Compass Software for Simple Western Size Assays

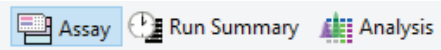
Quick Reference Guide

proteinsimple
a bio-techne brand

START YOUR ASSAY IN COMPASS

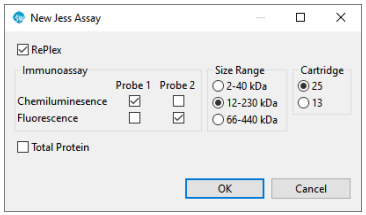
1. Load your assay

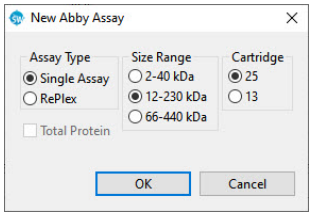
Open Compass software v6.0 or higher (Jess, Abby, or Wes) or Compass 5.0 (NanoPro 1000, Peggy Sue, Sally Sue) and click on the Assay icon.



From the File menu, click **New Assay** and select the template assay for your instrument by selecting the assay type, the size range and cartridge type (if running Jess, Abby, or Wes) or choose **Open Assay** to select from the menu of saved assays.

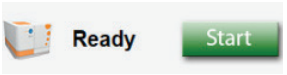
If you make changes to the assay, such as defining the wells in the plate, be sure to select **File** and **Save** before proceeding.



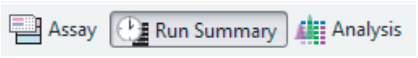


2. Run your assay

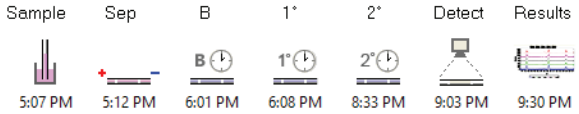
Prepare your assay following the Simple Western product insert. Once your plate and capillary cartridge are ready in the instrument, click **Start**."



To check the time remaining for your assay, click on the **Run Summary** icon…



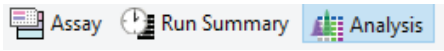
…and view the **Assay Scheduler** in the **Status** tab:



ANALYZE YOUR RESULTS

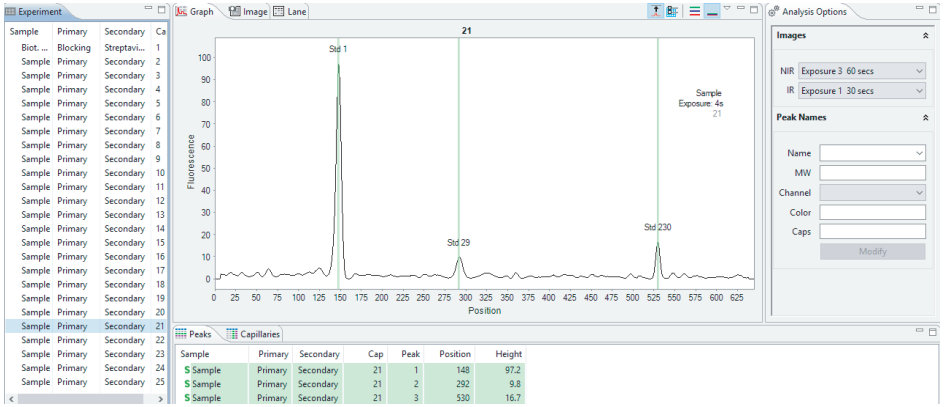
1. Verify your fluorescent standards

Click on the **Run Summary** icon. In the **Separation** tab, you can watch the movie showing separation of the fluorescent standards. Make sure that each of the markers (1, 29 and 230 kDa for the 12–230 kDa assays, 57 and 280 kDa for the 66–440 kDa assays, or 1 and 26 kDa for the 2-40 kDa) are visibly separated in each capillary. Click on the **Analysis** icon:



Then select the **Show Standards** and **View Selected** icons:

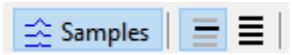
You can check if your standards are correctly identified in the **Graph View** tab. If they are incorrect, right-click on the correct peak and select **Force Standard**. Do this for each capillary.



