Leo Quick Reference Guide

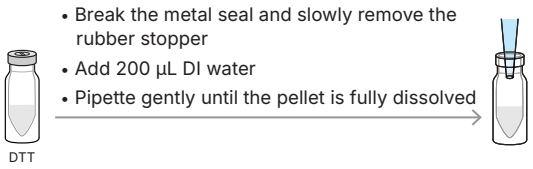
Introduction

This Quick Reference Guide walks you through how to set up a run on a Leo™ System and perform basic data analysis with Compass™ Software for Simple Western™ Platforms. Refer to the Leo Instrument User Guide and the Compass for Simple Western User Guide for more information.

1 // Prepare Your Reagents and Samples

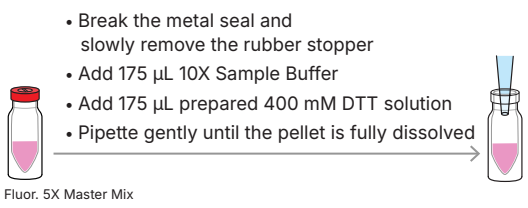
A. Prepare Your Standard Kit Reagents

DTT (Glass Vial, White Pellet)* – makes a 400 mM solution

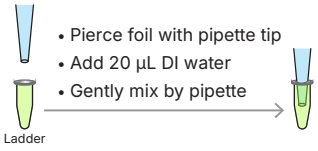


Fluorescent 5X Master Mix (Glass Vial, Pink Pellet):

- The size and morphology of the pink pellet will be different from vial to vial. These visual differences are normal and will not affect the product's performance or effectiveness.
- Store the reconstituted Fluorescent 5X Master Mix on ice until you are ready to prepare your sample.
- The remaining reconstituted Fluorescent 5X Master Mix can be aliquoted in microcentrifuge tubes and stored at -20 °C to -80 °C for up to 8 weeks. Each aliquot can undergo up to three freeze-thaw cycles.



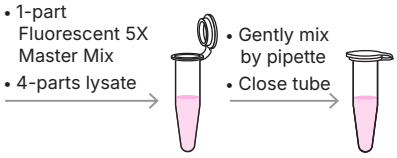
Biotinylated Ladder (Green Tube in sealed pack)



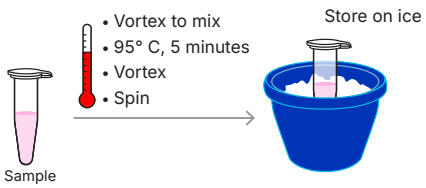
B. Prepare Your Samples

Prepare enough sample to pipette 3 µL/well

- The optimal protein concentration depends on the expression level of your target protein and the assay detection method used. Dilute lysates as needed with 0.1X Sample Buffer.



C. Denature Your Samples



*Contact your Field Application Scientist for guidance on running non-reduced samples.

1 // Prepare Your Reagents and Samples (continued)

D. Prepare Your Primary Antibodies

Prepare enough antibody to pipette:
10 µL/well for rows that will be used to load 1–2 cartridges
20 µL/well for rows that will be used to load 3–4 cartridges

- Dilute your primary antibody in Antibody Diluent 2.
  - If you're using a goat primary antibody, dilute with Milk-Free Antibody Diluent.
- If multiplexing, mix the primary antibodies into one solution.

Store the antibodies on ice until you are ready to pipette them into the Sample Plate.

E. Prepare Your Secondary Antibodies

Chemiluminescence

- The chemiluminescence Secondary-HRP Conjugate is provided ready-to-use (RTU) in the Detection Module.

If you are multiplexing consult ProteinSimple Technical Support or your Field Application Scientist for best practices when preparing the secondary antibody mix.

Fluorescence

- Secondary Dye Conjugates are provided as a 20X solution.
  - Add 15 µL of the Secondary Dye Conjugate to 285 µL of Milk-Free Antibody Diluent.
  - If multiplexing two Secondary Dye Conjugates, dilute 15 µL of each Secondary Dye Conjugation (totaling 30 µL) with 270 µL of Milk-Free Antibody Diluent into one solution.

Store the antibodies on ice until you are ready to pipette them into the Sample Plate.

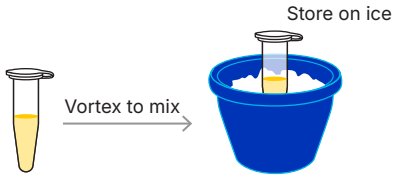
If you're using your own secondary antibody or multiplexing with chemiluminescence and fluorescence secondary antibodies, consult your Field Application Scientist or visit the Antibody Database at **bio-techne.com/sw-ab-database**.

