bio-techne® / proteinsimple

Leo Instrument

User Guide

Leo Instrument User Guide

P/N PL2-0026

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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 **www.bio-techne.com/support/instrument-support/intellectual-property**.

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Let’s Get Started

Chapter Overview

- Welcome
- Introduction to Simple Western
- Simple Western Size Assays
- Leo System – A High-Throughput Simple Western Platform
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- Simple Western Assays on Your Leo Platform

Welcome

Congratulations on bringing the Bio-Techne® Leo™ Platform into your lab! We welcome you as a new user and are excited to be a part of your work. This user guide will provide you with information on performing a Simple Western™ size assay and other useful operating and installation information.

To help you get the most from your Leo Platform, we've added some attention phrases to assist you through the user guide:

NOTE	Points out useful information.
IMPORTANT	Indicates information necessary for proper operation of your Leo System.
CAUTION	Cautions you about potentially hazardous situations that could result in injury to you or damage to your Leo Instrument.
!WARNING!	Warns you that serious physical injury can result if the listed precautions are not followed.

Introduction to Simple Western

Bio-Techne Simple Western Technology simplifies and automates traditional western blot processes to deliver sensitive, quantitative data. Simple Western assays are hands-free capillary immunoassays that combine protein separation with immunoassay-based detection using conventional antibodies.

Simple Western Size Assays

Simple Western fully automates your Immunoassay and Total Protein Normalization, which includes sample loading, protein separation, immunoprobing and labeling, washing, detection, and data analysis. Tiny amounts of sample are separated inside capillaries and cross-linked to the capillary wall, removing the inherent variability of traditional western blotting that occurs when proteins are transferred to a membrane. The system performs blocking and wash steps, then uses just a few microliters of your primary antibody and conjugated secondary antibodies to detect target proteins using chemiluminescence or fluorescence detection, or uses Total Protein or Protein Normalization reagents for chemiluminescence or fluorescence detection, respectively.

Simple Western assays are performed in a fully enclosed, controlled, and standardized system, delivering precise and quantitative data, high-throughput sample processing, and automated protein analysis.

Leo System – A High-Throughput Simple Western Platform

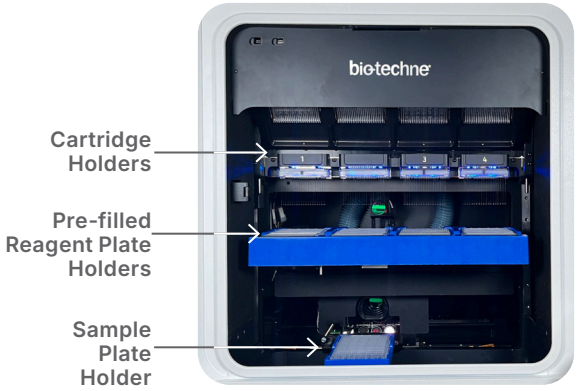
The Leo System maximizes your throughput, performing size-based capillary immunoassays (Simple Western assays) for up to 96 samples in just 3–5 hours. Just prepare your samples, antibodies, or Total Protein/Protein Normalization reagents (if needed), pipette them into a Simple Western Leo Sample Plate, load your Leo Instrument, and push **Start**. Your Leo Instrument does the rest. You'll get picogram-level sensitivity, built-in Total Protein Normalization, and highly reproducible quantitative results with molecular weight characterization.

The system runs up to four 25-capillary cartridges in parallel to boost the amount of data you can generate in one run. You have the flexibility to run just one, two, or three capillary cartridges depending on your throughput needs, or all four cartridges to maximize your data generation.

To operate your Leo System, you'll need the following:

- Leo Pre-filled Reagent Plate: The plates are ready-to-use and contain the wash buffer, and running buffer, separation and stacking matrix for the size range of your choice. You will need one Pre-filled Reagent Plate for every capillary cartridge used in the run.
- Leo 25-Capillary Cartridge or Leo Fluorescence 25-Capillary Cartridge for size-based separation, depending on the assay you are performing. Use a Leo Fluorescence 25-Capillary Cartridge if you are running an assay with both chemiluminescence and fluorescence detection, or an assay that uses the Protein Normalization detection channel.
- Leo Sample Plate: Where you will pipette your denatured sample, biotinylated ladder, diluted primary antibody, secondary antibody, wash buffer, antibody diluent, and Luminol-S/Peroxide (if needed). The Sample Plate is also where you will pipette your RePlex Reagent mix and Total Protein reagents if you plan to reprobe or perform a Total Protein assay, or your Protein Normalization reagent if you plan to perform a Protein Normalization assay.

See Chapter 4 "Operating Your Leo System" for more information.



Compass Software for Simple Western Platforms

Compass™ Software for Simple Western Platforms is used to run Simple Western assays on your Leo Instrument, monitor the run while in progress, and analyze resulting data. The software is available online at the Bio-Techne **Instrument Software Download Center** for installation on a separate workstation.

Simple Western Assays on Your Leo Platform

The following assays and assay combinations are currently validated on Leo Instruments; however, other combinations are possible.

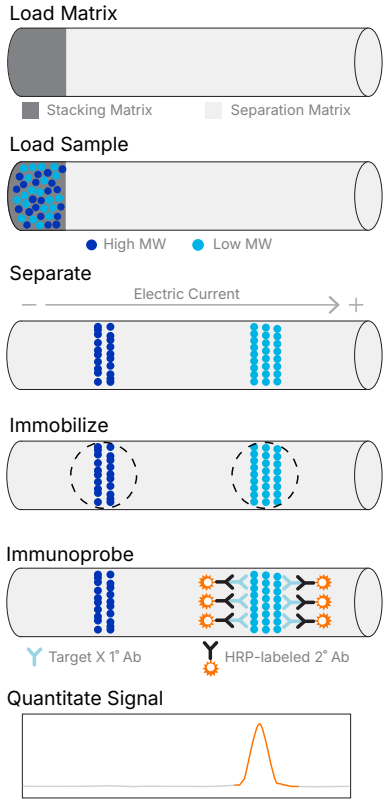
- Chemiluminescence Immunoassay
- Fluorescence Immunoassay

- Chemiluminescence Immunoassay and Fluorescence Immunoassay
- Chemiluminescence Immunoassay + Chemiluminescence Immunoassay using RePlex™
- Fluorescence NIR Immunoassay + Fluorescence NIR Immunoassay using RePlex
- Fluorescence NIR Immunoassay + Chemiluminescence Immunoassay using RePlex
- Chemiluminescence Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection
- Chemiluminescence and Fluorescence NIR Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection
- Chemiluminescence and/or Fluorescence Immunoassay + Total Protein Normalization using the PN Channel

Chemiluminescence Immunoassay

Precisely quantitate your protein of interest with as little as 3 µL of sample. Leo Instruments load separation and stacking matrix for the size range of your choice from the Pre-filled Reagent Plate(s) into the capillaries, followed by a denatured sample from the Sample Plate. Proteins are separated by size as they migrate through the stacking and separation matrices before the proteins are covalently bound to the capillary wall using proprietary, photoactivated capture chemistry. Primary antibodies that recognize your protein(s) of interest are incubated in the capillary followed by an HRP-conjugated secondary and Luminol-S/Peroxide. The chemiluminescent signal is detected and quantitated using Compass Software.

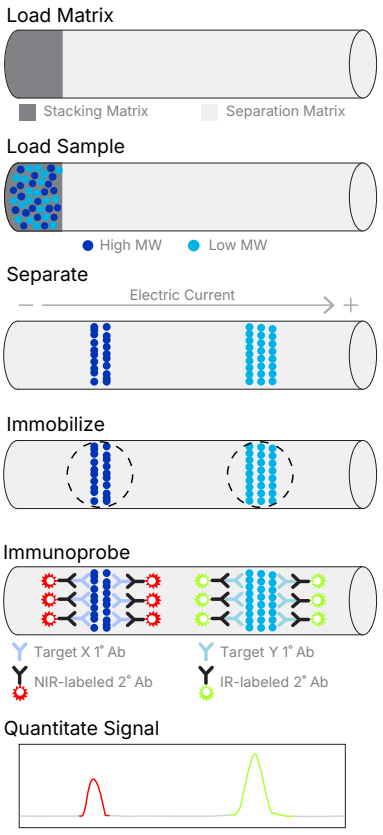
CHEMILUMINESCENCE IMMUNOASSAY WORKFLOW:



Fluorescence Immunoassay

Multiplex to maximize your time and sample with NIR or IR fluorescence detection. Similar to a chemiluminescence immunoassay, the Leo Instrument loads, separates, and immobilizes proteins to the capillary wall. Primary antibodies that recognize your protein(s) of interest are incubated in the capillary followed by secondary NIR/IR conjugates. The fluorescent signal is detected and quantitated using Compass Software.

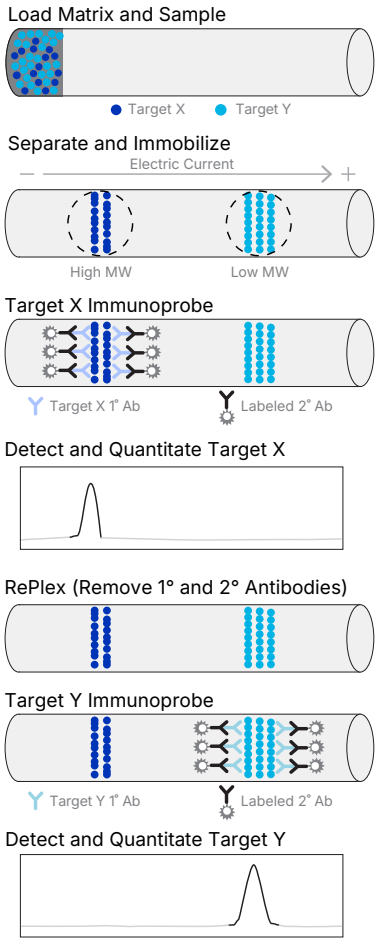
FLUORESCENCE IMMUNOASSAY WORKFLOW:



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

Get more data from your samples with RePlex. When you RePlex on a Leo System, you perform a chemiluminescence or fluorescence immunoassay in the first round of probing (Probe 1) before the RePlex Reagent mix removes the primary and secondary antibodies. You can then perform a second chemiluminescence or fluorescence immunoassay with another primary/secondary antibody set (Probe 2).

CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY + CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY USING REPLEX WORKFLOW:



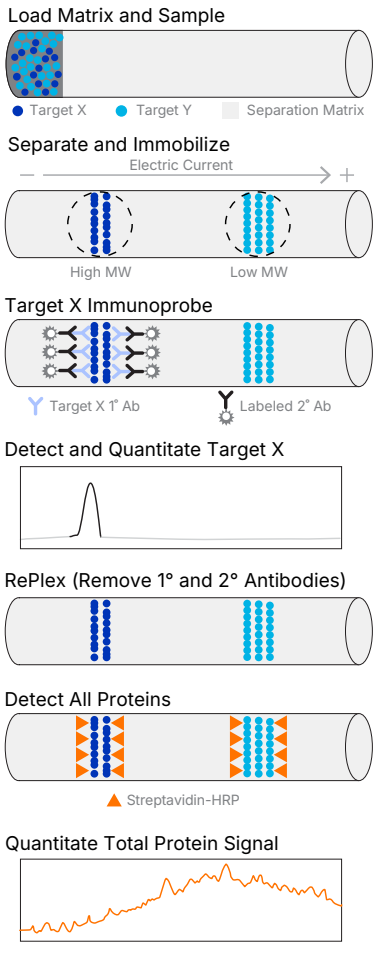
Chemiluminescence and/or Fluorescence Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection

The Total Protein Normalization Assay, performed on a Leo System as part of a RePlex assay, detects all the proteins in your sample to normalize your immunoassay data. After the proteins in your sample are separated and immobilized to the capillary wall, the Total Protein Biotin Labeling Reagent attaches biotin to all the proteins in your sample. The biotin is detected using Total Protein Streptavidin-HRP and Luminol-S/Peroxide to generate a chemiluminescent signal.

Total protein normalization is a preferred method to normalize data compared to housekeeping proteins. Compass for SW Software automatically normalizes the data for you, simplifying your analysis.

Visit the **Bio-Techne Academy** for more info about different Simple Western Normalization approaches to decide which is right for you.

CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY + TOTAL PROTEIN NORMALIZATION USING REPLEX AND CHEMILUMINESCENCE DETECTION WORKFLOW:



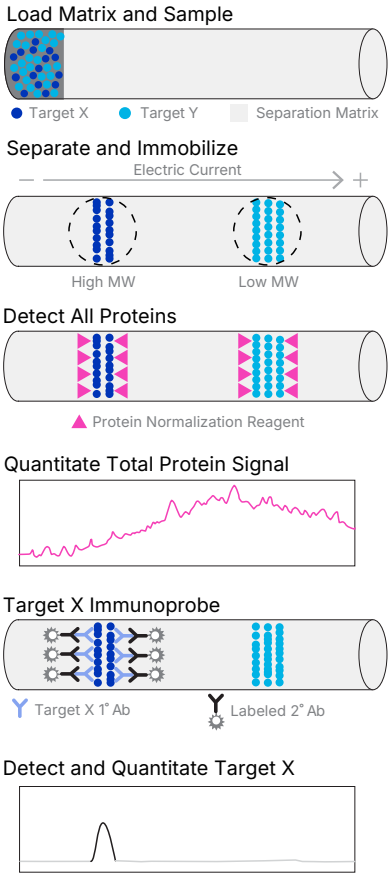
Chemiluminescence or Fluorescence Immunoassay + Total Protein Normalization using the PN Channel

Protein Normalization detects all the proteins in your sample without requiring a separate assay to normalize your immunoassay data. After the proteins in your sample are separated and immobilized to the capillary wall, the Protein Normalization Reagent attaches fluorophores to amines in all the proteins in your sample to generate a fluorescent signal. A chemiluminescence or fluorescence NIR/IR immunoassay can then be performed to detect your protein(s) of interest.