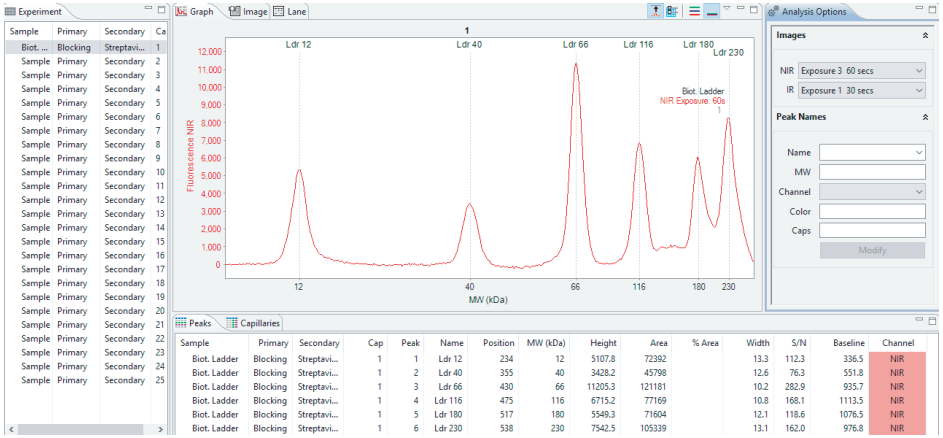
Graph View of fluorescent standards in the 12–230 kDa size assay. Standard peaks are labeled **Std 1**, **Std 29** and **Std 230** in the electropherogram.

2. Verify your biotinylated ladder

Click on the **Show Samples** and **View Selected** icons:



Then select the capillary containing the MW ladder (typically capillary 1) in the **Experiment** tab. Your biotinylated ladder should show the following sizing peaks: 12, 40, 66, 116, 180 and 230 kDa; or 66, 116, 200, 280 and 440 kDa; or 2, 5, 12, 26, and 40 kDa. Make sure these are correctly identified in the **Graph View** tab. If a peak is incorrectly identified, right click on it and select **Remove Peak**. If a ladder peak is not visible, select the appropriate detection channel or click **View**, then **View Region** and select **Full**. For fluorescent assays on Jess, the detection channel for the ladder is the NIR channel.



Graph View of the 12–230 kDa biotinylated ladder. Ladder peaks will be labeled **Ldr 12**, **Ldr 40**, **Ldr 66**, **Ldr 116**, **Ldr 180** and **Ldr 230** in the electropherogram.

3. Label your sample peaks

To toggle between or overlay multiple detection channels on Jess/Abby runs, click the Channel icon for each detection channel: Chemi (Jess/Abby) or NIR, IR, Protein Normalization (Jess only).

Peaks in **Graph** view or bands in **Lane** view are labeled automatically with the calculated protein size. Fully analyzed results including molecular weight, peak area, peak height and signal to noise (S/N) are shown in the **Peaks** table.

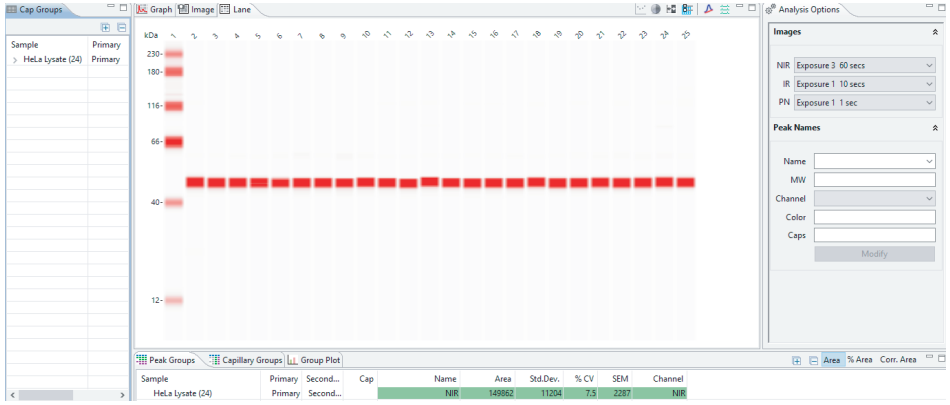
You can manually label specific sample peaks. In the Analysis Options panel, select the **Name** dropdown menu and select **[New]**. Fill in the **Name** and **MW** fields with desired name and molecular weight targets. Click on the **Channel** dropdown menu and select the desired detection channel. Fill in the desired color and applicable caps in the **Color** and **Caps** fields. Any peaks found within the default range setting of ± 10% of the entered molecular weight will automatically be labeled.

The bands and peaks in the sample are now labeled with your peak names.