*If multiple tubes are prepared, combine the reagent into one microcentrifuge and mix by pipette to avoid bubbles.

F. Prepare Luminol-S and Peroxide Mix (only if performing a Chemiluminescence Assay)

Combine in a microcentrifuge tube or conical tube:

- **Chemiluminescence Immunoassay**
  1–2 cartridges**:** 700 µL Luminol-S + 700 µL Peroxide
  3–4 cartridges: 950 µL Luminol-S + 950 µL Peroxide
- **RePlex™ Assay**
  1–2 cartridges: 950 µL Luminol-S + 950 µL Peroxide
  3–4 cartridges: 1300 µL Luminol-S + 1300 µL Peroxide



G. Prepare RePlex Reagent Mix (only if running a RePlex Assay)

Combine in a microcentrifuge tube or conical tube:

- 1–2 cartridges: 960 µL RePlex Reagent 1 + 240 µL RePlex Reagent 2
- 3–4 cartridges: 1760 µL RePlex Reagent 1 + 440 µL RePlex Reagent 2

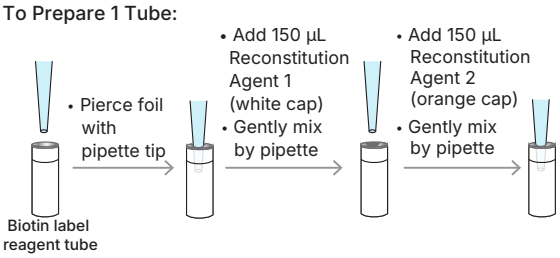
Vortex briefly to mix.

H. Prepare Biotin Labeling Reagent (only if running Total Protein Normalization using RePlex)

- Completely thaw the Reconstitution Agent 1 (white cap, recommend at least 30 minutes) before preparing the Biotin Labeling Reagent. Do NOT place the tube on ice.
- Prepare the Biotin Labeling Reagent immediately before pipetting it on the Sample Plate. Load the Biotin Labeling Reagent immediately prior to spinning the plate. Do NOT place the tube on ice at any time.

Prepare:

- 1 tube if running 1–2 cartridges
- 2 tubes* if running 3–4 cartridges



1 // Prepare Your Reagents and Samples (continued)

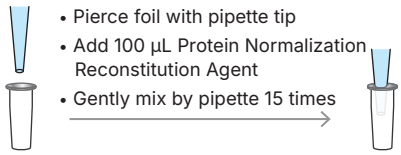
I. Prepare Protein Normalization Reagent (only if running a Total Protein Normalization Assay using the PN Channel)

- Prepare the Protein Normalization Reagent immediately before pipetting it on the Sample Plate. Load the Protein Normalization Reagent immediately prior to spinning the plate. Do NOT place the tube on ice at any time.

Prepare stock solution:

- 2 tubes if using the 12–230 kDa or 2–40 kDa Separation Module
- 3 tubes if using the 66–440 kDa Separation Module*

To Prepare 1 Tube:



Preparing working solution:

- Use the following table to prepare working solutions of the reconstituted reagents in a separate microcentrifuge tube based on the number of cartridges used and the Separation Module used.

Separation Module Molecular Weight Range	Stock Solution	Reconstitution Agent
2–40 kDa	200 µL	1000 µL
12–230 kDa	200 µL	1000 µL
66–440 kDa*	300 µL	600 µL

- Thoroughly mix the working solution by pipetting 15 times.

2 // Centrifuge the Pre-Filled Reagent Plate(s)

You'll need one Simple Western Leo Pre-filled Reagent Plate for every cartridge used in the run.

1. Centrifuge the plate(s) for 5 minutes at 1000 x *g* (~2500 rpm) at room temperature. Perform a visual check to ensure the liquid is fully down in all wells.
2. Store the plate on a flat surface with the plate seals still attached until you are ready to start your run.

*Contact your Field Application Scientist to discuss assay setup and optimization.

3 // Pipette Your Sample Plate

For more consistent results, keep the plate cover on the Simple Western Leo Sample Plate between reagent additions.