Total protein normalization is a preferred method to normalize data compared to housekeeping proteins. Compass for SW Software automatically normalizes the data for you, simplifying your analysis.

Visit the **Bio-Techne Academy** for more info about different Simple Western Normalization approaches to decide which is right for you.

CHEMILUMINESCENCE OR FLUORESCENCE IMMUNOASSAY + TOTAL PROTEIN NORMALIZATION USING THE PN CHANNEL WORKFLOW:



Chapter 2:

Getting Your Lab Ready

Chapter Overview

- Introduction
- Space Requirements
- Physical Specifications
- Electrical Requirements
- Environmental Requirements
- Software and Computer Requirements
- General Guidelines and Information

Introduction

This chapter will help you prepare the lab for your Leo System. Please have the space, electrical, and environmental requirements ready prior to installation.

!WARNING!
Please wait for the ProteinSimple representative to unpack and install the Leo Instrument. Don't try to lift or unpack the instrument on your own – you could cause injury to yourself or damage to the system.

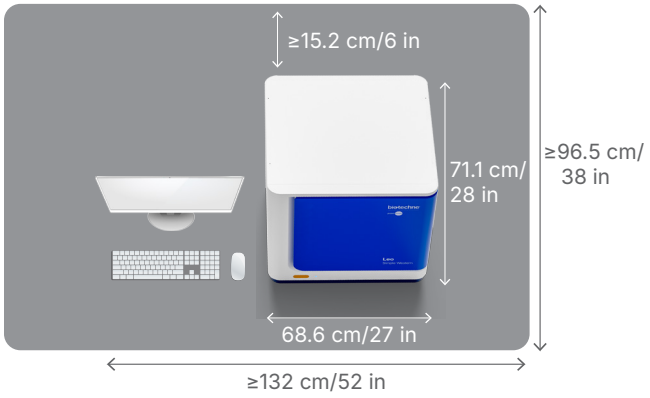
Space Requirements

You will need a lab bench or table without raised or rounded edges that can support 350 lbs (159 kg) and has enough space for both the Leo Instrument and a computer. There should be sufficient clearance for both heat ventilation and to provide access if the instrument needs service.

IMPORTANT:
A Leo Instrument needs a stable surface and must remain level to work properly. The lab bench or table can't shift or wobble under heavy weight.

The lab bench will need to be <3.3 ft. tall to be compatible with the lift tools used by the ProteinSimple representative during instrument installation.

RECOMMENDED BENCH TOP SPACE REQUIREMENTS FOR YOUR LEO SYSTEM



Dimension	Meters	Feet
Width	1.3	4.3
Depth	1.0	3.2
Bench Height	0.9	3.0
Space Above Bench	0.4	1.2

Physical Specifications

Description	Specification
Leo Instrument's Dimensions (Door Closed)	0.75 m H x 0.70 m W x 0.73 m D (2.5' H x 2.3' W x 2.4' D)
Leo Instrument's Dimensions (Door Open)	0.75 m H x 0.77 m W x 1.5 m D (2.5' H x 2.5' W x 4.9' D)
Leo Instrument's Weight	136 kg (300 lbs)
Computer Workstation Dimensions	Monitor/CPU: 0.43 m H x 0.57 m W x 0.21 m D (17" H x 22.4" W x 8.3" D) Keyboard: 0.03 m H x 0.43 m W x 0.13 m D (1" H x 17" W x 5" D) Mouse: 0.04 m H x 0.06 m W x 0.11 m D (1.4" H x 2.4" W x 4.5" D)

Electrical Requirements

A Leo Instrument requires a dedicated, grounded circuit capable of delivering the appropriate current and voltage for your country. The power requirements for selected regions are listed below:

Region	Volts (AC)	Frequency (Hz)	Amps
US and Canada	120	60	5.0
Europe	230	50	2.6
Japan	100	50/60	6.0

In addition to the requirements listed above, we recommend that the grounded circuits terminate at the receptacles, and receptacles must be located within 10 ft (3 m) of the instrument.

Environmental Requirements

Leo Instruments should be operated at a consistent temperature in the lab. It will work best when conditions stay within these ranges:

Requirements	Specification
Indoor or outdoor use	For indoor use only
Operating temperature range	18–23 °C (64–73 °F)
Operating humidity range	20–60% relative, non-condensing
Altitude	< 2000 m
Mains supply fluctuations	+/- 10%
Over voltage category	OVC II
Pollution degree	Pollution degree 2

NOTE: Leo Instruments should not be placed in a location with direct exposure to sunlight or placed on a bench with a piece of lab equipment that will induce vibrations during operation, like a centrifuge.

Software and Computer Requirements

Leo Systems come with a computer that has Compass Software for Simple Western Platforms pre-installed. The software is used to run assays and analyze resulting data.

Compass Software can be installed in a separate workstation, such as your laptop computer, if you don't want to analyze your data at the Leo Instrument workstation in the lab. The software is available on the Compass for Simple Western CD that comes with the Leo Instrument, and is available at the Bio-Techne **Instrument Software Download Center**.

Your computer must meet the minimum requirements listed below to run Compass Software and process data.

Component	Minimum Recommended
Operating system	Windows 11
Processor	Core i7 or better
Memory	16 GB
Free disk space	100 GB
USB ports	2 (Mouse and Keyboard)
Ethernet ports	2

General Guidelines and Information

Intended Use

Leo Systems are for research use only. Not for use in diagnostic procedures.

Lifting and Moving the System

!WARNING!
Do not attempt to lift or move your Leo Instrument by yourself. Contact ProteinSimple to arrange for a ProteinSimple representative to move the instrument for you.

Chapter 3:

Leo Systems

Chapter Overview

- Instrument Overview
- External Components
- Internal Components

Instrument Overview

Leo Instrument hardware components are described in this chapter.

External Components

System Door

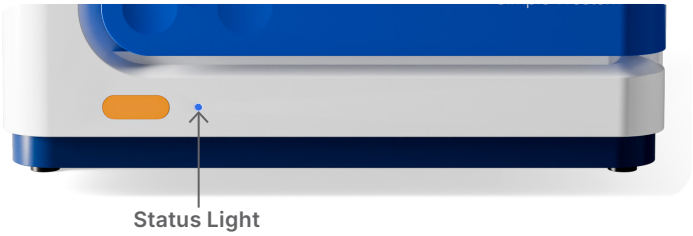
The door to the Leo Instrument gives you access to the inside of the system to load the capillary cartridge(s), Pre-filled Reagent Plate(s), and Sample Plate. To open the door, make sure the status light is a steady **blue**, then press the **orange** touch pad. To close the door, push the door until you hear the door latch “click”. The Leo Instrument will then pull the door closed.

NOTE: The Leo System door must be closed before starting a run.

Status Lights

The LED on the Leo Instrument front panel provides you with status updates.

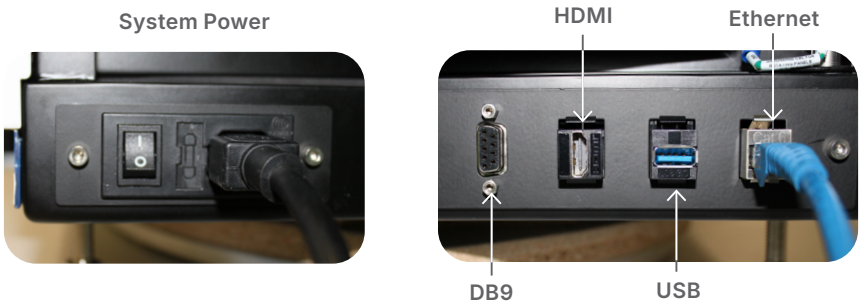
- **Start-up (magenta):** You've turned on the power and the instrument is warming up.
- **Ready (steady blue):** The Leo System is powered on and ready for use.
- **Opening Door (long blue flash followed by blue pulses):** The Leo Instrument door is opening.
- **Running (pulsing blue):** The Leo System is scanning cartridge and plate barcodes, or running an assay.
- **Trying to Open Door While Running (orange flash):** The Leo Instrument can't open the door when the instrument is running.
- **Temperature or Relative Humidity Warning When Idle (pulsing orange):** The ambient or instrument chamber temperature, or relative humidity is outside of the Leo System's operating range. The run can still be started, but a warning message will display in the run file and the data generated may be impacted.
- **Error (steady red):** The Leo System has detected an error. To get more information, check the Status window of the Run Summary Screen in Compass for Simple Western.



NOTE: For more information about operating the instrument when there is a temperature or relative humidity warning, or it is in an error state, 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.

Rear Panel

The power entry, power switch, and network connection port are located on the Leo Instrument's rear panel.



- **System Power** - The main system power components consist of the power On/Off switch next to an AC power input port.
- **Connect to PC** - A 10/100/BASE-T Ethernet (RJ-45 connector) is used to connect your Leo Instrument to a computer.

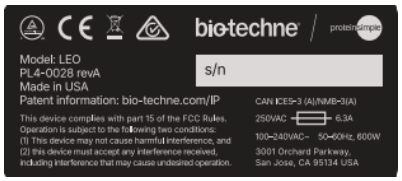
!WARNING!
Only use the power supply cord provided with your Leo Instrument. If the cord is damaged, please contact ProteinSimple Technical Support toll-free in the US and Canada at 1-888-607-9692, and in Europe at +44 1235 529449.

!WARNING! SHOCK HAZARD
Disconnect the power cord from the power input to disconnect power to the instrument.

The rear panel also includes a DB9 port, an HDMI port, and a USB 3.0 port. Do not connect cables to them as they should only be used by a ProteinSimple representative when the system is being serviced.

System Label

A system label is also located on the Leo Instrument's rear panel. It includes the ProteinSimple location, system model, power requirements, serial number, and certification markings.

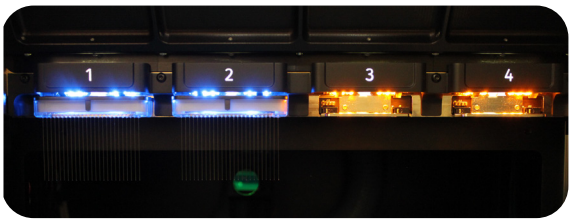


NOTE: Serial numbers are used to identify individual instruments on the network.

Internal Components

Capillary Cartridge Holders

There are 4 capillary cartridge holders that will hold the Leo 25-Capillary Cartridge (P/N 009-651) or Leo Fluorescence 25-Capillary Cartridge (P/N 009-701). White-lighted numbers on the cartridge holders indicate the position number, with Cartridge 1 positioned to the left and Cartridge 4 positioned to the right.

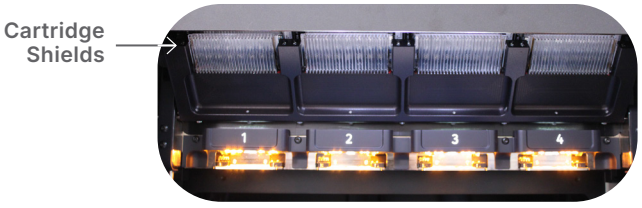


NOTE:
The 13-Capillary Cartridge (P/N 009-081), Fluorescence 13-Capillary Cartridge (P/N 009-400), 25-Capillary Cartridge (P/N 009-050), Fluorescence 25-Capillary Cartridge (P/N 009-401), and Blot Cartridge (P/N 004-651) are not supported on Leo Instruments.

Use only Leo-specific capillary cartridges (P/N 009-651 or P/N 009-701) when performing Simple Western size assays on your Leo System. Jess and Abby capillary cartridges are not supported on Leo Instruments.

Cartridge Shields

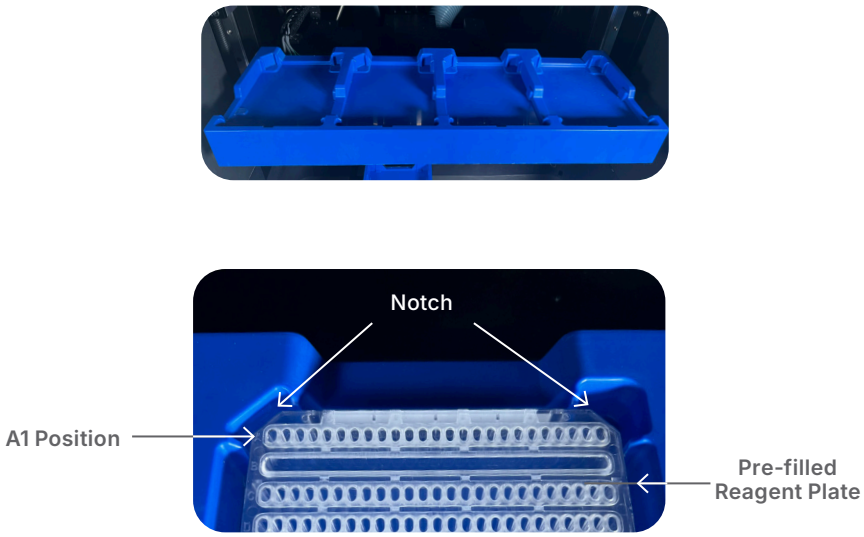
Cartridge shields appear above the cartridge holders and are not user-serviceable parts.



!WARNING!:
Avoid touching the shields in general and when inserting capillary cartridges into the cartridge holders.

Pre-filled Reagent Plate Tray

The Pre-Filled Reagent Plate tray contains four plate holders. The Pre-filled Reagent Plates come with assay reagents for the size range you are running.



A Pre-filled Reagent Plate holder has notches at the upper right and upper left corner that correspond to notches on the plate. Plates should be inserted into the plate trays so that the A1 position is aligned with the upper left corner of the holder.

