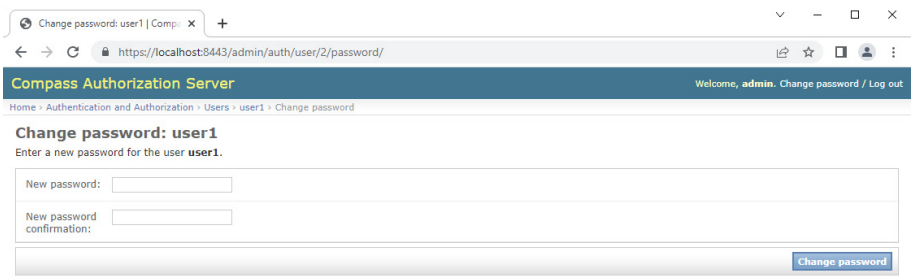
**NOTE:** Users are blocked after the number of login failures defined in the Password policy setting.

To reset a user password:

1. Select **Users** from the Site Administration home page, then select the user to change. The following screen displays:



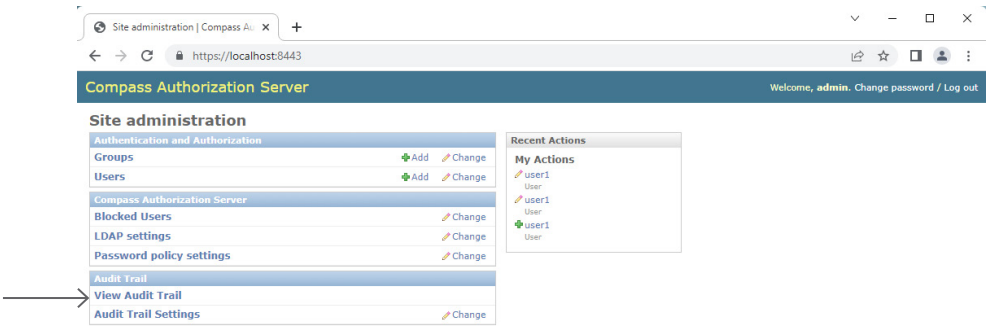
2. Click the text link to access the password change form:




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.



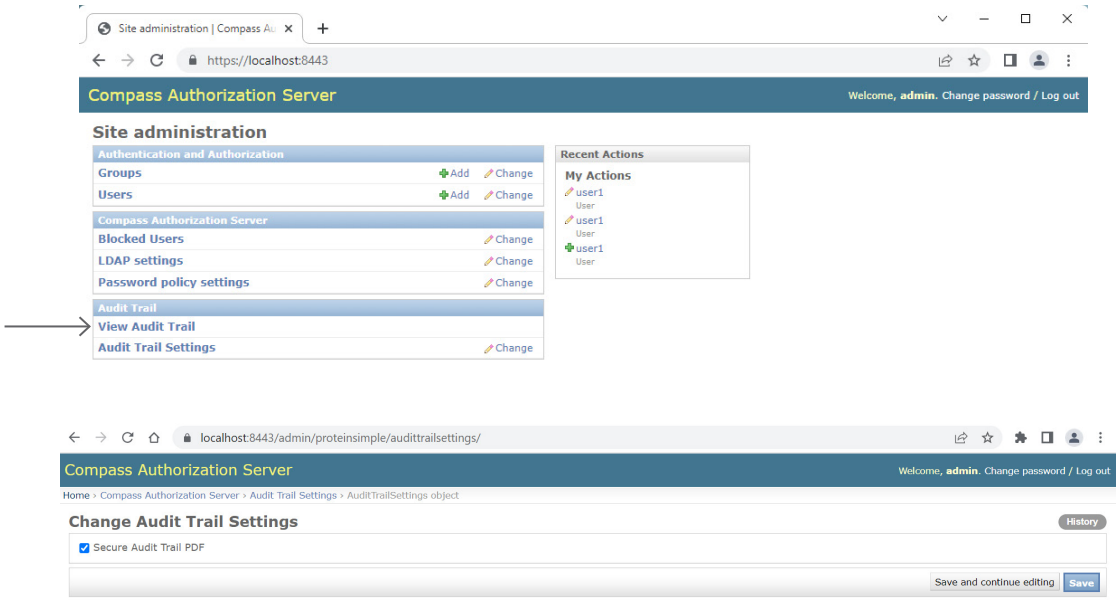

Here are the actions currently logged in the Audit Trail:

- **Login.** User logged into CAS or Compass.
- **Logout.** User logged out of CAS or Compass.
- **Start run.** User started a run in Compass. Barcodes for cartridges and all plates will be logged when the run starts.
- **Stop run.** User stopped a run in Compass.
- **Delete run.** Run file was deleted.
- **Self test.** User initiated a self-test on an instrument.
- **Open runfile.** User opened a run file (*.cbz) in Compass.
- **Save runfile.** User saved a run file (*.cbz) in Compass to the file location shown.
- **Save assayfile.** User saved an assay file (*.assay) in Compass to the file location shown.
- **Instrument upgrade.** User initiated an update of instrument software from Compass.
- **Change Control Settings.** User disabled Access Control from Compass.
- **Generate Run Report.** User generated Run Report from run in Compass.
- **Export spectra ANDI format.** User exported the raw and analyzed data traces and background for each capillary in the run in .cdf format from Compass.
- **Export spectra text format.** User exported the raw and analyzed data traces and background for each capillary in the run in .txt format from Compass.
- **Export tables.** User exported the results for all capillaries in the run in .txt format from Compass.
- **User created.** A new user was created on CAS.
- **User modified.** A user was modified on CAS.
- **User deleted.** A user was deleted on CAS.
- **Group created.** A group was created on CAS.
- **Group modified.** A group was modified on CAS.
- **Group deleted.** A group was deleted on CAS.
- **Change password.** A user's password was changed.
- **Sign off.** A user signed off on changes to the file location shown utilizing e-signatures in Compass.
- **Edit LDAP settings.** LDAP settings in CAS were modified.
- **Edit password policy.** Password policy settings were modified in CAS.
- **Install.** CAS software was installed.

- **Upgrade.** CAS software was upgraded to new version.
- **Blocked.** A user has been blocked from logging in.
- **Unblock.** A user has been unblocked, allowed to log in.
- **Stop events.** User initiated a stop to the instrument from Compass.

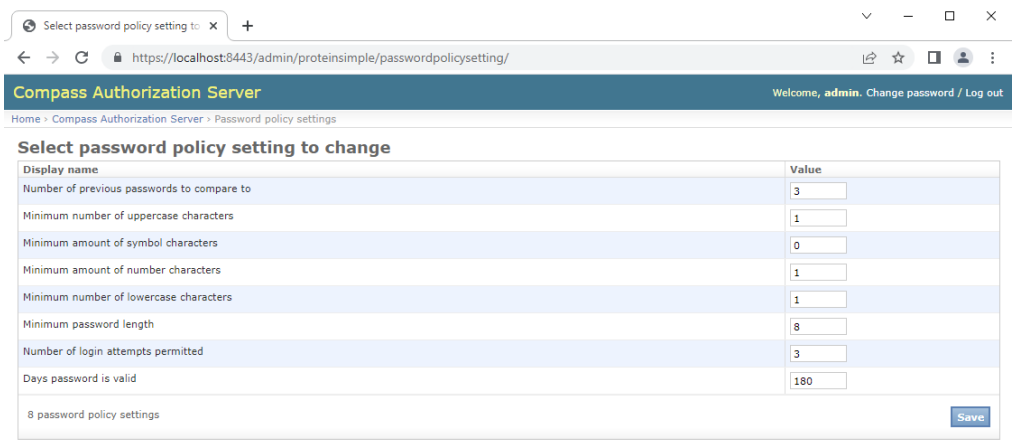
### Audit Trail Settings

The Secure Audit Trail PDF setting (selected by default), allows users to download audit trail PDFs securely. Secure audit trail PDFs can be viewed and printed, but content cannot be copied or modified.