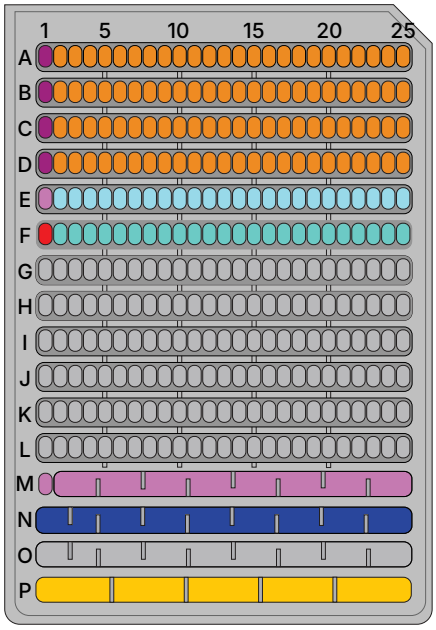
1. Dispense reagents into Sample Plate using the volumes shown in the tables below. When dispensing into the troughs, move the pipette tip across them to ensure an even distribution and avoid creating bubbles.

   NOTE: After reagents have been dispensed into the troughs, keep the Sample Plate flat during transfer to avoid spills and prevent shifting of reagents.

2. Cover the plate with the plate cover and centrifuge the plate(s) for 5 minutes at 1000 x *g* (~2500 rpm) at room temperature. Perform a visual check to ensure the liquid is fully down in all wells.
3. Pop large bubbles with a pipette tip.
4. Store the plate on a flat surface with the cover on at room temperature until you are ready to start your run.

Some example plate layouts with four rows of sample are shown below.

Chemiluminescence Immunoassay

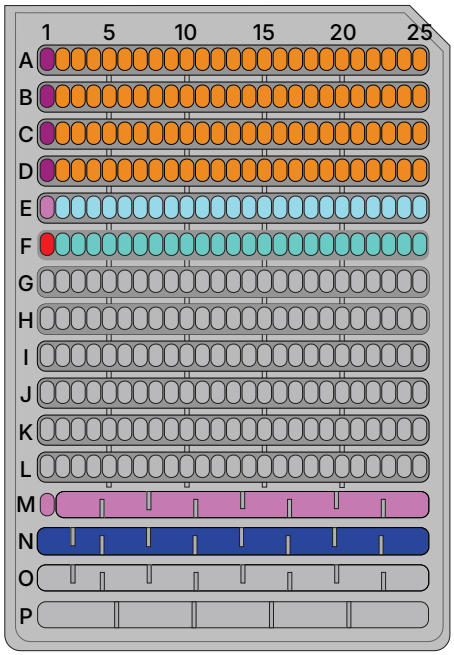


*Sampled = number of times a row is used to load cartridges.

Well Reagent (Row A–L)	Sampled* 1–2 times (µL/well)	Sampled* 3–4 times (µL/well)
Biotinylated Ladder	3	3
Sample	3	3
Antibody Diluent	10	20
Primary Antibody	10	20
Streptavidin-HRP	10	20
Secondary Antibody	10	20
Well Reagent (M1)	**1–2 Cartridges (µL)**	**3–4 Cartridges (µL)**
Antibody Diluent	30	30
Trough Reagent (Row M–P)	**1–2 Cartridges (µL)**	**3–4 Cartridges (µL)**
Antibody Diluent	1500	1500
Wash Buffer	2000	2000
Luminol-S/Peroxide (per compartment)	250	350

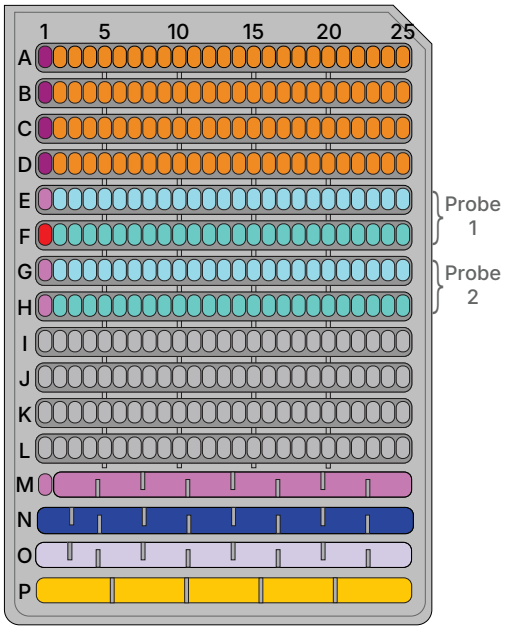
3 // Pipette Your Sample Plate (continued)