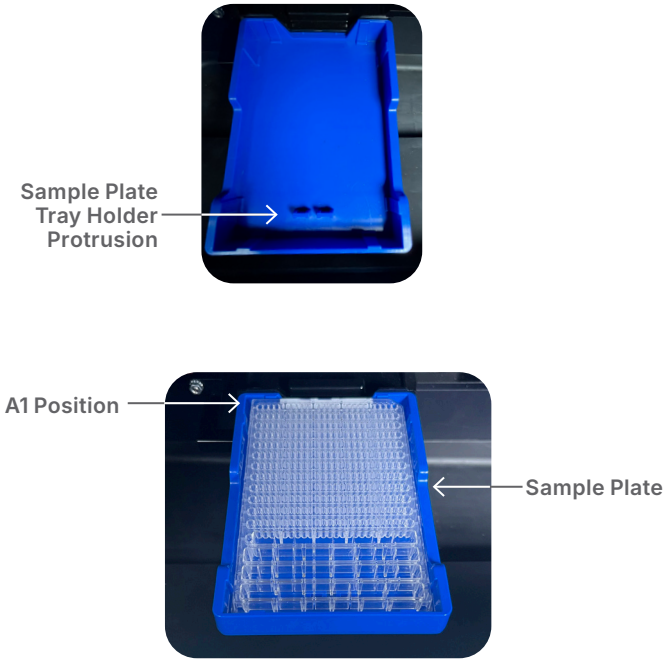
NOTES:
Do not remove the seals from the Pre-filled Reagent Plate(s) until you are ready to load them into the instrument.

Pre-filled Reagent Plate(s) should be inserted in your Leo Instrument right before starting a run.

When inserting Pre-filled Reagent Plate(s), make sure the plate is firmly seated and level in the tray.

Sample Plate Tray

The Sample Plate tray contains one plate holder that holds the Leo Sample Plate. The Sample Plate is where you will pipette your samples and assay reagents.



The Sample Plate holder has a protrusion at the bottom center of the tray to ensure that a Pre-filled Reagent Plate is not accidentally placed in the Sample Plate tray. The plate should be inserted into the holder so that the A1 position is aligned with the upper left corner of the holder.

NOTES:
When inserting the Sample Plate, make sure the plate is firmly seated and level in the tray.

The Sample Plate bottom notch should be flush with the small protrusion in the Sample Plate holder.

Inner Door Liner

Avoid touching the Leo Instrument inner door liner to keep it clean from debris and fingerprints. See “Maintenance” on page 141 for more information on cleaning the door liner.

Chapter 4:

Operating Your Leo System

Chapter Overview

- Before You Throw the Switch
- Power Up
- Self Test
- Starting a Run
- Stopping a Run
- Viewing and Changing System Properties
- Viewing Errors and Test Logs
- Status Modes
- Shutdown

Before You Throw the Switch

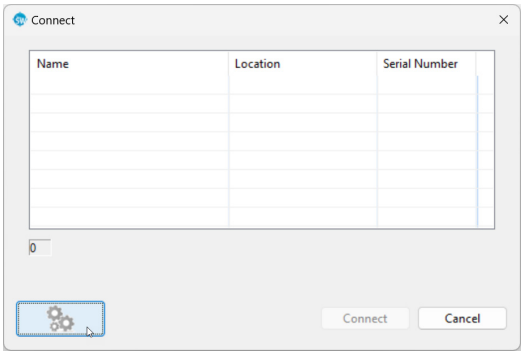
Ensure that everyone using your Leo System has:

- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for the Leo System.
- Received instruction on handling of biohazards (if biohazardous materials are to be used on your Leo Instrument).
- Read and understood all related Product Inserts and related Safety Data Sheets (SDS) documentation.
- Completed in-person or virtual training provided by ProteinSimple.
- Review door opening/closing instructions.
- Visited the **Bio-Techne Training Academy** and completed the Leo Learning Plan.

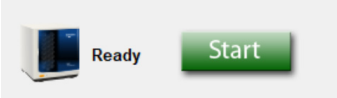
Power Up

NOTE: Turn the Leo Instrument on with the door closed for at least 1.5 hours before starting a run to ensure the instrument reaches its temperature setpoint.

1. Turn on the computer connected to your Leo Instrument.
2. Turn on the main power switch located on the right rear panel.
3. Wait for the Leo Instrument to initialize. The status light will turn solid blue when the instrument is ready.
4. Double-click the Compass for Simple Western icon to open the application.
5. Connect Compass Software to your Leo Instrument.
   a. Select **Instrument** in the main menu and click **Connect...**. Then select your Leo Instrument and click **Connect**.
   b. If you don't see your instrument, click on the **Advanced Connect** button in the bottom left corner of the Connect window. Type in the network address for your instrument and click **Connect**.

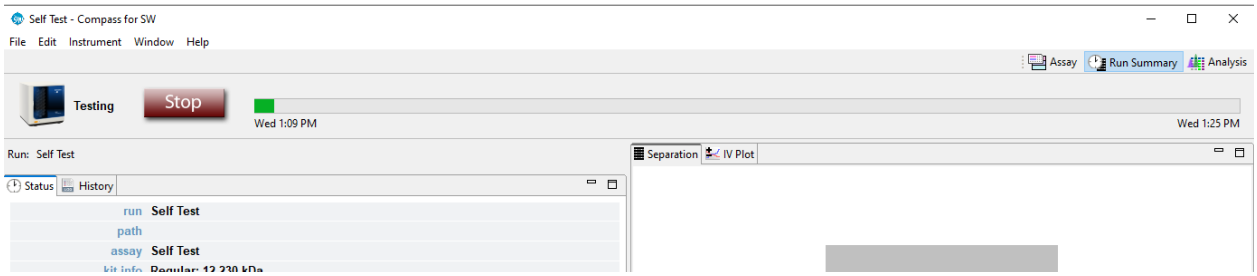


c. The instrument status bar will appear after Compass for Simple Western Software is connected to your Leo Instrument.



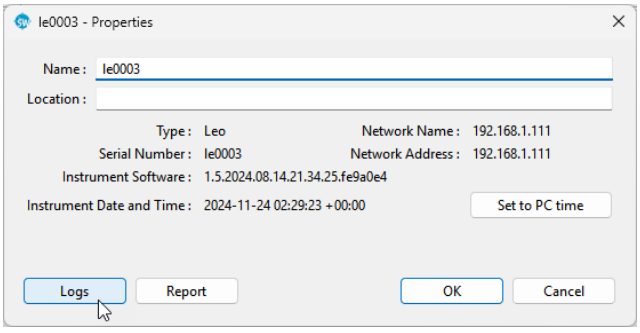
Self Test

The Leo Instrument can perform a set of self tests to make sure it is operating properly. To start the testing, ensure all Leo cartridges, Pre-filled Reagent Plates, and the Sample Plate are removed from the instrument. Ensure Compass Software is connected to the system and then select **Instrument** in the main menu and click **Self Test**. A prompt will appear for confirmation to start the test. Click **OK**. Testing will take about 40 minutes.

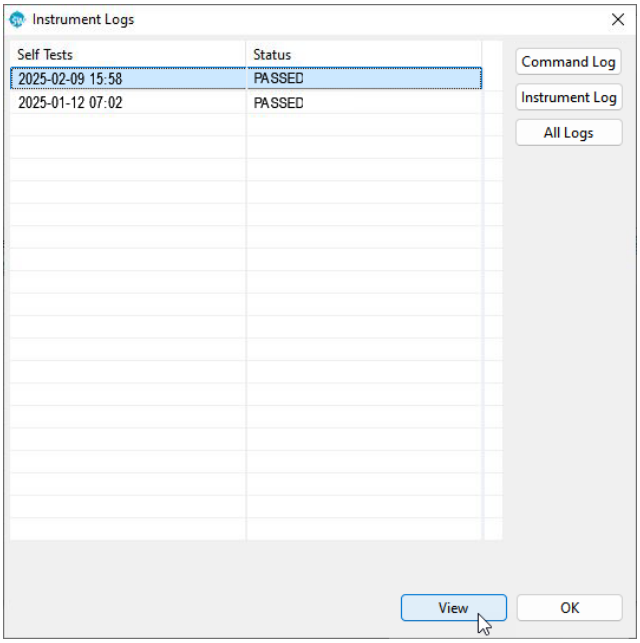


NOTE: We recommend running the self test monthly or when encountering issues with the system. When performing the self test, start the test before preparing your sample, reagents, and Sample Plate, and before peeling the two seals off the Pre-filled Reagent Plate(s).

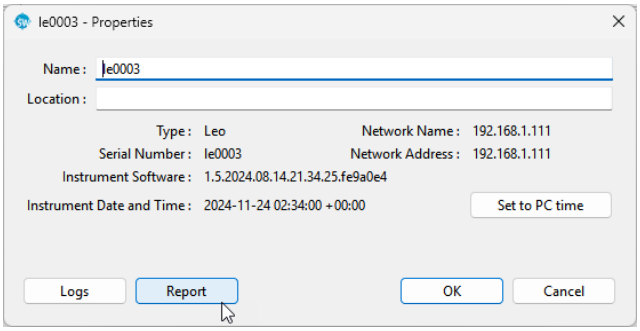
To view the log when the test is done, select **Instrument** in the main menu, click **Properties**, and click **Logs**.



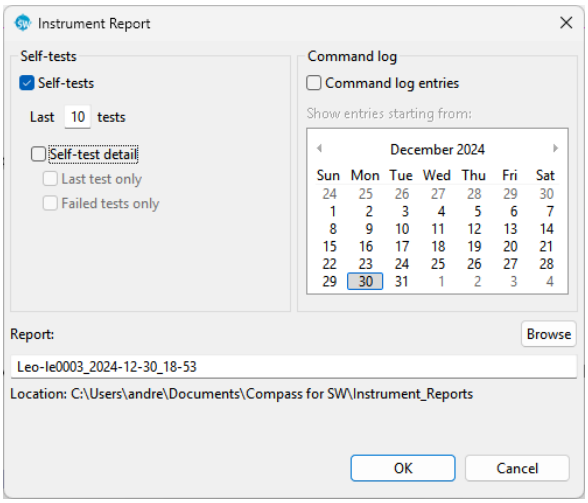
Select the test log you would like to view and click **View**.



To export and save self test results, click **Report**.



Select the number of self tests to download and the details to include. Click **OK** to save the self test report(s) to the designated file directory.



Starting a Run

NOTES:
Turn the Leo System on with the door closed for at least 1.5 hours before starting a run to ensure the instrument reaches its temperature setpoint.

Keep the Leo Instrument door closed when the cartridge holders and plate trays are not being accessed to keep the instrument chamber temperature at the optimal operating range.

To start a run after loading the 25-Capillary Cartridge(s), Pre-filled Reagent Plate(s), and prepared Sample Plate in the Leo System, click the **Start** button on the Assay screen. You can also start a run by selecting **Instrument** in the main menu and clicking **Start**.

For more information on setting up a run, including sample and reagent preparation, refer to Chapter 5: “Running Size Assays”.

Stopping a Run

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

A prompt will appear asking if you really want to stop the run. Click **Yes**.

When the run stops, the Start button will reappear, and the instrument will end the run as soon as possible.

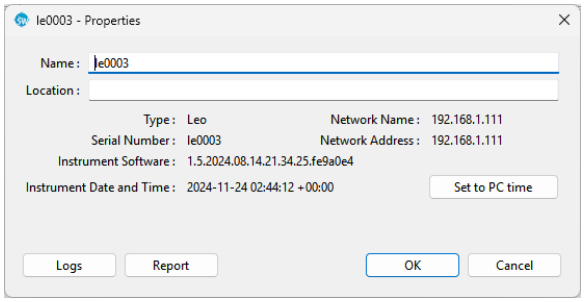
When the run has stopped, you can remove the capillary cartridge(s), Pre-filled Reagent Plate(s), and the Sample Plate and discard them. The stopped status, date, and time will be displayed on the Run Summary screen.

IMPORTANT: Do not re-use the Leo capillary cartridges, Pre-filled Reagent Plates, or Sample Plates.

Viewing and Changing System Properties

From the Compass for Simple Western main menu, select **Instrument** and click **Properties** to see properties for the Leo System:

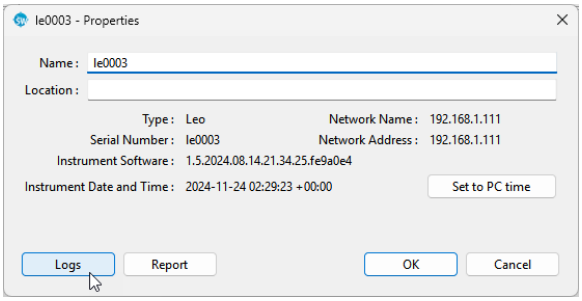
- Name
- Location
- Type
- Serial number
- Instrument software version (embedded)
- Network name and address
- Date and time of the instrument clock



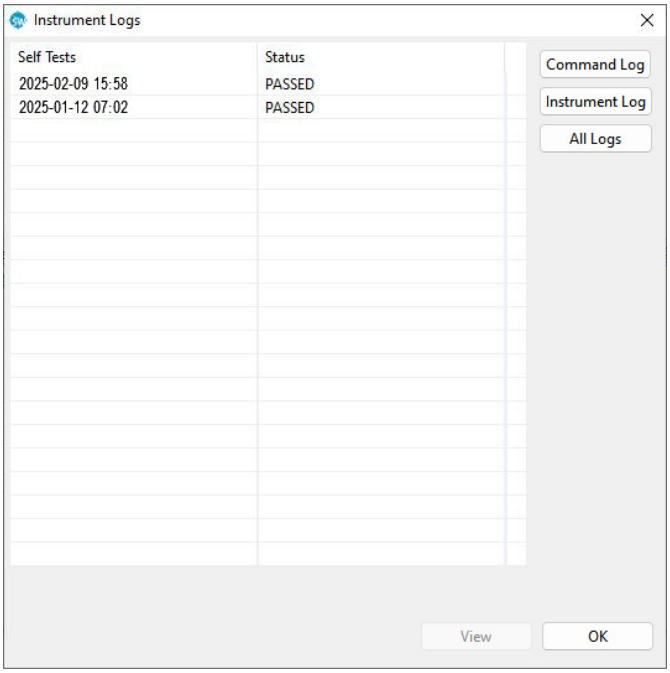
- **To change the system name or location:** Click in the name or location box and enter the new information.
- **To sync the instrument clock with the computer:** Click **Set to PC time**.