## Password Policy Settings

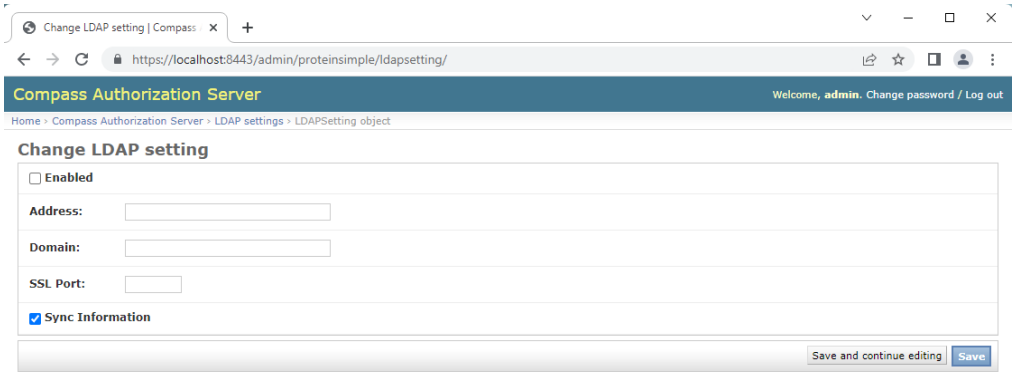
These settings let administrators set password policies. Select **Password policy settings** from the Site Administration home page to make changes.




## LDAP Setting

The LDAP setting allow you to connect the Compass Authorization Server for Simple Western 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 for Simple Western 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 346 and select the LDAP User checkbox. Passwords aren't required for LDAP users.

**NOTE:** When enabling the LDAP setting, ensure the SSL port number reflects a secure LDAP port (636 recommended). Compass Authorization Server for Simple Western doesn't support non-secure connections.

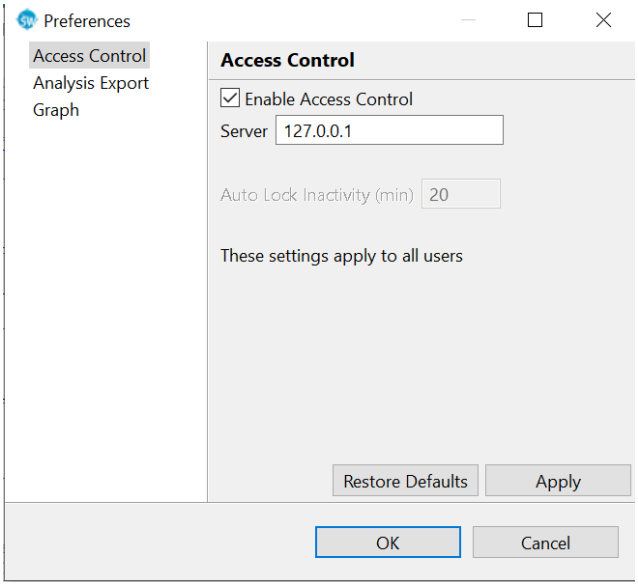
## Encryption Details

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 for Simple Western 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 for Simple Western can open any file.

# 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 Access Control** box.
2. Enter the IP address of the Authorization Server for Simple Western. Use format X.X.X.X or localhost if installing the server on the local machine.
3. Close Compass for Simple Western. The next time the software is launched, a user log in will be required.

**NOTES:**
Access Control can only be disabled by logging into Compass for Simple Western and deselecting the **Enable Access Control** box in the Access Control page of Preferences.

If your Jess, Abby, or Leo system is running a controlled assay, you cannot connect to the instrument with Compass unless Access Control is enabled. If your Jess, Abby, or Leo system is running an uncontrolled assay, you will not be able to connect to the instrument if Access Control is enabled on Compass Software.

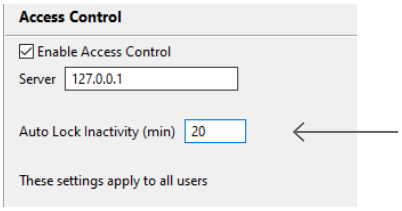
Leo systems will remain in controlled mode once they are connected to Compass Software with Access Control enabled. You will need to turn the instrument off and then back on again before the instrument will go back to being in non-controlled mode.

Jess and Abby instruments will go back to being in non-controlled mode once Access Control is disabled.

# Changing the Software Inactivity Auto Lock

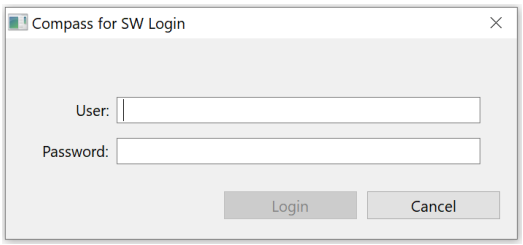
Compass for Simple Western software automatically locks to prevent user access after a period of inactivity. Once the software is locked, users must log in again. Only users with instrument administrator permission can set the auto lock inactivity time.