Fluorescence Immunoassay



Well Reagent (Row A–L)	Sampled* 1–2 times (µL/well)	Sampled* 3–4 times (µL/well)
Biotinylated Ladder	3	3
Sample	3	3
Antibody Diluent	10	20
Primary Antibody	10	20
Streptavidin-NIR	10	20
Secondary Antibody	10	20
Well Reagent (M1)	**1–2 Cartridges (µL)**	**3–4 Cartridges (µL)**
Antibody Diluent	30	30
Trough Reagent (Row M–P)	**1–2 Cartridges (µL)**	**3–4 Cartridges (µL)**
Antibody Diluent	1500	1500
Wash Buffer	2000	2000

Chemiluminescence and/or Fluorescence Immunoassay + Chemiluminescence and/or Fluorescence Immunoassay using RePlex

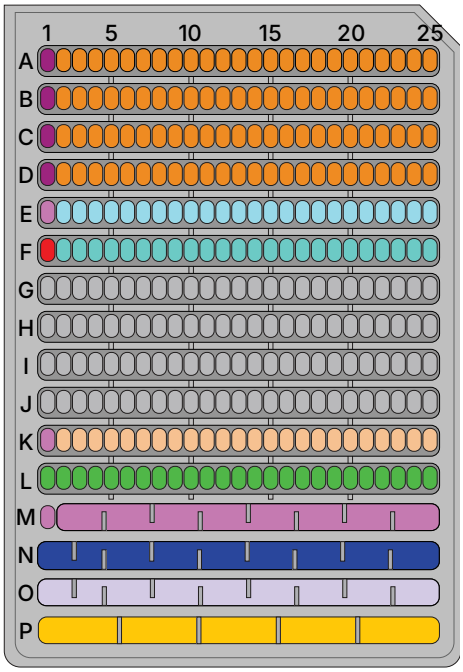


*Sampled = number of times a row is used to load cartridges.

Well Reagent (Row A–L)	Sampled* 1–2 times (µL/well)	Sampled* 3–4 times (µL/well)
Biotinylated Ladder	3	3
Sample	3	3
Antibody Diluent	10	20
Primary Antibody	10	20
Streptavidin-HRP or NIR	10	20
Secondary Antibody	10	20
Well Reagent (M1)	**1–2 Cartridges (µL)**	**3–4 Cartridges (µL)**
Antibody Diluent	30	30
Trough Reagent (Row M–P)	**1–2 Cartridges (µL)**	**3–4 Cartridges (µL)**
Antibody Diluent	1500	1500
Wash Buffer	2500	2500
RePlex Reagent	1000	2000
Luminol-S/Peroxide (per compartment, for chemiluminescence only)	350	500

3 // Pipette Your Sample Plate (continued)

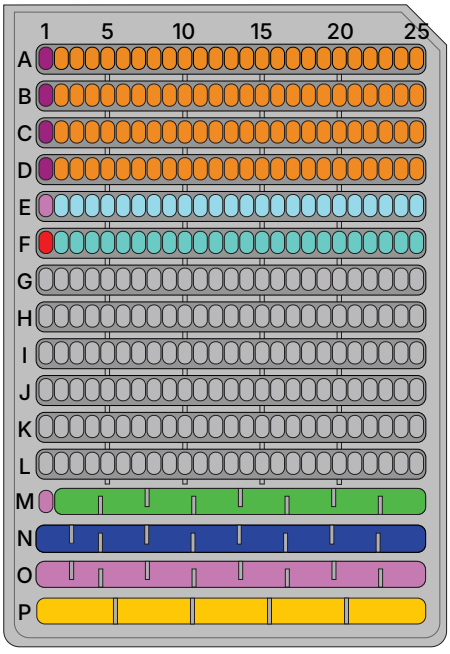
Chemiluminescence and/or Fluorescence Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection



Well Reagent (Row A–L)	Sampled* 1–2 times (µL/well)	Sampled* 3–4 times (µL/well)
Biotinylated Ladder	3	3
Sample	3	3
Antibody Diluent	10	20
Primary Antibody	10	20
Streptavidin-HRP or NIR	10	20
Secondary Antibody	10	20
Biotin Labeling Reagent	10	20
Total Protein Streptavidin-HRP	10	20
Well Reagent (M1)	**1–2 Cartridges (µL)**	**3–4 Cartridges (µL)**
Antibody Diluent	30	30
Trough Reagent (Row M–P)	**1–2 Cartridges (µL)**	**3–4 Cartridges (µL)**
Antibody Diluent	1500	1500
Wash Buffer	2500	2500
RePlex Reagent	1000	2000
Luminol-S/Peroxide (per compartment)	350	500

Chemiluminescence and/or Fluorescence Immunoassay + Total Protein Normalization using the PN Channel

NOTE: Protein Normalization Assays use row O for the Antibody Diluent by default, unlike other Leo assays.