4. Analyze your final results

You can group your results and view the associated statistics by selecting **View** and clicking on **Grouping**.

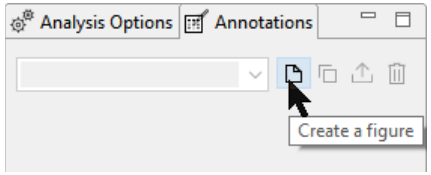
Grouped data, including peak area (Area), standard deviation (Std.Dev), % CV and standard error (SEM), can be viewed under the **Peak Groups** (data shown), **Capillary Groups** and **Group Plot** tabs. You can also copy and paste the data from the table into Excel or other graphing programs.



Lane View of grouped data.

5. Create an annotated Lane View figure (optional), Compass 6.0 and higher.

The **Annotations** pane lets you create annotated figures of the Compass Lane view for use in presentations or publications. Select the lanes you want to create a figure from. For multi-channel runs, select the channel(s) to include too. 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. Drag the box until the desired range is displayed in the table.

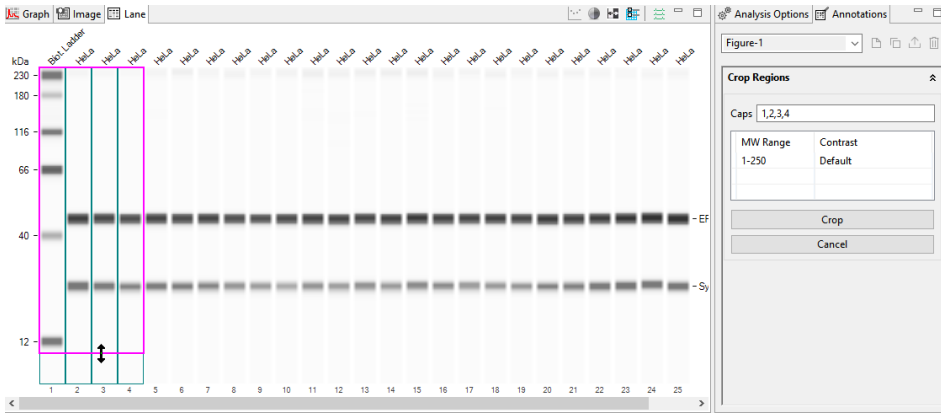
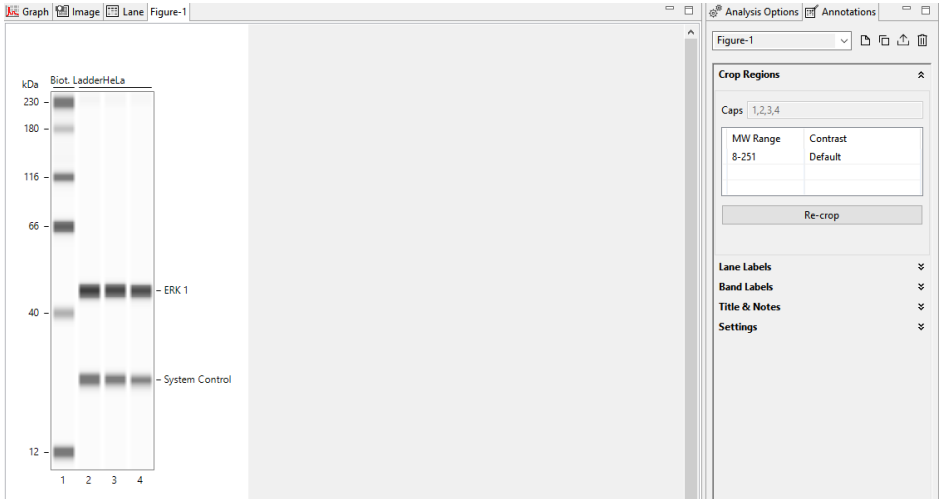
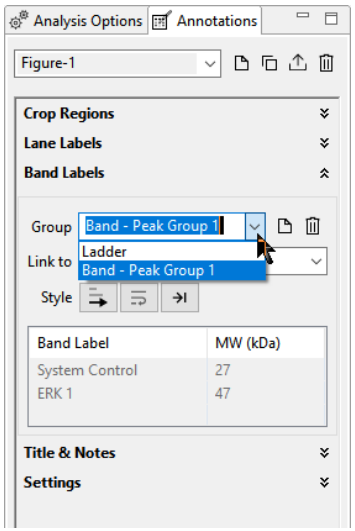