1. Select **Edit** > **Preferences** > **Access Control**.
2. Enter an **Auto Lock Inactivity** time in minutes. The default setting is 20 minutes.




# Logging In to Compass for Simple Western

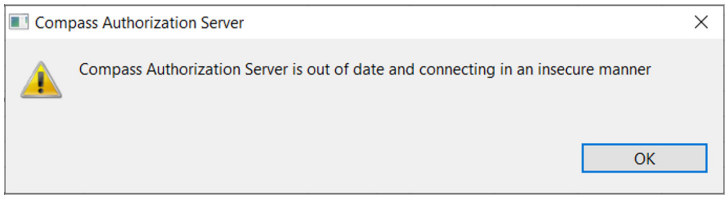
With Access Control enabled, all users must log in to Compass for Simple Western whenever the software is launched.



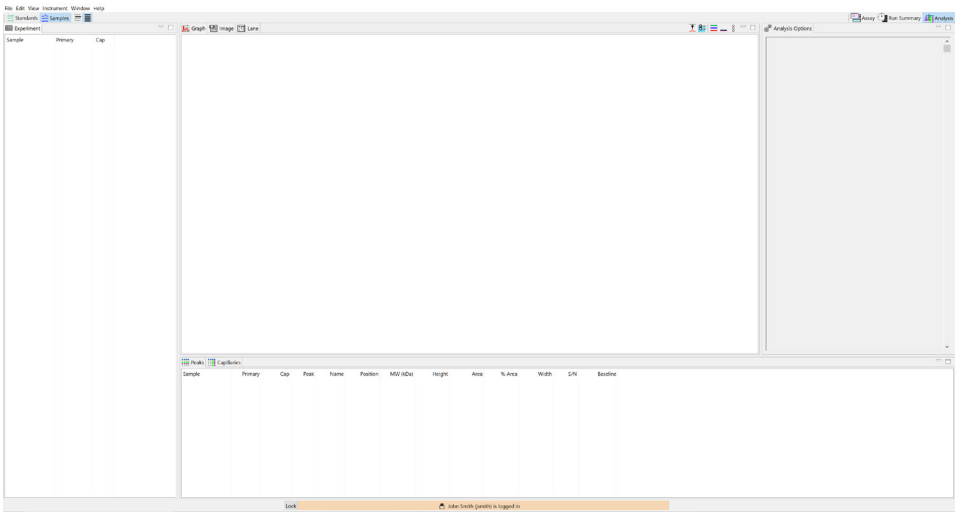
Enter your username and password previously setup by your Compass for Simple Western Administrator.

**NOTE:** Your account will be blocked after a certain number of login failures. If this happens, contact your administrator to unblock the account.

A window will appear if Compass for Simple Western is connecting to a previous version of the Compass Authorization Server that does not support a secure connection.




A successful log in will display the Compass for Simple Western main window with the user information in the lower status bar. The full username is displayed with the unique user ID in parenthesis:



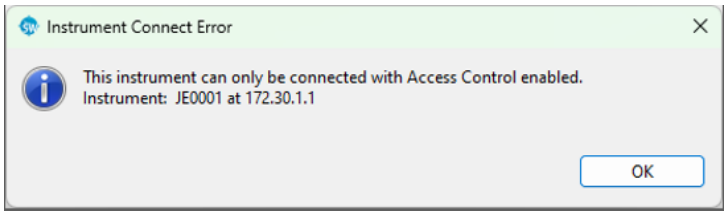
## Connecting Compass to Systems with Access Control Enabled

Access Control checks are in place that could impact your ability to connect to Compass for Simple Western software.

**Jess/Abby**

- When Compass has access control enabled, you will not be able to connect to an instrument running an assay in non-CFR mode.