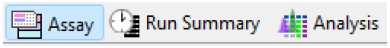
*Sampled = number of times a row is used to load cartridges.

Well Reagent (Row A–L)	Sampled* 1–2 times (µL/well)	Sampled* 3–4 times (µL/well)
Biotinylated Ladder	3	3
Sample	3	3
Antibody Diluent	10	20
Primary Antibody	10	20
Streptavidin-HRP	10	20
Secondary Antibody	10	20
Well Reagent (M1)	**1–2 Cartridges (µL)**	**3–4 Cartridges (µL)**
Antibody Diluent	30	30
Trough Reagent (Row M–P)	**1–2 Cartridges (µL)**	**3–4 Cartridges (µL)**
Protein Normalization Reagent	900	900
Wash Buffer	1500	1500
Antibody Diluent	2000	2000
Luminol-S/Peroxide (per compartment, for chemiluminescence only)	250	350

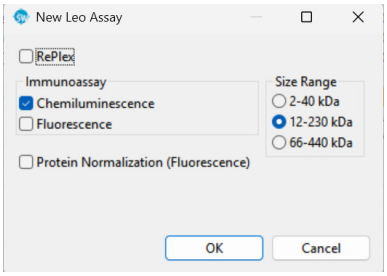
4 // Set Up Your Assay in Compass for SW

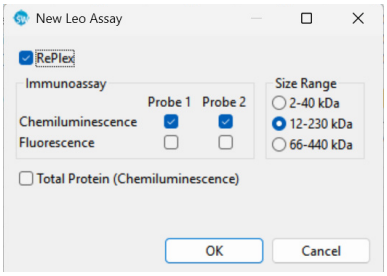
1. Open Compass for SW Software v7.1 or higher.
2. Confirm the software is connected to the Leo System. You'll see a Leo Instrument icon next to a green start button.

3. Click on the **Assay** icon.

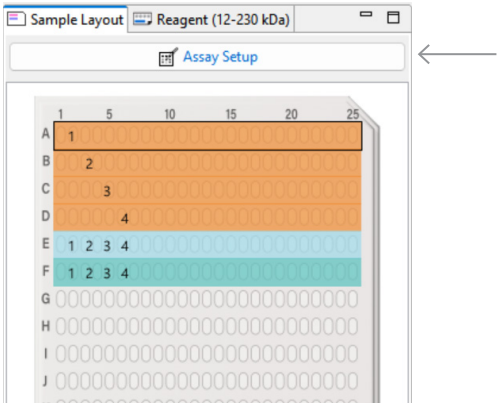


4. From the File menu, click **New Assay** and select the assay type and size range for your run, or choose **Open Assay** to select from a menu of saved assays.

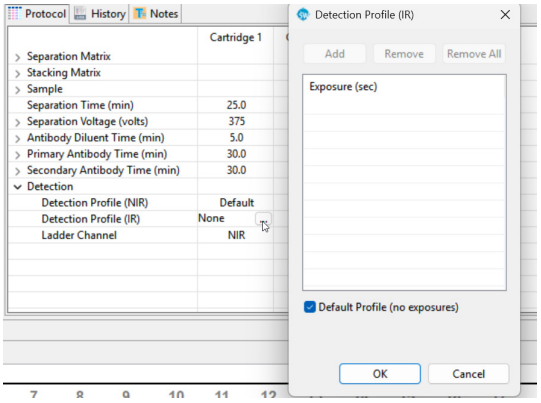




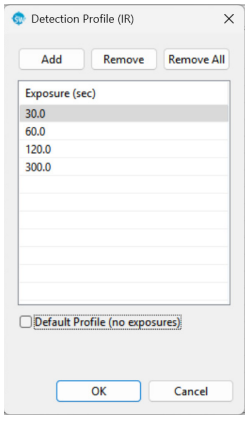
5. Click on the **Assay Setup** button under the Sample Layout or Reagent tab to define assay parameters and assign reagent locations. Click **File** and **Save** after making a change.