Viewing Errors and Test Logs

From the Compass for Simple Western main menu, select **Instrument** and click **Properties**. To view a log, click **Logs**.



The table that displays shows the list of Self Test logs. You can also select **Command Log** or **Instrument Log** to view and save logs related to the instrument and software.



Status Modes

The instrument status bar displays status, buttons, warnings, and progress bars depending on what the Leo Instrument is doing.

- **Ready/Start button** - The Leo Instrument is ready and an assay is loaded. The status light will be solid **blue**. Click **Start** to begin a run.
- **Not Ready/Reset button** – The Leo Instrument is not ready and must reinitialize. The status light will be solid **red**/flashing **magenta**. Click **Reset** to start the initialization protocol if the status light is solid **red**.
- **Running/Stop button** - The Leo Instrument is running an assay. The status light will be pulsing **blue**. The run name, time it started, and when it will be done are shown in the run progress bar. Click **Stop** to stop the run.
- **Error/Reset button** - The Leo System has detected an error. Go to the **Status** window in the **Run Summary** screen to get more details. The status light will be **red**. Once you've been able to correct the error, click **Reset**.

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

A warning message will appear in the instrument status bar if the ambient temperature, chamber temperature, or relative humidity is outside the optimal operating range.

Shutdown

1. Make sure to remove all plates and cartridges before shutting down the system.

IMPORTANT: Liquid may leak out of the cartridge if left in the instrument after your Leo System is powered down.

2. Close Compass for Simple Western Software and shut down the computer connected to your Leo Instrument.
3. The Leo Instrument should remain on unless you won't use it for more than a week. In that case, you can just turn the power off.

Chapter 5:

Running Size Assays

Chapter Overview

- Overview
- Starting a Run
- Creating a New Assay: Immunoassay
- Creating a New Assay: RePlex
- Creating a New Assay: Protein Normalization
- Opening and Modifying an Existing Assay
- Creating a Custom Template Assay
- Installing the Cartridges and Plates
- Starting the Assay
- Monitoring a Run
- Post-Run Procedures
- Size Assay Data Analysis

Overview

The Leo System is an open platform, giving you flexibility in your Simple Western assay setup.

Throughput

Leo Systems can perform parallel immunoassays in 4 capillary cartridges to generate 100 data points in one run. When your assay throughput needs are lower, you can also just run 1 cartridge, 2 cartridges, or 3 cartridges in a run.

Resampling Rows

Sample rows and antibody rows can be resampled, so that one sample row or one antibody row can be used to load multiple capillary cartridges in one run. This increases the number of data points you get per sample, increases your sample and/or antibody savings, and decreases the time it takes to set up a run.

Leo Assay Combinations

The following assay combinations are validated on Leo Instrument; however, other combinates are possible based on your experimental needs:

- **Chemiluminescence Immunoassay:** Perform a chemiluminescence immunoassay on 1–4 capillary cartridges.
- **Fluorescence Immunoassay:** Perform a fluorescence NIR/IR immunoassay on 1–4 capillary cartridges.
- **Chemiluminescence Immunoassay + Chemiluminescence Immunoassay using RePlex:** Perform a chemiluminescence immunoassay on 1-4 capillary cartridges, then use the RePlex Reagent mix to remove antibodies from the first round of probing. Perform a second chemiluminescence immunoassay with a fresh set of antibodies in a second probe.
- **Fluorescence NIR Immunoassay + Fluorescence NIR Immunoassay using RePlex:** Perform a fluorescence NIR immunoassay on 1–4 capillary cartridges, then use the RePlex Reagent mix to remove antibodies from the first round of probing. Perform a second fluorescence NIR immunoassay with a fresh set of antibodies in a second probe.
- **Fluorescence NIR Immunoassay + Chemiluminescence Immunoassay using RePlex:** Perform a fluorescence NIR immunoassay on 1–4 capillary cartridges, then use the RePlex Reagent mix to remove antibodies from the first round of probing. Perform a second chemiluminescence immunoassay with a fresh set of antibodies in a second probe.
- **Chemiluminescence Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection**: Perform a chemiluminescence immunoassay on 1–4 capillary cartridges, then use the RePlex Reagent mix to remove antibodies from the first probe. Perform sequential protein normalization, detecting total protein chemiluminescence signal in the same capillary.
- **Chemiluminescence and Fluorescence NIR Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection:** Perform a chemiluminescence and fluorescence NIR immunoassay on 1–4 capillary cartridges, then use the RePlex Reagent mix to remove antibodies from the first probe. Perform sequential protein normalization, detecting total protein chemiluminescence signal in the same capillary.
- **Chemiluminescence and/or Fluorescence Immunoassay + Total Protein Normalization using the PN Channel:** Detect total protein in the PN channel using the Protein Normalization Reagent on 1–4 capillary cartridges, then perform a chemiluminescence and/or fluorescence immunoassay.

NOTE: The Leo Instrument should be on with the door closed for at least 1.5 hours before starting a run to ensure the instrument reaches its temperature setpoint. See “Power Up” on page 28 for instructions on turning on your Leo Instrument.

Starting a Run

What You’ll Need

- Leo Separation Module for the size range and detection method of your choice.
  - Simple Western 12–230 kDa Separation Module for Chemiluminescence

Components
Leo 25-Capillary Cartridges
Leo 12–230 kDa Pre-filled Reagent Plates
Leo Sample Plates
10X Sample Buffer
Wash Buffer
Plate Cover
Standard Kit 1
12–230 kDa Fluorescent 5X Master Mix 1
DTT
12–230 kDa Biotinylated Ladder

  - Simple Western 12–230 kDa Separation Module for Fluorescence

Components
Leo Fluorescence 25-Capillary Cartridges
Leo 12–230 kDa Pre-filled Reagent Plates
Leo Sample Plates
10X Sample Buffer
Wash Buffer
Plate Cover
Standard Kit 1
12–230 kDa Fluorescent 5X Master Mix 1
DTT
12–230 kDa Biotinylated Ladder

  - Simple Western 66–440 kDa Separation Module for Chemiluminescence

Components
Leo 25-Capillary Cartridges
Leo 66–440kDa Pre-filled Reagent Plates
Leo Sample Plates
10X Sample Buffer
Wash Buffer
Plate Cover
Standard Kit 3
66–440 kDa Fluorescent 5X Master Mix 3
DTT
66–440 kDa Biotinylated Ladder

  - Simple Western 66–440 kDa Separation Module for Fluorescence

Components
Leo Fluorescence 25-Capillary Cartridges
Leo 66–440kDa Pre-filled Reagent Plates
Leo Sample Plates
10X Sample Buffer
Wash Buffer
Plate Cover
Standard Kit 3
66–440 kDa Fluorescent 5X Master Mix 3
DTT
66–440 kDa Biotinylated Ladder

- Simple Western 2–40 kDa Separation Module for Chemiluminescence

Components
Leo 25-Capillary Cartridges
Leo 2–40 kDa Pre-filled Reagent Plates
Leo Sample Plates
10X Sample Buffer
Wash Buffer
Plate Cover
Standard Kit 5
2–40 kDa Fluorescent 5X Master Mix 5
DTT
2–40 kDa Biotinylated Ladder

- Simple Western 2–40 kDa Separation Module for Fluorescence

Components
Leo Fluorescence 25-Capillary Cartridges
Leo 2–40 kDa Pre-filled Reagent Plates
Leo Sample Plates
10X Sample Buffer
Wash Buffer
Plate Cover
Standard Kit 5
2–40 kDa Fluorescent 5X Master Mix 5
DTT
2–40 kDa Biotinylated Ladder

NOTES:
Leo Pre-filled Reagent Plates are preloaded with separation matrix, stacking matrix, running buffer, matrix removal buffer, and wash buffer.

Capillary Cartridge Kits, Pre-filled Reagent Plates and Reagent Kits for your size range of choice, Standard Kits, and Sample Plate Kits are available as à la carte products as well.

- Leo Detection Module for the detection method of your choice.
  - Chemiluminescence Detection Module

Components
Luminol-S
Peroxide
Streptavidin-HRP
Antibody Diluent 2 or Milk-Free Antibody Diluent
One of the following Secondary Antibodies*: Gt Anti-Rabbit Secondary HRP Antibody Gt Anti-Mouse Secondary HRP Antibody Secondary Streptavidin HRP Gt Anti-Human IgG Secondary HRP Antibody Dk Anti-Goat Secondary HRP Antibody

  - Fluorescence Detection Module

Components
Streptavidin-NIR
Milk-Free Antibody Diluent
One of the following Secondary Antibodies: Dk Anti-Rabbit Secondary NIR Antibody Dk Anti-Rabbit Secondary IR Antibody Dk Anti-Mouse Secondary NIR Antibody Dk Anti-Mouse Secondary IR Antibody

*No secondary antibody is provided with the “No Secondary Detection Module”

NOTES:
Use the **Simple Western Kit Builder** to identify the exact kits needed to perform your experiment.

Jess and Abby Detection Modules are also compatible with your Leo System.

- Bio-Techne RePlex Module, (optional)
- Bio-Techne Simple Western Total Protein Detection Module, (optional)
- Bio-Techne Simple Western Protein Normalization Module (optional)
- Protein samples
- Primary antibodies
- System Control Primary Antibody (optional)
- Water, 0.22 µm-filtered and deionized (molecular biology grade or better)
- Pipettes and tips
- Microcentrifuge and tubes
- Ice and ice bucket
- Vortex
- Heat block/thermocycler/water bath
- Centrifuge with plate adaptor

NOTE: Visit the **Simple Western Antibody Database** to find commercial primary and secondary antibodies validated for Simple Western Technology.

Preparing Standard Kit Reagents

Open the Standard Kit box and remove the required number of vials and Ladder Pack.

Prepare 400 mM DTT (Glass Vial, White Pellet)

1. Open the vial of lyophilized DTT by lifting the center tab and gently pulling it back to break the metal seal. Carefully remove the rubber stopper.
2. Add 200 µL deionized water.
3. Gently mix by pipetting up and down until the pellet is fully dissolved.

> **NOTE:** If you want to run non-reduced samples on a Leo System, reach out to ProteinSimple Technical Support 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.

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

1. Open the vial of lyophilized Fluorescent 5X Master Mix by lifting the center tab and gently pulling it back to break the metal seal. Carefully remove the rubber stopper.

> **NOTE:** The morphology of the pink pellet will be different from vial to vial.

2. Add 175 µL 10x Sample Buffer.
3. Add 175 µL of the prepared 400 mM DTT solution.
4. Gently mix by pipetting up and down to avoid creating bubbles until the pellet is fully dissolved.
5. Store the reconstituted Fluorescent 5X Master Mix on ice until you are ready to prepare your samples.

> **NOTE:** The remaining reconstituted Fluorescent 5X Master Mix can be aliquoted into microcentrifuge tubes and stored at -20 °C to -80 °C for up to 8 weeks. Each aliquote can undergo up to three freeze-thaw cycles.

Prepare the Biotinylated Ladder (Green Tube with Pink Pellet in Sealed Pack)

1. Pierce the foil with a pipette tip.
2. Add 20 µL deionized water.
3. Gently mix by pipetting up and down.

Prepare Your Samples

NOTES:
Prepare enough sample to pipette 3 µL per well.

The optimal protein concentration depends on your protein's expression level. Dilute lysates as necessary with 0.1X Sample Buffer. For a new assay measuring endogenous protein levels, we recommend starting around a final concentration of 0.4 mg/mL for a chemiluminescence immunoassay and 1.0 mg/mL for a fluorescence immunoassay. Refer to the **Bio-Techne Academy** for information on determining optimal lysate concentrations.

Bicine/CHAPS lysis buffer (P/N 040-764) and RIPA lysis buffer (P/N 040-483) are optimized for use with Simple Western systems and can be used to lyse your cells. If you would prefer to use your own buffer, refer to the **Simple Western Size Assay Buffer Compatibility Matrix** to check for the compatibility of commonly used commercial buffers or buffer components with Simple Western systems.

1. In a microcentrifuge tube, combine 1-part Fluorescent 5X Master Mix with 4-parts diluted lysate.
2. Close the microcentrifuge tube.
3. Pipette or gently vortex the sample to mix thoroughly.
4. Centrifuge the microcentrifuge tube and heat the sample in a water bath, heat block, or thermocycler at 95 °C for 5 minutes.

NOTE: Some proteins may require other denaturation conditions. Denature your sample at the optimal temperature for your target of interest.

5. Vortex the sample.
6. Spin down the sample.
7. Store the sample on ice until you are ready to pipette the Sample Plate.

Preparing Your Assay Reagents

Primary Antibody

NOTES:
Prepare enough diluted primary antibody to pipette 10 µL per well if that row will be sampled 1–2 times or 20 µL per well if that row will be sampled 3–4 times.

The primary antibody will need to be titrated to determine the optimal dilution. We recommend starting with 1:10, 1:50, and 1:250 dilutions. Refer to the **Bio-Techne Academy** for information on determining optimal primary antibody dilutions.

An optional System Control Primary Antibody (P/N 042-196 or 042-191) can be mixed with your primary antibody in the assay to calculate inter-assay and inter-instrument variability.

Refer to the **Simple Western Antibody Database** to search for commercial antibodies validated for Simple Western systems.