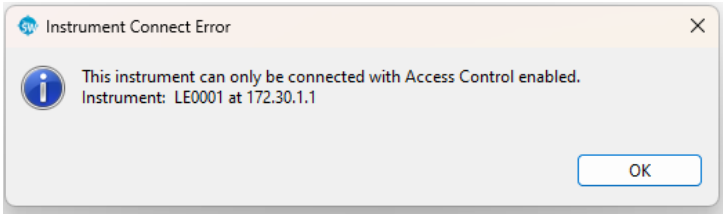

- When Compass does not have access control enabled, you will not be able to connect to an instrument running an assay in CFR mode.

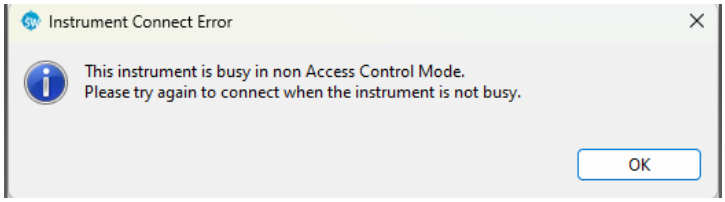



**Leo**

- When Compass has access control enabled:
  - The Leo instrument will lock into CFR mode the first time Compass for Simple Western connects to the instrument. The instrument switching into CFR will be recorded in the instrument command log.
  - Compass must have access control enabled to connect to an instrument that is in CFR mode, even if it is not running an assay.
  - The Leo instrument will reset to a non-CFR mode when the system is turned off and then back on.
- When Compass does not have access control enabled, it will not be able to connect to an instrument locked in CFR mode.

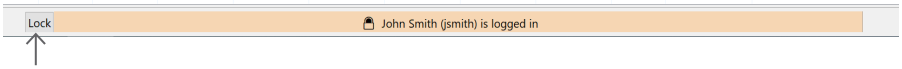
A warning will appear if it is unable to connect to the instrument. The warning will not impact any assay or test that the instrument is in the middle of performing.





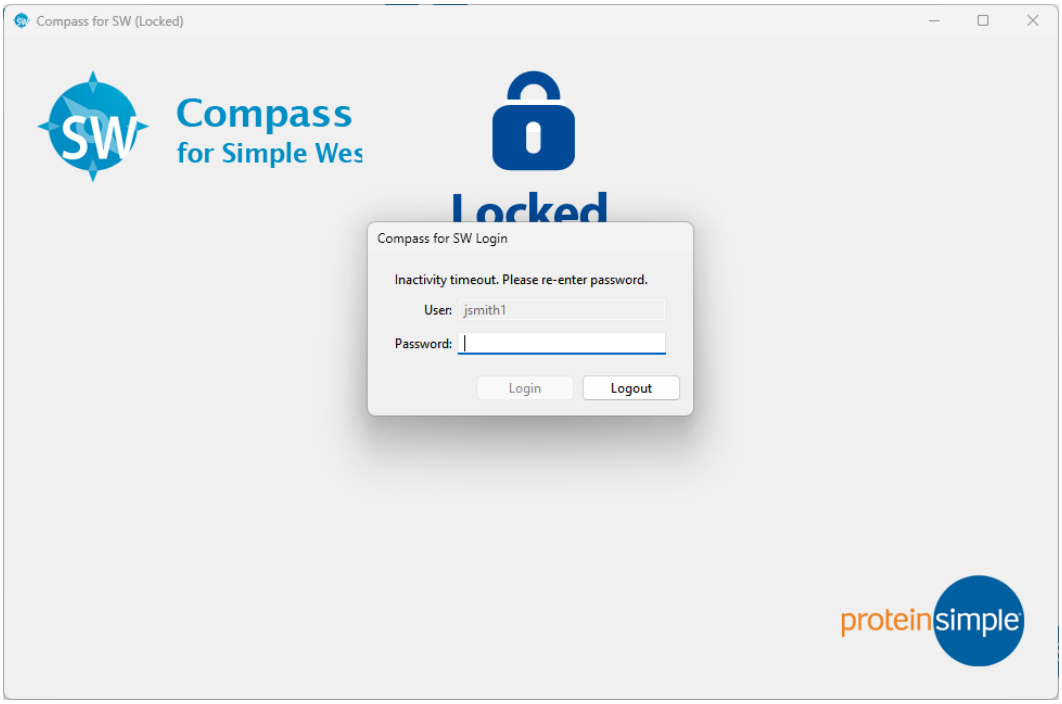

## Locking and Unlocking the Application

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.