6. The default detection profile for a Fluorescence Immunoassay is in the NIR channel. To add a detection profile to the IR channel:
   a. Click the gray arrow next to Detection in the Protocol pane to expand the row.
   b. Open the Detection Profile window for the IR channel.
      - **For a Fluorescence Immunoassay:** Select the **None** cell in the 'Cartridge 1' column next to Detection Profile (IR) and click the **...** button that appears to open the Detection Profile window.
      - **For a Fluorescence Immunoassay using RePlex:** Select the **None** cell in the 'P1 Cart 1' (for Probe 1) or 'P2 Cart 1 (for Probe 2) column next to Detection Profile (IR). Click the **...** button that appears to open the Detection Profile window.



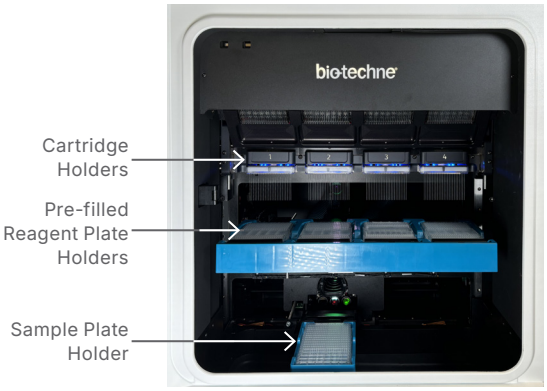
   c. Deselect the **Default Profile (no exposures)** checkbox. Exposure times will appear. Click **OK**.



7. Select **File** and click **Save Assay**.

5 // Start a Run on Your Leo System

1. Open the Leo System's door to access the inside the instrument.



2. Remove the evaporation seals from the Pre-filled Reagent Plate(s).
   a. Hold the plate firmly on the bench.
   b. Carefully peel the upper seal from the top corner.
   c. Carefully peel the bottom seal from the side.
3. Place the Pre-filled Reagent(s) Plate in the Pre-filled Reagent Plate holder(s). Insert the bottom of the plate first, then push the top of the plate down.

   NOTE: Keep the Pre-filled Reagent Plate flat during transfer to avoid spills and shifting of reagents.

4. Remove the capillary cartridge(s) from the packaging and insert them into the cartridge holder(s). The interior light(s) will change from **orange** to **blue.**

   NOTES:
   Use only capillary cartridges specifically designated for use with you Leo Instrument.

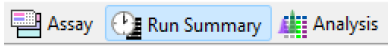
   If you are running an assay with both chemiluminescence and fluorescence detection use a Leo Fluorescence 25-Capillary Cartridge.

   If you are running an assay that using the Protein Normalization detection channel, use a Leo Fluorescence 25-Capillary Cartridge.

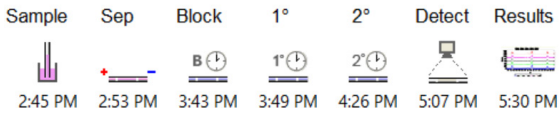
5. Remove the cover and place the Sample Plate in the Sample Plate holder.

   NOTE: Keep the Sample Plate flat during transfer to avoid spills and prevent shifting of reagents. Visually confirm even distribution of reagents in the troughs after placement in the instrument.

6. Close the Leo System's door.
7. Wait for the Leo System's status light to turn solid **blue**.
8. Click the **Start** button in Compass.
9. To check the remaining time for your assay, click on the **Run Summary** icon...



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

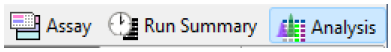


10. When the run is done, discard the capillary cartridges and plates.

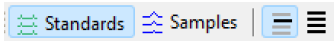
6 // Analyze Your Results

Verify Your Fluorescent Standards

1. Click on the **Run Summary** icon.
2. Watch the movie in the Separation tab showing the fluorescent standard separation. Make sure that each of the markers are visibly separated in each capillary:
   - 12–230 kDa assay: 1, 29, and 230 kDa
   - 66–440 kDa assay : 57 and 280 kDa
   - 2–40 kDa assay : 1 and 26 kDa
3. Click on the Analysis icon:

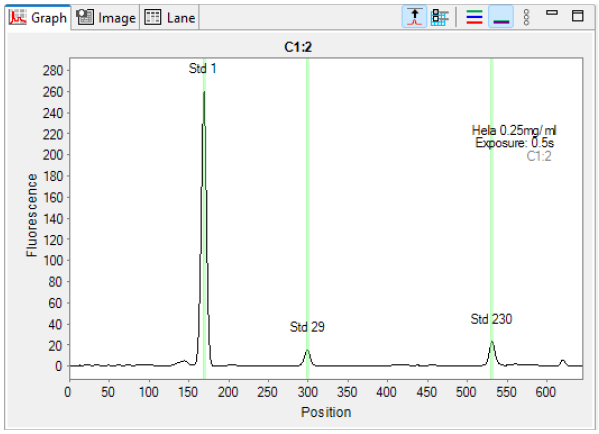


Then select the **Show Standards** and **View Selected** icons.