1. Dilute your Primary Antibody into Antibody Diluent 2. If you are using a Goat Primary Antibody with the Simple Western Goat Secondary Antibody, dilute your Primary Antibody with Milk-Free Antibody Diluent.
2. If you're using the System Control, dilute the System Control (10X) to 1X in your diluted Primary Antibody.
3. Store the Primary Antibody on ice until you are ready to pipette the Sample Plate.

NOTES: You can perform sized-based multiplexing in one capillary by probing for two different proteins at the same time. The molecular weights of the proteins you are simultaneously detecting will need to be far apart enough that they can be resolved from each other. For multiplexing, just dilute both Primary Antibodies into the appropriate Antibody Diluent. Contact Technical Support or your local Field Application Scientist to learn more.

The reported molecular weight for sample proteins in Compass for Simple Western may vary from apparent molecular weights based on other techniques as well as 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.*

Secondary Antibody

The chemiluminescence Secondary HRP Conjugate in the Simple Western Detection Modules for Chemiluminescence are ready-to-use. Store them on ice until you are ready to pipette them into the Sample Plate.

The fluorescence Secondary Dye Conjugates in the Simple Western Detection Modules for Fluorescence are provided as a 20X solution. To dilute, add 15 µL of the Secondary Dye Conjugate to 285 µL of the Milk-Free Antibody Diluent. If multiplexing with two Secondary Dye Conjugates, prepare the multiplex mix by diluting 15 µL of each selected Secondary Dye Conjugate (totaling 30 µL) with 270 µL of Milk-Free Antibody Diluent into one solution. Store the prepared multiplex mix on ice until you are ready to pipette them into the Sample Plate.

NOTES:
If you use your own HRP-labeled secondary antibody, dilute the antibody with the same Antibody Diluent used for your primary antibody. If you use your own secondary dye conjugate, dilute the antibody with Milk-Free Antibody Diluent. The secondary antibody will need to be titrated to determine the optimal dilution. Refer to the **Bio-Techne Academy** for information on determining optimal secondary antibody dilutions.

Refer to the **Simple Western Antibody Database** to search for commercial antibodies validated for Simple Western systems.

Luminol-S/Peroxide (only if performing a Chemiluminescence Assay)

NOTE: The volume of prepared Luminol-S/Peroxide you need will depend on the number of capillary cartridges you are running in one run and the type of assay being performed.

1. Combine Luminol-S and Peroxide in a microcentrifuge tube or conical tube according to the following table:

Assay	Cartridge #	Luminol-S	Peroxide	Total
Non-RePlex Chemiluminescence Immunoassay	1–2	700 µL	700 µL	1400 µL
	3–4	950 µL	950 µL	1900 µL
RePlex Assay	1–2	950 µL	950 µL	1900 µL
	3–4	1300 µL	1300 µL	2600 µL

2. Mix thoroughly by pipette or vortex.
3. Store the Luminol-S/Peroxide mix on ice until you are ready to pipette the Sample Plate.

RePlex Reagent (only if performing a RePlex Assay)

The RePlex Reagent mix removes primary and secondary antibodies from the first probe so you can perform a second immunoassay or Total Protein assay as a second probe in a single run.

NOTE: The volume of prepared RePlex Reagent mix you need will depend on the number of capillary cartridges you are running in one run.

1. Combine RePlex Reagent 1 and RePlex Reagent 2 in a microcentrifuge tube or conical tube according to the following table:

Cartridge #	RePlex Reagent 1	RePlex Reagent 2	Total
1–2	960µL	240 µL	1200 µL
3–4	1760 µL	440 µL	2200 µL

2. Close the tube and vortex briefly.
3. Store the prepared RePlex Reagent mix at room temperature until you are ready to pipette the Sample Plate.

Total Protein Biotin Labeling Reagent (only if performing a Total Protein Normalization Assay using RePlex and Chemiluminescence Detection)

The Total Protein assay enables total protein normalization within a capillary. Total Protein Biotin Labeling Reagent adds a biotin molecule to all the samples' proteins before detection with the Total Protein Streptavidin-HRP.

NOTES:
Prepare 1 tube of Total Protein Biotin Labeling Reagent if you are running 1–2 cartridges, and 2 tubes of Total Protein Biotin Labeling Reagent if you are running 3–4 cartridges.

The Total Protein Biotin Labeling Reagent should be prepared right before pipetting it into the Sample Plate, after all other reagents have already been loaded on the plate.

The Total Protein Assay is compatible with lysate concentrations ranging from 0.05 to 0.2 mg/mL. For new samples, we recommend titrating lysates to ensure total protein signal linearity within the desired concentration range. Refer to the **Bio-Techne Academy** for more information about Total Protein Assay optimization.

The Total Protein Streptavidin-HRP is different from the Streptavidin-HRP used for the biotinylated ladder in an immunoassay.

1. Completely thaw the Reconstitution Reagent 1 (white cap, recommended at least 30 minutes) before preparing the Total Protein Biotin Labeling Reagent. Do NOT place the tube on ice.
2. Pierce the foil of the Total Protein Biotin Labeling Reagent tube with a pipette tip.
3. For each tube of Total Protein Biotin Labeling Reagent:
    a. Add 150 µL of the Total Protein Reconstitution Agent 1 (white cap) to each tube of Total Protein Biotin Labeling Reagent.
    b. Gently mix by pipette.
    c. Add 150 µL of the Total Protein Reconstitution Agent 2 (orange cap) to each tube of reconstituted Total Protein Biotin Labeling Reagent.
4. Gently mix by pipette until mixed thoroughly.
5. If multiple tubes were prepared, combine the Total Protein Biotin Labeling Reagent into one microcentrifuge tube and mix by pipette.
6. Load the reconstituted Total Protein Biotin Labeling Reagent on the Sample Plate immediately prior to spinning the plate. Do NOT place the tube on ice at any time.

Protein Normalization Reagent (only if performing a Protein Normalization Assay using the PN Channel)

The Protein Normalization assay enables total protein normalization within a capillary without a RePlex assay. Protein Normalization Reagent adds a fluorophore to all the samples' proteins before detection in the PN channel. Protein Normalization can be used in combination with a Leo Fluorescence Separation Module and a Chemiluminescence or Fluorescence Detection Module.

NOTES:
The Protein Normalization Reagent should be prepared right before pipetting it into the Sample Plate, after all other reagents have already been loaded on the plate.

The Protein Normalization Assay is compatible with lysate concentrations ranging from 0.2 to 1.2 mg/mL. For new samples, we recommend titrating your lysate to ensure protein normalization signal linearity in desired concentration range. Refer to the **Bio-Techne Academy** for information on optimizing Protein Normalization assays.

Reconstitute the following number of Protein Normalization Reagent tubes based on the Separation Module used:

- 2 tubes: 12–230 kDa and 2–40 kDa Separation Modules:
- 3 tubes: 66–440 kDa Separation Module

NOTE: Contact your Field Application Sceintist to discuss assay setup and optimization if you want to use the Protein Normalization Reagent with a 66–440 kDa Separation Module.

Prepare Protein Normalization Reagent Stock Solution

1. Pierce the foil of the Protein Normalization Reagent tube with a pipette tip.
2. For each tube of Protein Normalization Reagent tube:
   a. Add 100 µL of Protein Normalization Reconstitution Agent to the tube.
   b. Thoroughly mix the stock solution by pipetting 15 times.

Prepare Protein Normalization Reagent Working Solution

1. Use the following table to prepare in a separate microcentrifuge tube working solutions of the reconstituted Protein Normalization Reagent based on the Separation Module used for the assay.

Separation Module Molecular Weight Range	Stock Solution	Reconstitution Reagent	Total
2–40 kDa	200 µL	1000 µL	1200 µL
12–230 kDa	200 µL	1000 µL	1200 µL
66–440 kDa	300 µL	600 µL	900 µL

2. Thoroughly mix the working solution by pipetting 15 times.
3. Load the Protein Normalization Reagent working solution on the Sample Plate immediately prior to spinning the plate.

Preparing Your Plates

Pre-filled Reagent Plate(s)

NOTES:
You will need one Pre-filled Reagent Plate for every Capillary Cartridge used in the run.

Pre-filled Reagent Plates should be stored at room temperature for at least 24 hours after receiving them before running an assay.

The separation range for the Pre-filled Reagent Plates used in a single Leo Instrument run all must be the same.

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

Sample Plate

NOTES:
For more consistent results, keep the plate cover on the Leo Sample Plate between reagent additions and minimize bubble formation when adding reagents to the plate.

The first capillary in the cartridge has been optimized and should only be used for running the ready-to-use biotinylated ladder.

If a row in the Sample Plate is used for the assay, don't leave any empty wells in the row. Fill empty wells in rows used in the assay with the same diluent used for the entire row. For rows filled with ready-to-use reagents, fill the empty wells with either Antibody Diluent 2 or Milk-free Antibody Diluent. For sample rows, fill empty wells with 0.1X Sample Buffer.

1. Dispense reagents into Sample Plate wells (rows A–L and well M1) using the volumes shown in the tables below.
2. Carefully dispense the reagents in the Sample Plate troughs (rows M-P) 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 to avoid creating bubbles.

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

3. Cover the plate with the plate cover and centrifuge the plate 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.
4. Pop any bubbles with a pipette tip.
5. Store the plate on a flat surface with the plate cover on at room temperature until you are ready to start your run.

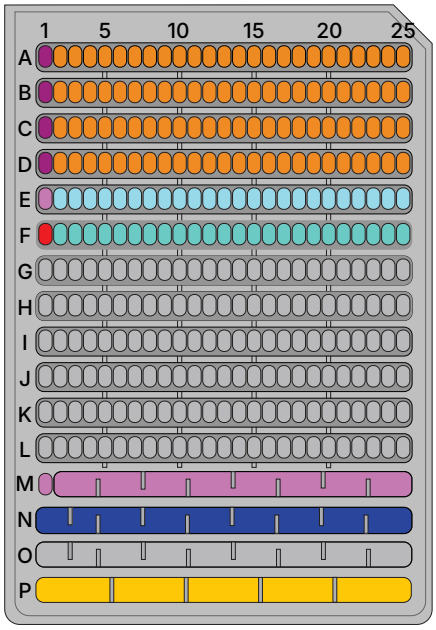
Some example plate layouts with four rows of samples are shown below.

CHEMILUMINESCENCE IMMUNOASSAY

IMPORTANT: Row N must be filled with Wash Buffer and Row P must be filled with Luminol-S/Peroxide mix.

NOTES:
The user defines where reagents are pipetted in Rows A–L.

Reagent locations will be defined in Compass for Simple Western Software before starting the run. See "Creating a New Assay: Immunoassay" on page 53 for more information. Make sure to select the desired assay layout before adding names and attributes to the Sample Template.



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

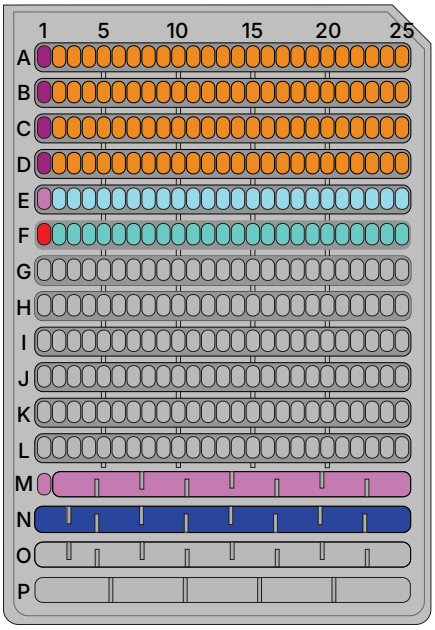
Well Reagent (Row A–L)	Sampled* 1–2 times (µL/well)	Sampled* 3–4 times (µL/well)
Biotinylated Ladder	3	3
Sample	3	3
Antibody Diluent	10	20
Primary Antibody	10	20
Streptavidin-HRP	10	20
Secondary Antibody	10	20
Well Reagent (M1)	**1–2 Cartridges (µL)**	**3–4 Cartridges (µL)**
Antibody Diluent	30	30
Trough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

FLUORESCENCE IMMUNOASSAY

IMPORTANT: Row N must be filled with Wash Buffer.

NOTES:
The user defines where reagents are pipetted in Rows A–L.

Reagent locations will be defined in Compass for Simple Western Software before starting the run. See “Creating a New Assay: Immunoassay” on page 53 for more information. Make sure to select the desired assay layout before adding names and attributes to the Sample Template.



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

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

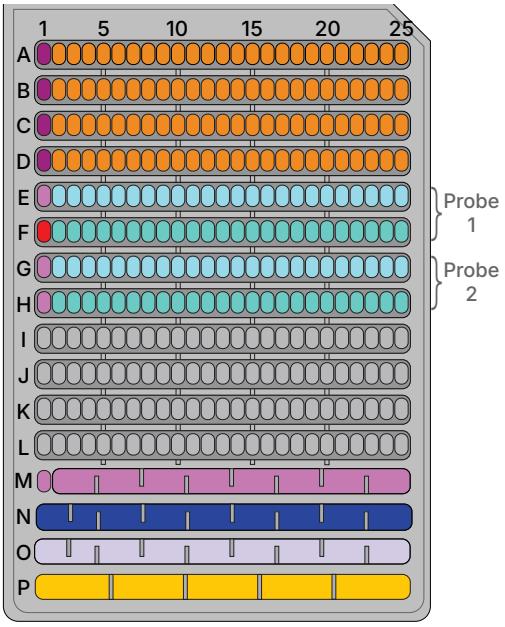
CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY + CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY USING REPLEX:

IMPORTANT: Row N must be filled with Wash Buffer and Row O must be filled with RePlex Reagent mix. If a chemiluminescence assay is being performed, Row P must be filled with Luminol-S/Peroxide mix.

NOTES:
The user defines where reagents are pipetted in Rows A-L.