If there is no activity in Compass for Simple Western for 20 minutes, the application automatically locks. Users must re-enter their passwords to perform any controlled actions:

**NOTE:** The default software inactivity lock-out time is 20 minutes. See “Changing the Software Inactivity Auto Lock” on page 357 for information on how to modify the lock-out timing.



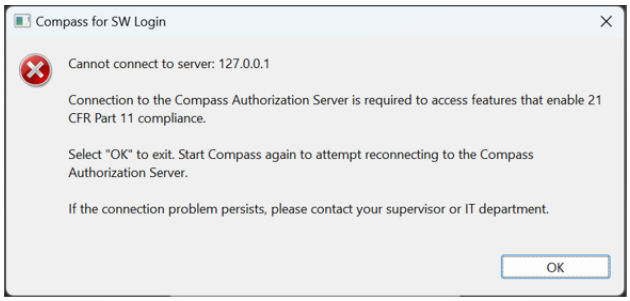
## Resolving Log In Issues

Log in failures may occur when:

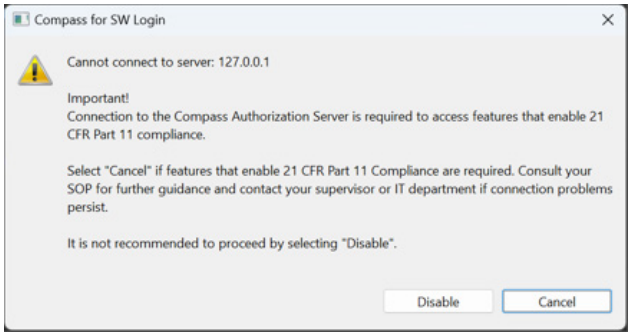
- The server is temporarily unavailable
- Compass for Simple Western is using the wrong IP address

When this happens, the following message displays:

**USER:**



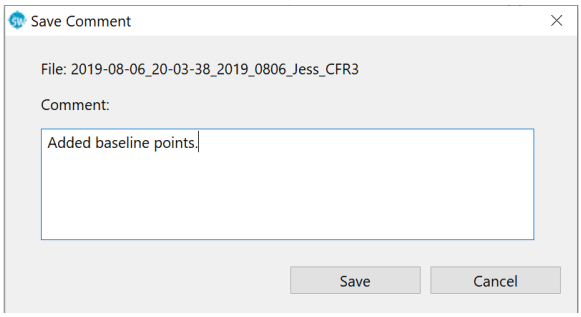

**ADMIN USER:**



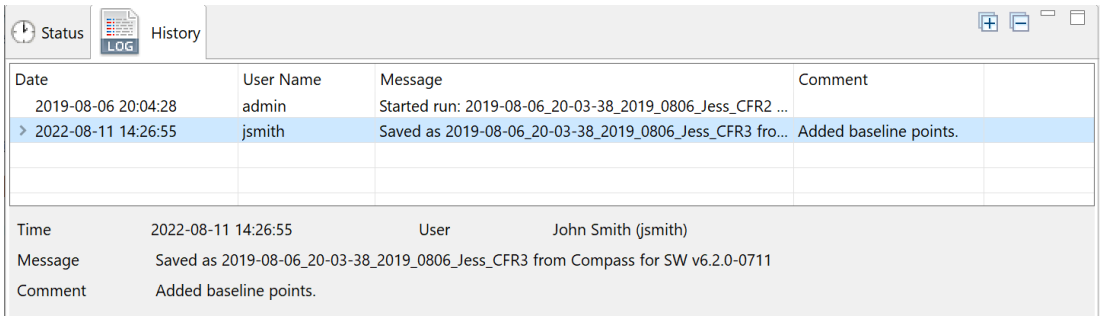

Click **Disable** to restart Compass for Simple Western with Access Control disabled. Verify or correct the server IP address then close and restart the software to log in with Access Control enabled.