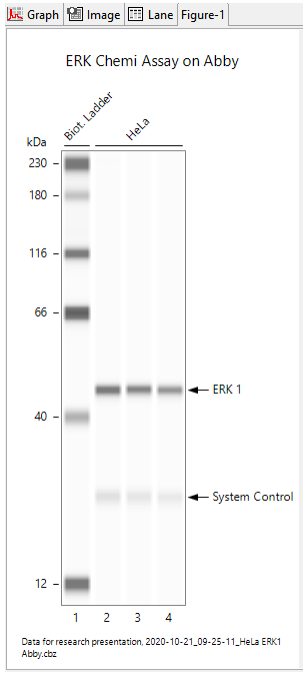
Figure preview for a standard immunoassay.

Click **Crop** to create the figure file. The figure displays in a new tab.



Add lane and band labels, a title, notes and adjust font settings as needed. For more details, see the Compass for Simple Western user guide.



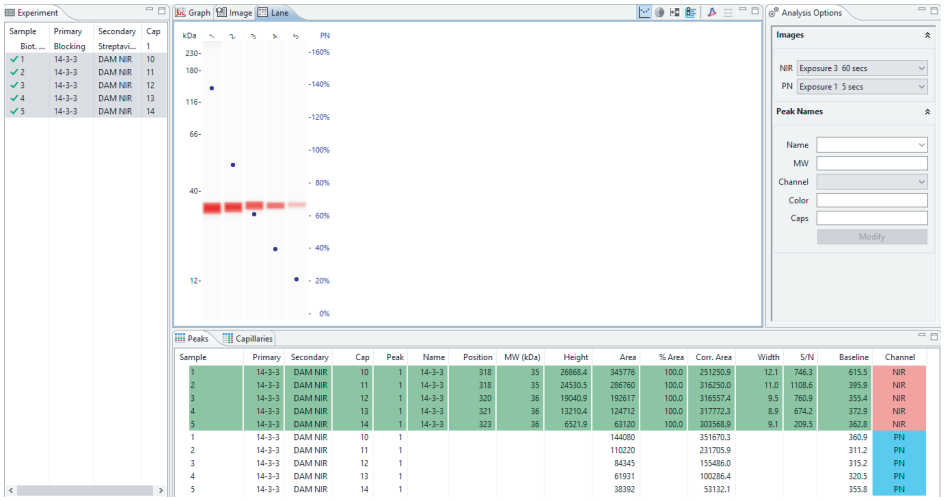


When you're done, right click the figure and select **Export**. Browse to the directory you want to save the file to and type a file name. Click the down arrow in **Save as Type** to choose a file format: .png, .jpg or a 300 dpi .tiff (recommended for submitting data to journals for publication).

6. Total Protein Normalization (if applicable, Jess and Abby only)

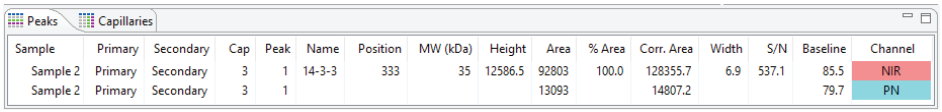
To view Normalization data, ensure the channel overlay for either Protein Normalization (Jess only) or Total Protein in Probe 2 (Jess and Abby) is on, and toggle the **Protein Overlay** icon in the Lane View.

Total Protein Normalization data will be shown as peak area counts, or peak area count percentages relative to a selected reference capillary (Normalization must be enabled to display peak count percentage values) on the secondary y-axis in lane view.



By default, Normalization data transformations are enabled for Jess runs that include Protein Normalization or RePlex runs with Total Protein in Probe 2 (Jess and Abby). To enable/disable Normalization, select the **Edit** menu and click on **Analysis**. On the Analysis screen, select **Normalization** and click on **Enable**. Compass will use capillary 2 as the reference capillary by default. To choose a different reference capillary, on the Analysis screen under Normalization, use the dropdown menu to choose a **Reference Capillary**.

When Normalization is enabled, Compass will automatically display 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 the Protein Normalization channel overlay is on (Jess only), the total peak area used for normalization is listed in the Corr. Area column. 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.



If you need assistance, please contact ProteinSimple Technical Support toll-free in the US and Canada at (888) 607-9692 (option 3), support@proteinsimple.com or visit http://www.proteinsimple.com/resources.html?product=simple-western. You can also contact your local Field Application Scientist for help.