Reagent locations will be defined in Compass for Simple Western Software before starting the run. See "Creating a New Assay: RePlex" on page 74 for more information. Make sure to select the desired assay layout before adding names and attributes to the Sample Template.

The chemiluminescence or fluorescence immunoassay selected for Probe 1 is run first. When Probe 1 is complete, the Leo Instrument performs a RePlex purge to remove the bound antibodies from Probe 1 before beginning the chemiluminescence or fluorescence immunoassay selected for Probe 2.



*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, chemiluminescence only)	350	500

CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY + TOTAL PROTEIN NORMALIZATION USING REPLEX AND CHEMILUMINESCENCE DETECTION:

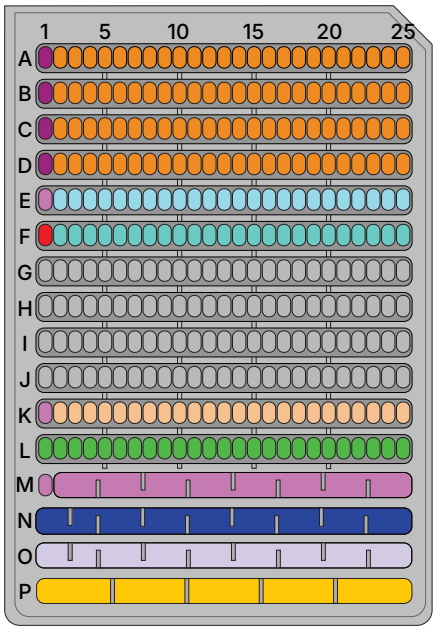
IMPORTANT: Row N must be filled with Wash Buffer, Row O must be filled with RePlex Reagent mix, and Row P must be filled with Luminol-S/Peroxide mix.

NOTES:
Total Protein assay reagents are assigned to row K and L by default. You can reassign them to any location between rows A–L.

The user defines where samples and antibodies are pipetted in Rows A–L.

Reagent locations will be defined in Compass for Simple Western Software before starting the run. See "Creating a New Assay: RePlex" on page 74 for more information. Make sure to select the desired assay layout before adding names and attributes to the Sample Template.

The chemiluminescence or fluorescence immunoassay selected for Probe 1 is run first. When Probe 1 is complete, the Leo Instrument performs a RePlex purge to remove the bound antibodies from Probe 1 before detecting the Total Protein assay signal in Probe 2.



*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
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:

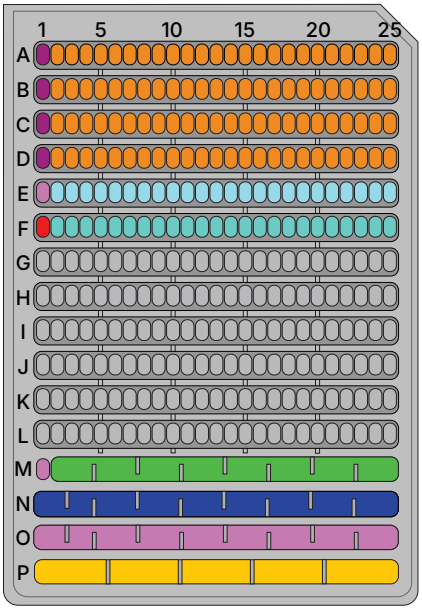
IMPORTANT: Row N must be filled with Wash Buffer. If a chemiluminescence assay is being performed, Row P must be filled with Luminol-S/Peroxide mix.

NOTES:
Protein Normalization assay reagents are assigned to row M by default and should not be moved to another trough.

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

The user defines where samples and antibodies are pipetted in Rows A–L.

Reagent locations will be defined in Compass for Simple Western Software before starting the run. See “Creating a New Assay: Protein Normalization” on page 92 for more information. Make sure to select the desired assay layout before adding names and attributes to the Sample Template.



*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)**
Protein Normalization Reagent	900	900
Wash Buffer	2000	2000
Antibody Diluent	1500	1500
Luminol-S/Peroxide (per compartment, chemiluminescence only)	250	350

Creating a New Assay: Immunoassay

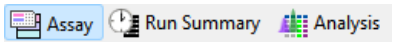
Create an assay using Compass for Simple Western Software. To connect your Leo Instrument to Compass, select **Instrument** in the main menu and click **Connect...**. Then select your instrument and click **Connect**.

IMPORTANT: If you plan on running a Fluorescence Immunoassay on a Leo Instrument initially configured for chemiluminescence only, contact ProteinSimple Technical Support to enable fluorescence functionality. A warning will appear if you try to run a Fluorescence Assay before your Leo Instrument is updated.

NOTES:
If you are using a Wes™ System, Peggy Sue™ System, Sally Sue™ System, or NanoPro™ 1000 System, 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 a Peggy Sue, Sally Sue, or NanoPro 1000 Systems and software versions 6.x and lower for Wes Systems to create, load, and run assays on these systems, and to analyze data.

To learn more about Compass for Simple Western, refer to the **Compass for Simple Western User Guide**.

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

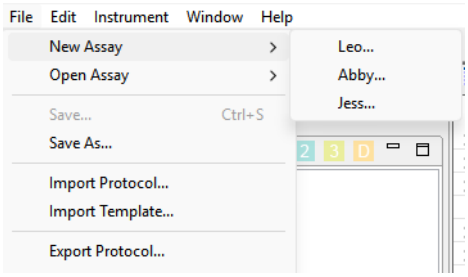


You can run an existing assay or create a new assay. See “Opening and Modifying an Existing Assay” on page 106.

To create a new assay, we recommend using one of the default template assays and making any necessary modifications from there. See “Creating a Custom Template Assay” on page 107 for more information.

Step 1: Open a Template Assay

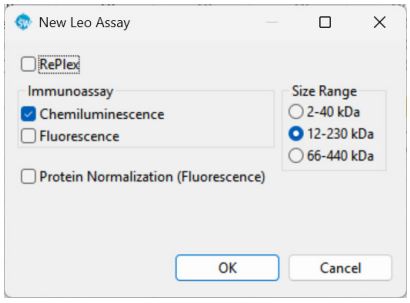
1. Select the **Assay** screen.
2. If your Leo Instrumentis not connected to Compass for Simple Western, select **File** in the main menu, click **New Assay**, and select **Leo**.



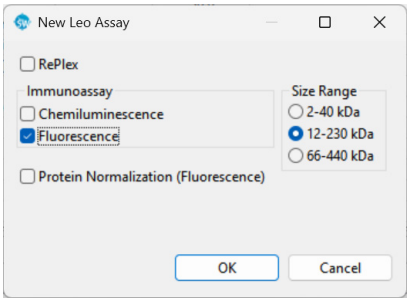
If your Leo Instrument is connected to Compass for Simple Western, select **File** in the main menu and click **New Assay**.

3. Select the appropriate **Assay Type** or **Immunoassay** and **Size Range**.

CHEMILUMINESCENCE IMMUNOASSAY:

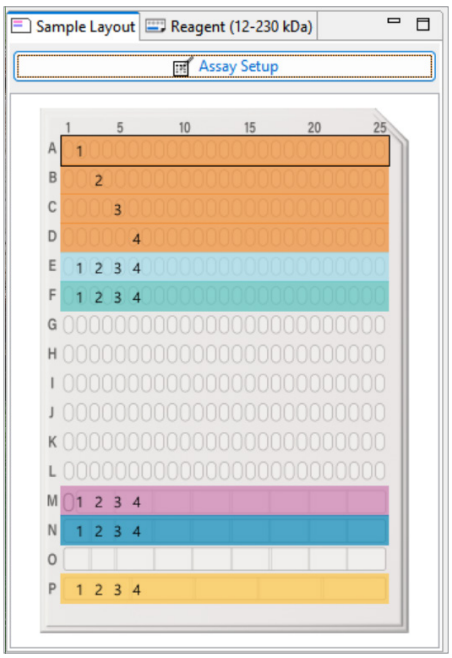


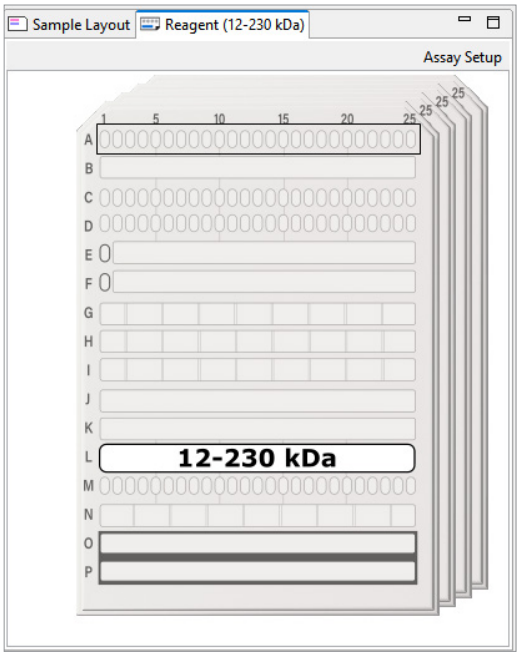
FLUORESCENCE IMMUNOASSAY:



4. Click **OK**.

5. A template assay with default reagent locations will display in the Sample Layout pane of the Assay screen. The Reagent pane will display the size range selected. The Sample Layout pane example shown below is for a Chemiluminescence Immunoassay, and the Reagent pane is for an assay using the 12–230 kDa Size Range.



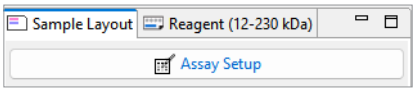


The numbers on the Sample Plate indicate the row each cartridge will load reagents from.

NOTE: If you make changes to the assay, such as adding rows or changing the reagent assigned to a row, be sure to select **File** and **Save** before proceeding.

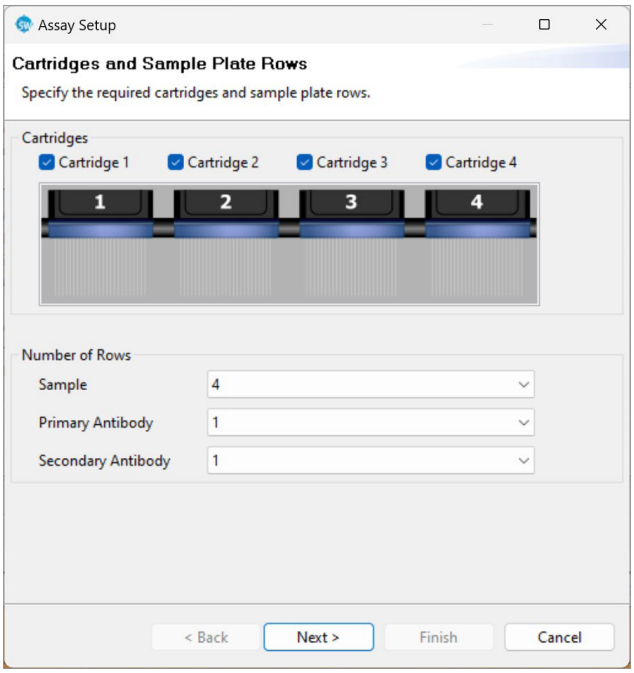
Step 2: Assign Sample Plate Reagents

1. Click on the **Assay Setup** button under the Sample Layout or Reagent tab.



2. Define the assay parameters.
   a. Deselect the boxes next to the cartridge holder as needed 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-downs.

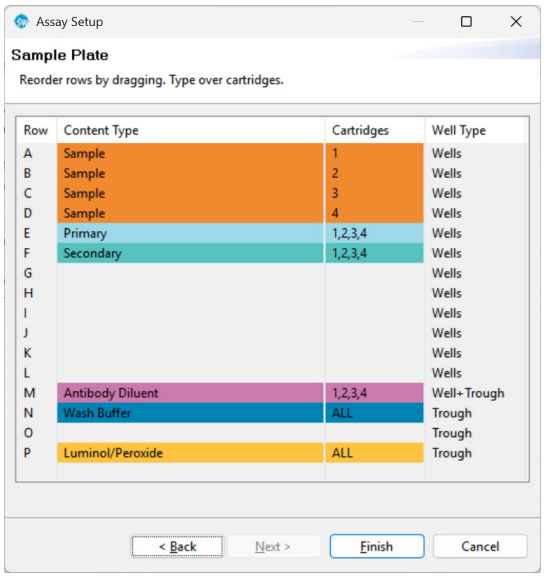
      Default parameters with four rows of samples are shown. The number of Sample rows will automatically adjust if less than four cartridges are selected.



3. Click **Next**.

4. An ‘Assay Setup - Sample Plate’ prompt will appear. Color coding is used to identify various assay reagents.
    - **Orange** – Samples and Biotinylated ladder
    - **Light Teal** – Primary antibody
    - **Teal** – Secondary conjugate
    - **Magenta** – Antibody Diluent (2 or Milk-free)
    - **Blue** – Wash Buffer
    - **Gold (if applicable)** – Luminol-S/Peroxide mix

CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY:



NOTE: Luminol-S/Peroxide will only appear in Row P if a Chemiluminescence Immunoassay is being performed.

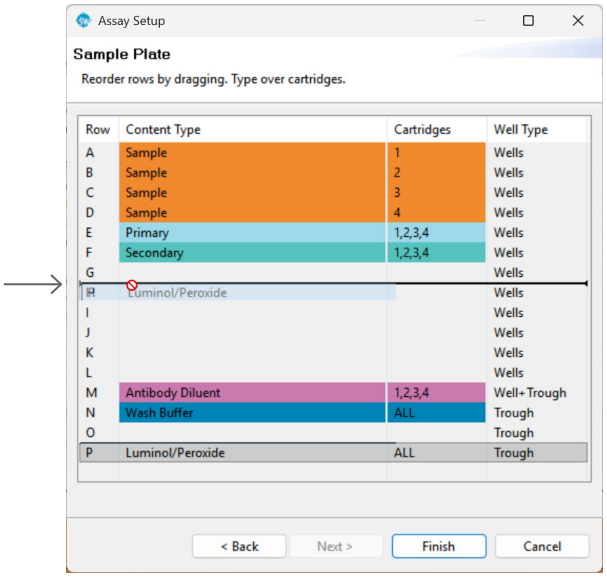
5. The default protocol automatically assigns all reagent locations for the assay.