# 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:

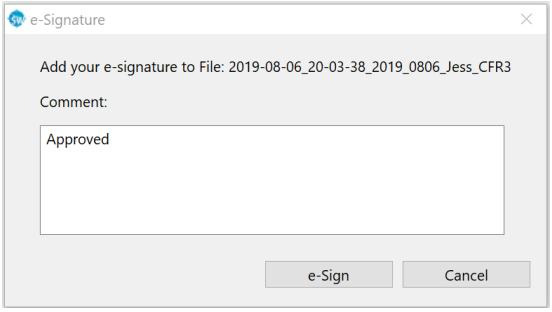



The comment is added to the signature entry in the file History:

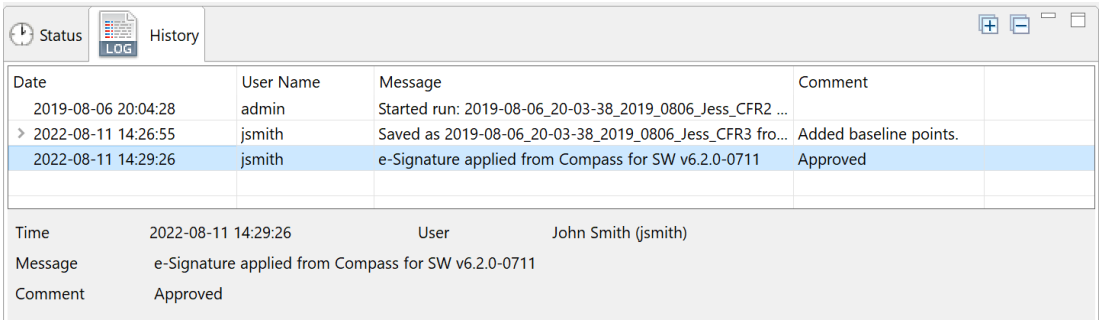



# Signing Files

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*.

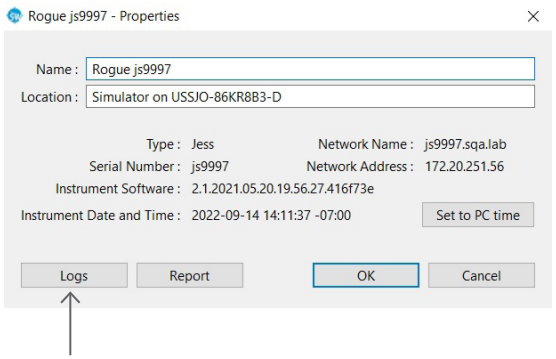


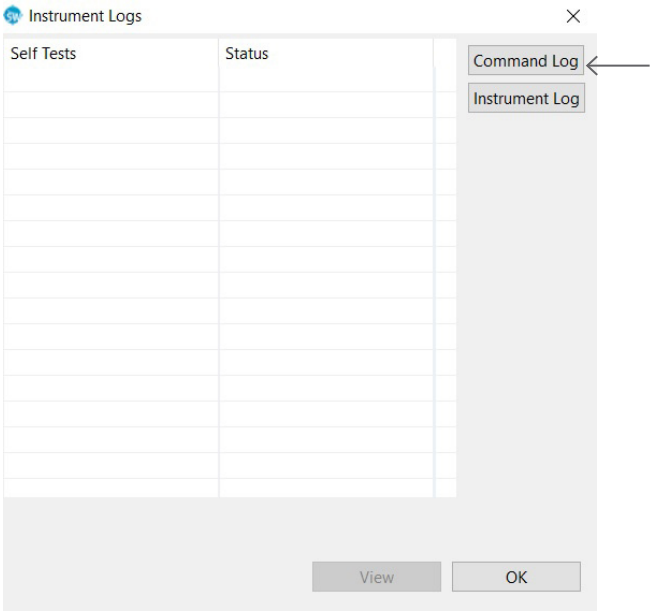

# Exporting Uncontrolled Files

You can export an uncontrolled file when in the Analysis screen by selecting **File** > **Export Uncontrolled...**. The export will also be logged in the Audit Trail and the History log for the exported file.