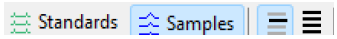
4. Check if your standards are correctly identified in the **Graph View** tab. If they are incorrect, right-click on the correct peak and select **Force Standard**. Do this for each capillary.

The example below: **12–230 kDa** size assay. Standard peaks are labeled **Std 1**, **Std 29**, and **Std 230**.



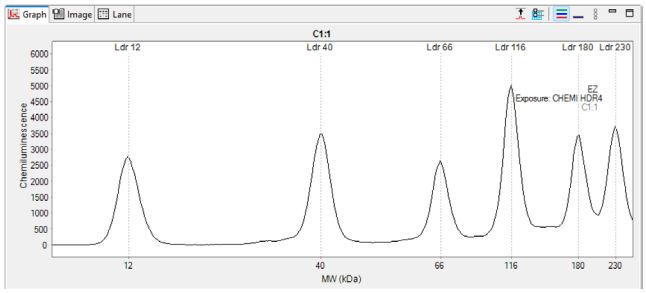
Verify Your Biotinylated Ladder

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



2. Select the capillary containing the biotinylated MW ladder (capillary 1) in the **Experiment** tab.
3. Your biotinylated ladder contains the following size peaks:
   - 12–230 kDa assay: 12, 40, 66, 116, 180, and 230 kDa
   - 66–440 kDa assay : 66, 116, 200, 280, and 440 kDa
   - 2–40 kDa assay : 2, 5, 12, 26, and 40 kDa
4. Confirm 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, click **View**, then **View Region**, and select **Full**.

The example below: **12–230 kDa Biotinylated Ladder**. Ladder peaks are labeled **Ldr 12**, **Ldr 40**, **Ldr 66**, **Ldr 116**, **Ldr 180**, and **Ldr 230**.

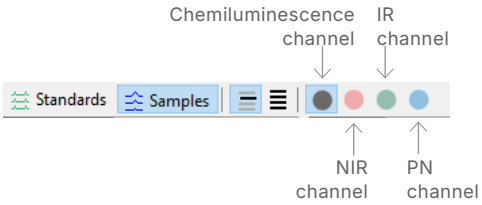


6 // Analyze Your Results (continued)

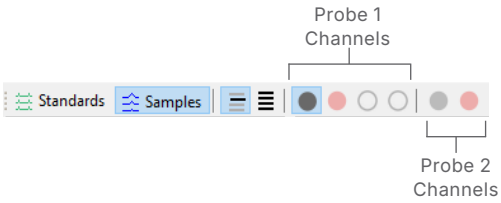
Label Your Sample Peaks

To toggle between or overlay multiple detection channels, click the Channel icon for each detection channel: Chemiluminescence, NIR, IR, Protein Normalization.

CHANNEL VIEWS



REPLEX CHANNEL VIEWS



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.

To manually label specific sample peaks:

1. In the **Analysis Options** pane, select the **Name** drop-down menu and select **[New]**. Fill in the **Name** and **MW** fields with desired name and molecular weight targets.
2. Click on the **Channel** drop-down menu and select the desired detection channel.
3. Fill in the desired color and applicable caps in the **Color** and **Caps** fields.
4. Any peaks found within the default range setting of ±10% of the entered molecular weight will automatically be labeled.