    Reagent assignments to rows M–P should not be modified.

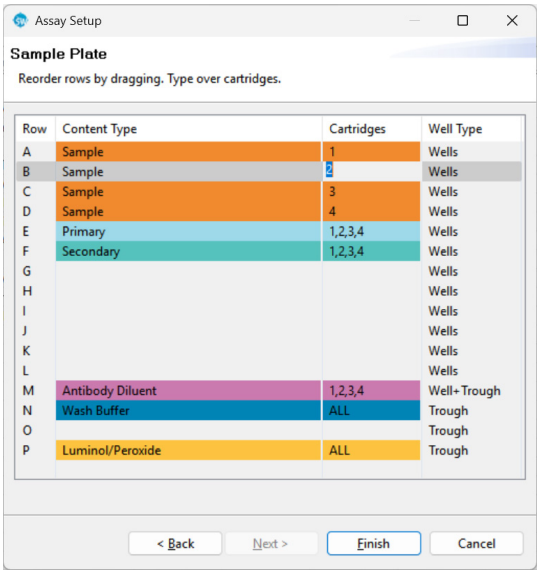
6. Modify reagent assignments as needed for rows A–L.

   a. Reorder row locations by clicking on the row and dragging it to the desired location. The examples below are from setting up a Chemiluminescence Immunoassay.

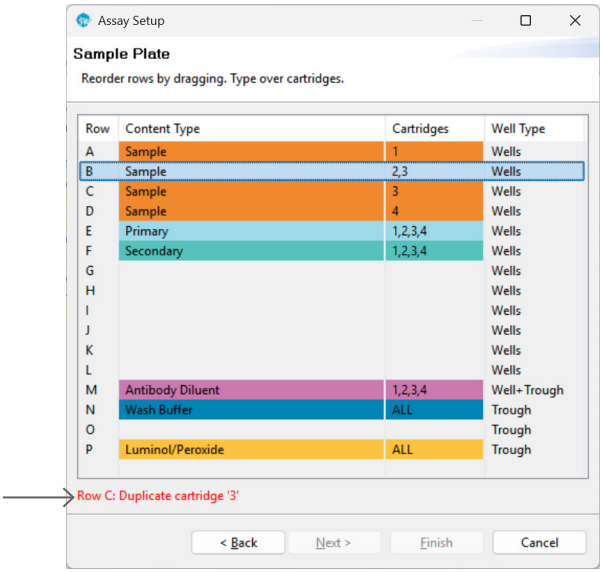
      A warning will appear if you try to move the reagent to a row that is not allowed.

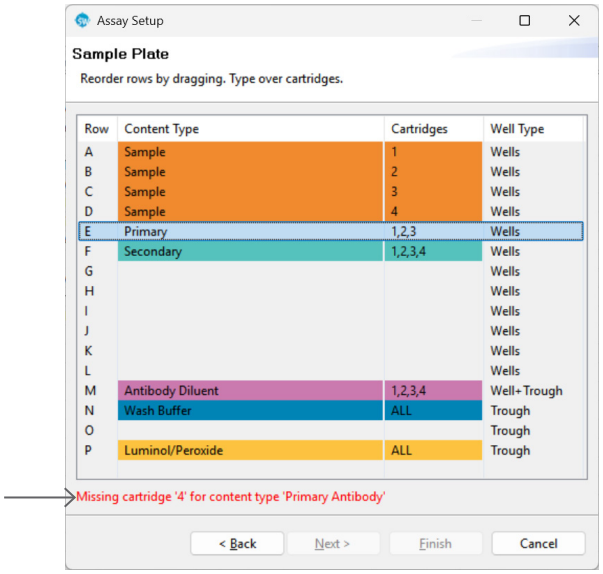


   b. Change the row a cartridge will load a sample, primary antibody, or secondary antibody from by clicking on the cell in the Cartridges column and typing in the cartridge number.



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

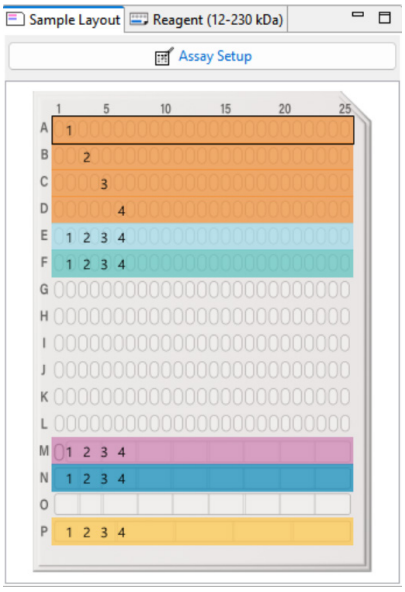




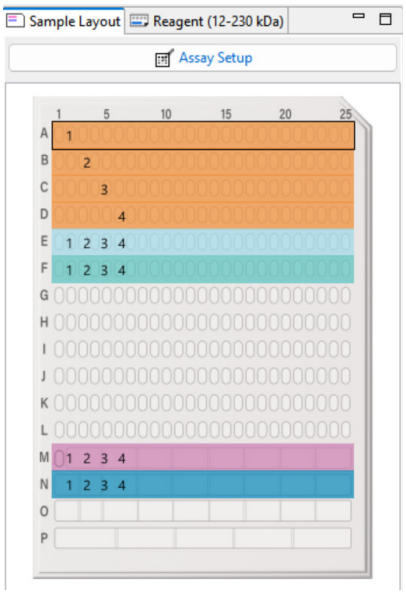
7. To add or remove a row, click the **Back** button and adjust the number of rows using the drop-down menus.

8. Click **Finish**. You can also adjust the row a cartridge will load from later in the Protocol pane.
9. A Sample Plate with updated reagent locations will display in the Assay screen. Numbers on the Sample Plate indicate the row each cartridge will be loaded from.

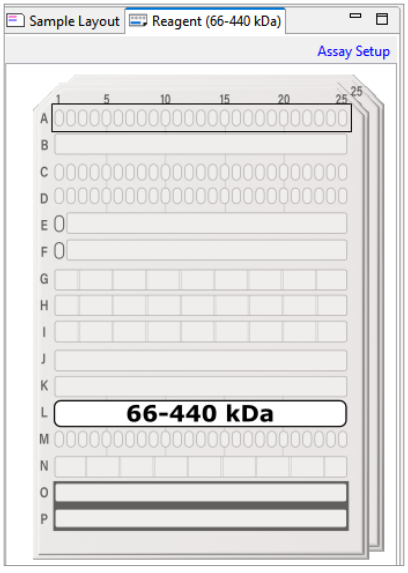
CHEMILUMINESCENCE IMMUNOASSAY



FLUORESCENCE IMMUNOASSAY



10. The Reagent pane will display the size range selected and the number of Pre-filled Reagent Plates required to perform your run. The Reagent pane example below is for a run using 66–440 kDa plates that is running two capillary cartridges.

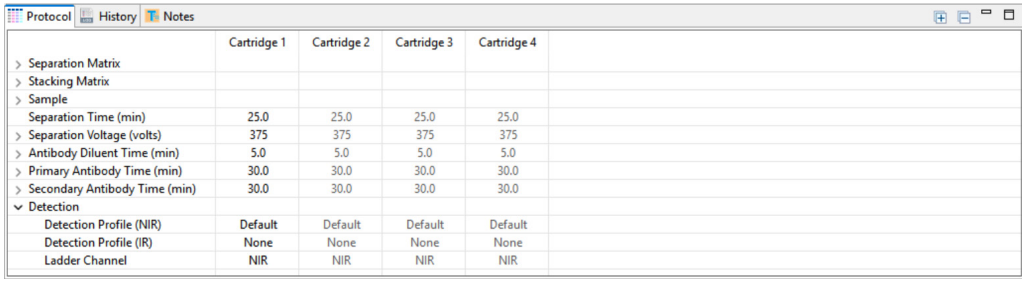


Step 3: Modifying the Assay Protocol (Optional)

NOTE: The capillary cartridges are all run in parallel, so voltage- and time-based parameters for all the cartridges will 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.

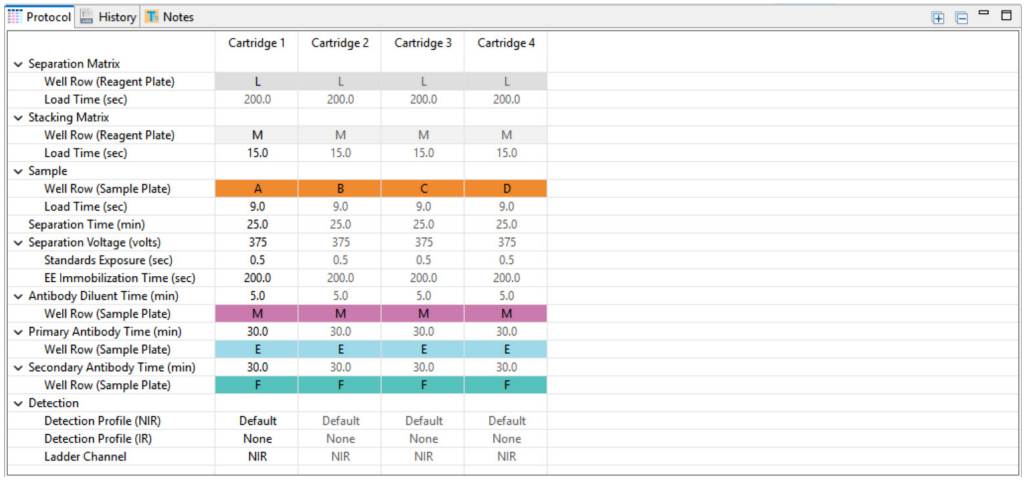
To Modify an Assay Protocol:

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 Fluorescence Immunoassay using 4 cartridges.



	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				
Detection Profile (NIR)	Default	Default	Default	Default
Detection Profile (IR)	None	None	None	None
Ladder Channel	NIR	NIR	NIR	NIR

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				
Detection Profile (NIR)	Default	Default	Default	Default
Detection Profile (IR)	None	None	None	None
Ladder Channel	NIR	NIR	NIR	NIR

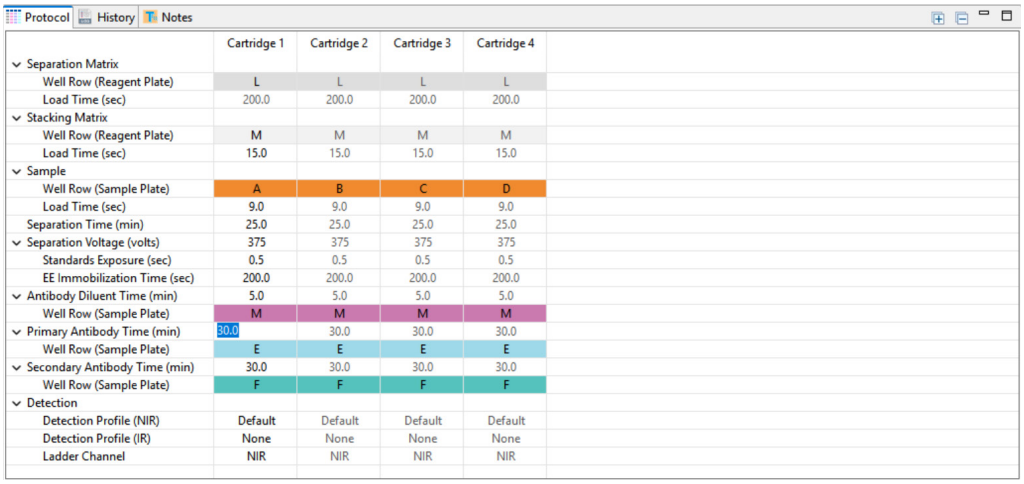
NOTE: To expand all parameters, click the ⊞ button at the upper right of the pane. To collapse all parameters, click the ⊟ button at the upper right of the pane.

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

To change the primary antibody incubation time:

a. Click the cell with the numerical value next to Primary Antibody Time (min) in the 'Cartridge 1' column.

b. Enter a new value in minutes.



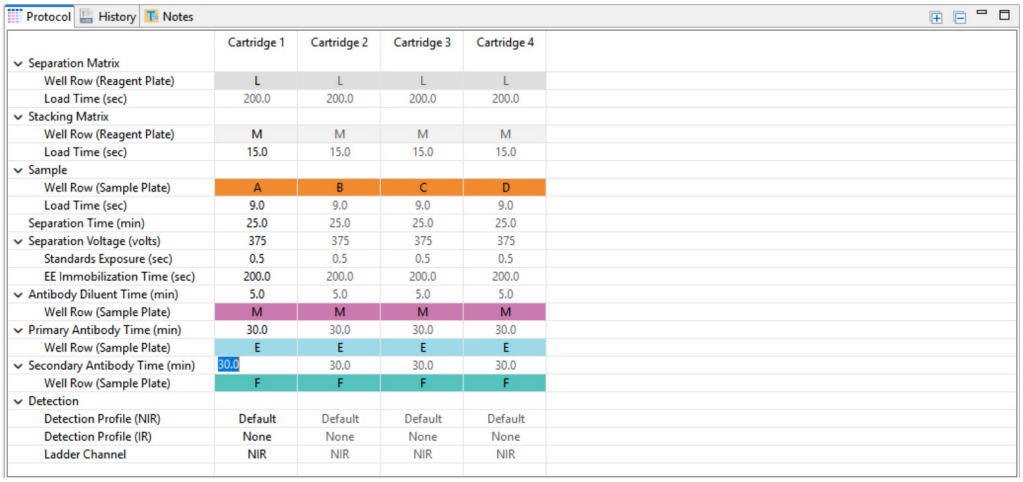
	Cartridge 1	Cartridge 2	Cartridge 3	Cartridge 4
Separation Matrix				
Well Row (Reagent Plate)	L	L	L	L
Load Time (sec)	200.0	200.0	200.0	200.0
Stacking Matrix				
Well Row (Reagent Plate)	M	M	M	M
Load Time (sec)	15.0	15.0	15.0	15.0
Sample				
Well Row (Sample Plate)	A	B	C	D
Load Time (sec)	9.0	9.0	9.0	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				
Detection Profile (NIR)	Default	Default	Default	Default
Detection Profile (IR)	None	None	None	None
Ladder Channel	NIR	NIR	NIR	NIR

c. The values for other cartridges will automatically update.