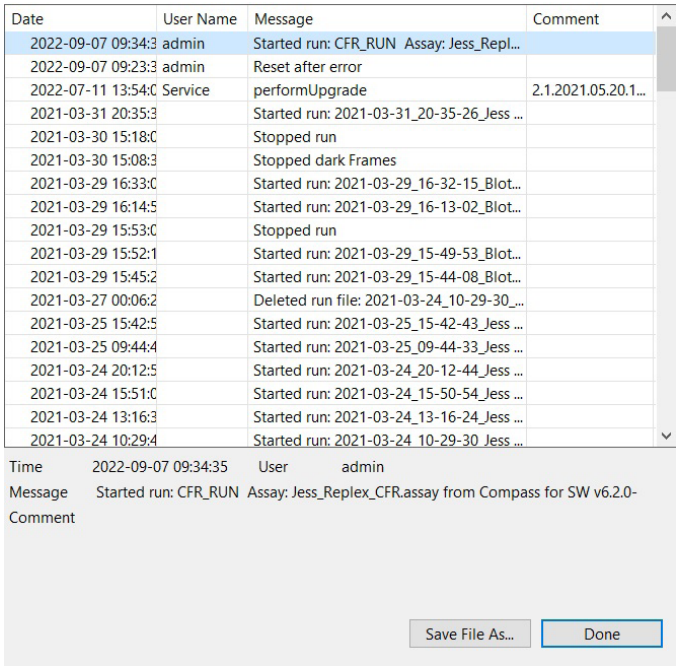
# Instrument Command Log

The Instrument Command Log can be viewed at any time by selecting the **Instrument** menu and clicking **Properties**. Click the **Logs** button and then in the Instrument Logs box click **Command Log**:



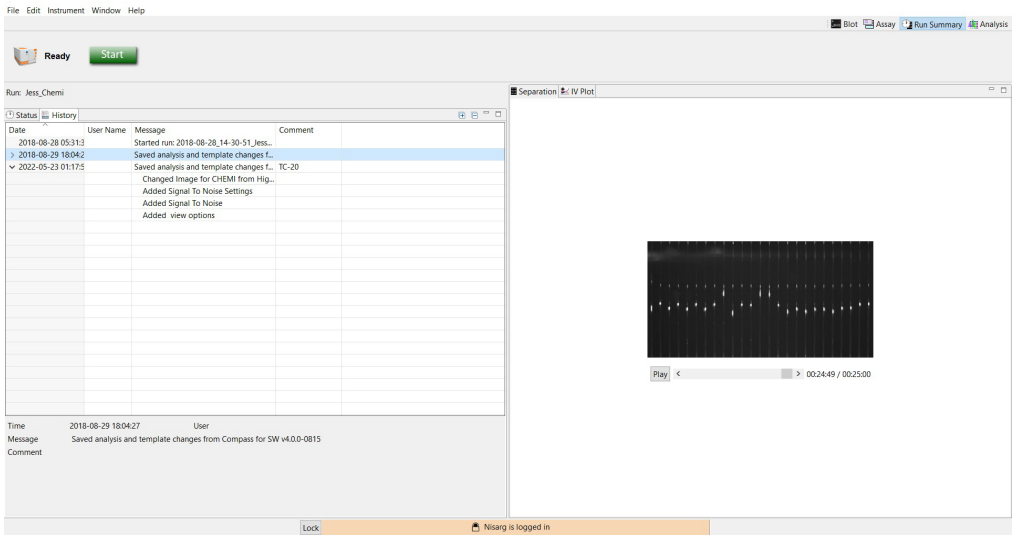



The Command Log lists all the commands sent to the instrument that were signed by the user who sent the command. If you want to copy the Command Log at any time, right-click in the table and select **Copy**, then paste into another document.




# Run File History

Select the **Run Summary** screen tab and then the **History** tab to see the file History.



# Troubleshooting Problems and Suggested Solutions

If any of the following error messages are encountered, follow the recommended steps below to resolve the issue.

- **Unknown username or password.**
  - Check if the Caps Lock is on, username and password are case sensitive.
  - Ask a Compass for Simple Western administrator to confirm your username. If your password is unknown then the administrator can reset your password (see “Resetting User Passwords” on page 351 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 for Simple Western address. Compass for Simple Western 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 Access Control**, close Compass for Simple Western, then re-launch the software 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 Access Control**, close Compass for Simple Western then re-launch the software.

**NOTES:**
Only users with Instrument Administrator permission can turn Access Control on or off. This event will also be logged in the Audit Trail.

Uncontrolled files cannot be opened when Compass 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 for Simple Western web interface at the address shown on the **Access Control** page in **Preferences**, such as: 127.0.0.1:8443/.
- **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 Access Control**, close Compass for Simple Western, then re-launch the software. You should now be prompted for a log in.

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