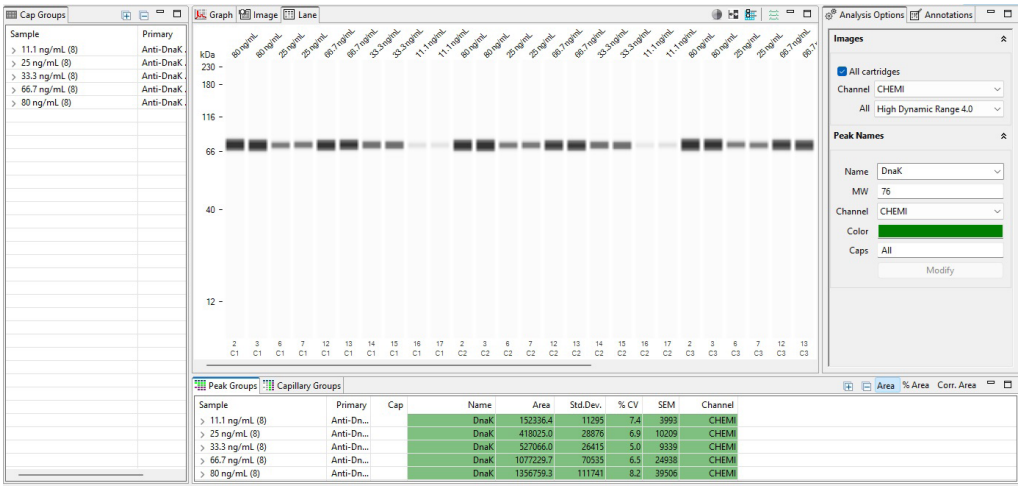
The bands and peaks in the sample are now labeled with your peak names.

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** or **Capillary Groups** tabs. You can also copy and paste the data from the table into Excel or other graphing programs.

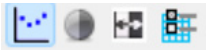
GROUPED DATA



6 // Analyze Your Results (continued)

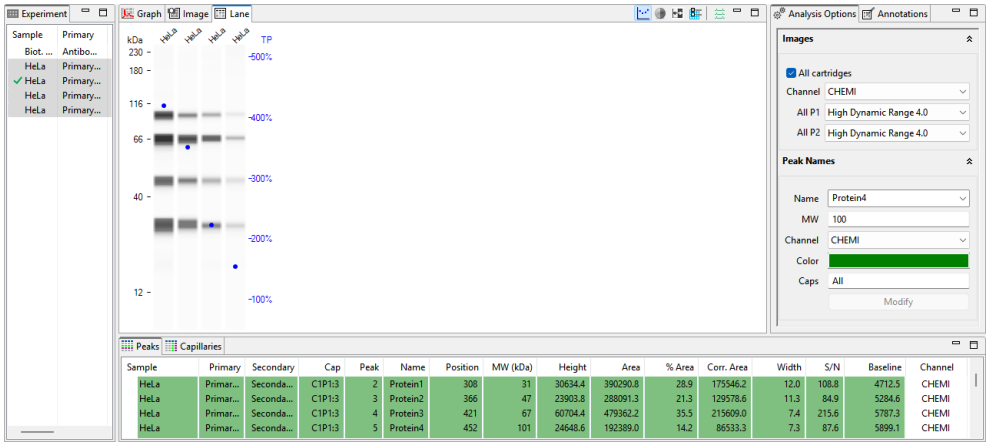
Total Protein Normalization (if applicable)

To view Normalization data, ensure the channel overlay for either Protein Normalization or Total Protein in Probe 2 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.

TOTAL PROTEIN NORMALIZATION DATA

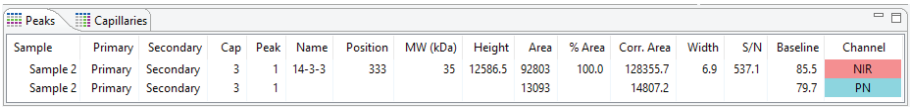


By default, Normalization data transformations are enabled for runs that include Protein Normalization or RePlex runs with Total Protein in Probe 2.

To enable/disable Normalization:

1. Select the **Edit** menu and click on **Analysis**.
2. On the Analysis screen, select **Normalization** and click on **Enable**.
3. Compass will use capillary 2 within each cartridge as the reference capillary by default. To choose a different reference capillary, on the Analysis screen under Normalization, use the drop-down menu to choose a **Reference Capillary**. To use a single reference capillary for the entire run, use the drop-down menu to choose a **Cartridge**.

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, 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.



Peaks | Capillaries

Sample	Primary	Secondary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Corr. Area	Width	S/N	Baseline	Channel
Sample 2	Primary	Secondary	3	1	14-3-3	333	35	12586.5	92803	100.0	128355.7	6.9	537.1	85.5	NIR
Sample 2	Primary	Secondary	3	1					13093		14807.2			79.7	PN

If you have any questions or concerns, please 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 contact your local Field Application Scientist.

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