To change the secondary antibody incubation time:

a. Click the cell with the numerical value next to Secondary Antibody Time (min) in the 'Cartridge 1' column.

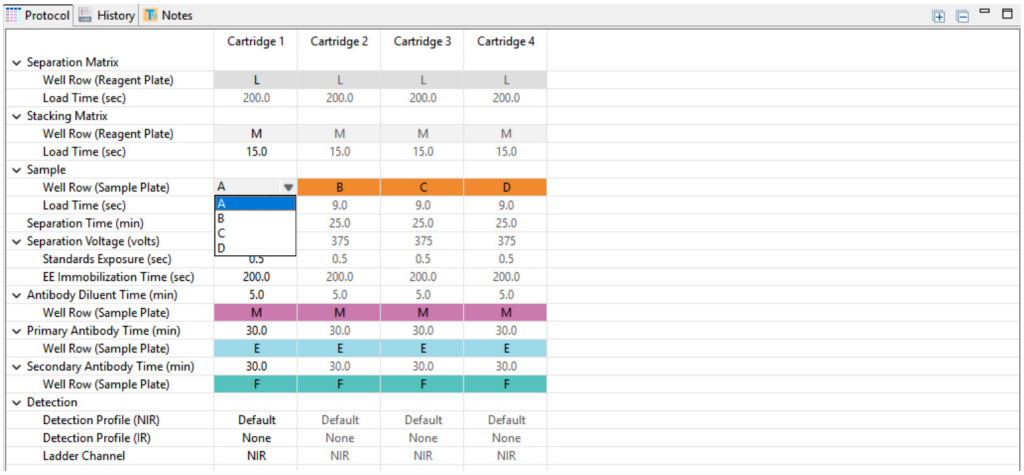
b. Enter a new value in minutes.



	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				
Detection Profile (NIR)	Default	Default	Default	Default
Detection Profile (IR)	None	None	None	None
Ladder Channel	NIR	NIR	NIR	NIR

c. The values for the other cartridges will automatically update.

3. **For reagents loaded in rows A–L:** You can change the row in the Sample Plate that a cartridge will load a reagent from. Click the cell in the column next to the Well Row and select a different row:



	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				
Detection Profile (NIR)	Default	Default	Default	Default
Detection Profile (IR)	None	None	None	None
Ladder Channel	NIR	NIR	NIR	NIR

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

4. Leo immunoassay protocols use default detection profiles.

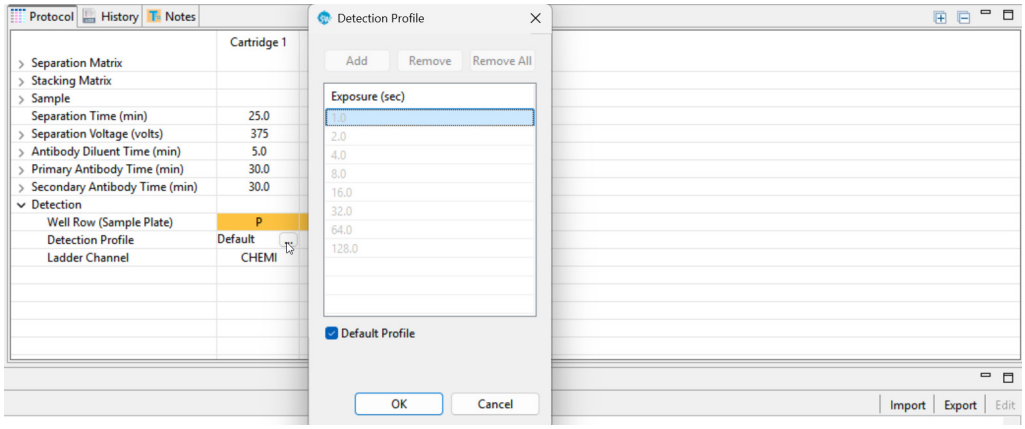
CHEMILUMINESCENCE DETECTION PROFILE

The default detection profile is similar to the High Dynamic Range (HDR) exposure series used for Jess and Abby assays, but removes the 512 second exposure. See the **Compass for Simple Western User Guide** for more information about HDR detection profiles.

To adjust exposures in the Detection Profile:

a. If needed, click the gray arrow next to Detection to expand the row.

b. Select the Default cell in the 'Cartridge 1' column next to Detection Profile and click the **...** button that appears to open the Detection Profile window.



c. Deselect the **Default Profile** checkbox.

d. To remove times, click the **Remove** button.

e. To add additional times to the protocol, click the **Add** button and enter the values.

f. Select **OK**.

g. The number of exposures in the modified chemiluminescence Detection Profile will appear.

NOTES:
You can only use the Remove All button to remove all exposure times if the Ladder Channel is different from the Detection Profile channel you are modifying.

No more than two exposures can be longer than 32 seconds in Chemiluminescence Immunoassays.

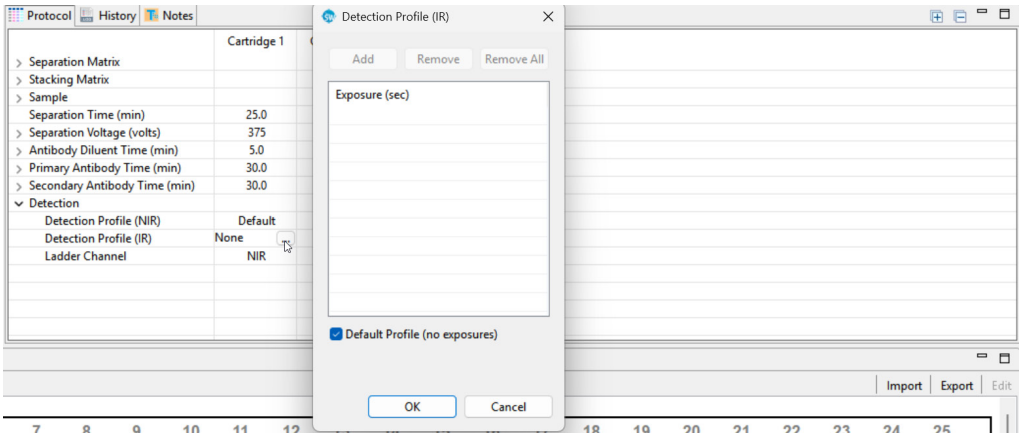
FLUORESCENCE DETECTION PROFILES

The default detection profile is in the NIR channel with 6 standard exposures.

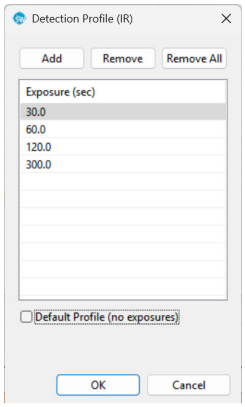
To add a Detection Profile to the IR channel:

a. If needed, click the gray arrow next to Detection to expand the row.

b. 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.



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



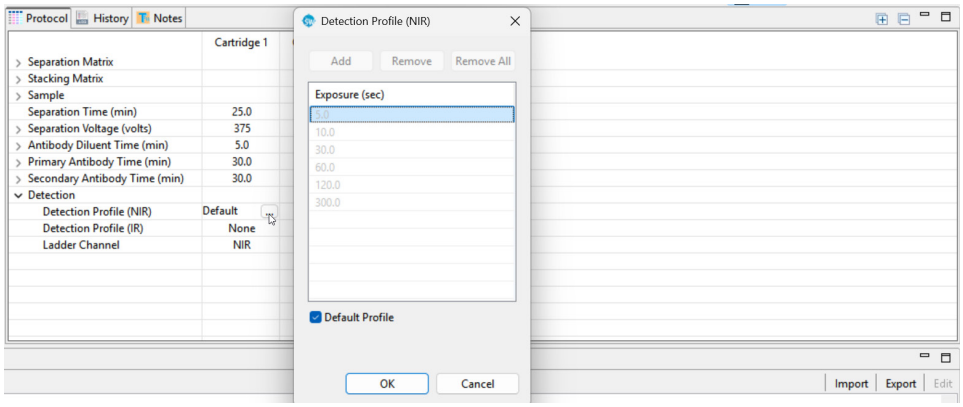
d. Select **OK**.

e. The number of exposures in the IR channel Detection Profile will appear.

To remove the Detection Profile from the NIR channel:

a. If needed, click the gray arrow next to Detection to expand the row.

b. Select the Default cell in the 'Cartridge 1' column next to Detection Profile (NIR) and click the **...** button that appears to open the Detection Profile window.



c. Deselect the Default Profile checkbox.

d. Click **Remove All**.

NOTE: You can only use the Remove All button to remove all exposure times if the Ladder Channel is different from the Detection Profile channel you are modifying.

e. Select **OK**.

f. 'None' will appear in the column next to Detection Profile (NIR) in the modified protocol.

To adjust exposures in the NIR or IR channel:

a. If needed, click the gray arrow next to Detection to expand the row.

b. Select the Default cell or None cell in the 'Cartridge 1' column next to Detection Profile (NIR) or Detection Profile (IR), respectively. Click the **...** button that appears to open the Detection Profile window.

c. Deselect the Default Profile or Default Profile (no exposures) checkbox.

d. To remove times, click the **Remove** button or **Remove All**.

NOTE: You can only use the Remove All button to remove all exposure times if the Ladder Channel is different from the Detection Profile channel you are modifying.

e. To add additional times to the protocol, click the **Add** button and enter the values.

f. Select **OK**.

g. The number of exposures in the modified Detection Profile(s) will appear.

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

Exporting and Importing Assay Protocols

A modified assay protocol can be exported for later use. Exporting an assay protocol exports information from the Protocol pane only.

To export an assay protocol:

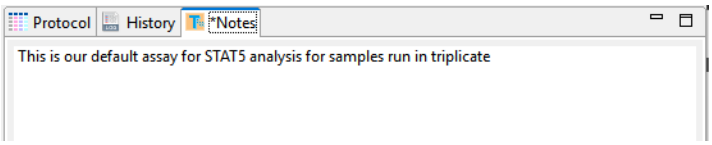
1. Select **File** in the main menu and click **Export Protocol...**.
2. The default directory is Compass for SW/Assays. Change the directory if needed.
3. Enter a protocol name and click **Save**. The protocol will be saved as a *.protocol file. The protocol file will include the information in the Protocol pane (steps and row locations), but will not include information in the Sample Template pane.

To import a previously saved assay protocol:

1. Select **File** in the main menu and click **Import Protocol...**.
2. Select the *.protocol file.
3. Click **Open**.

Step 4: Add Assay Notes (Optional)

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



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

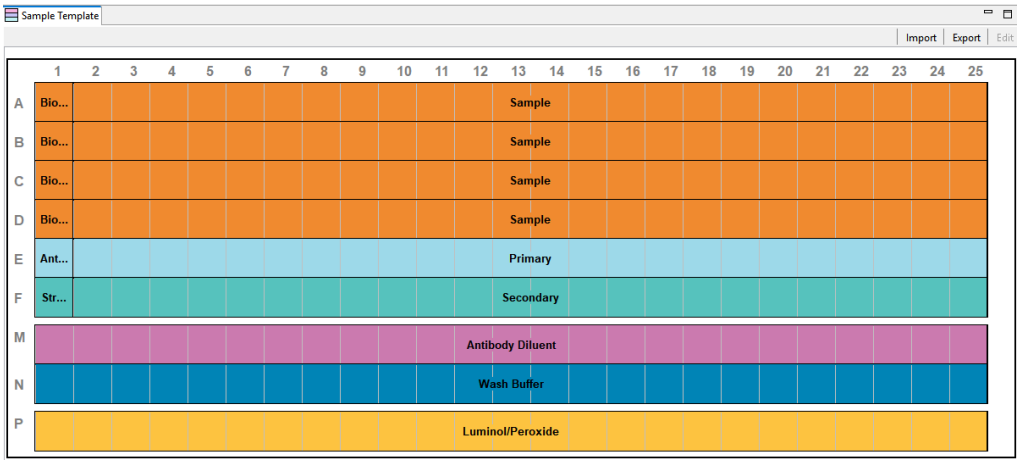
Step 5: Add Sample Template Annotations (Optional)

Custom reagent names, notes, and attributes can be entered for individual rows and wells on the Sample Plate. This information will be stored with the assay and run data. During the post-run analysis, this information can be used to apply custom analysis settings specific to sample names, primary antibody and secondary antibody names, blocking reagent names, and/or sample, primary antibody, and secondary antibody attributes. To learn more, refer to "Analysis Settings Overview" in the **Compass for Simple Western User Guide**.

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

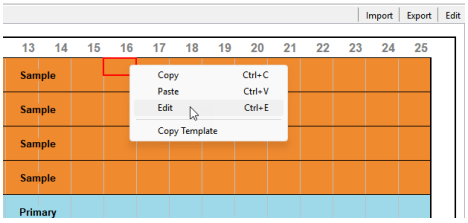
To enter annotations:

1. Click on the **Sample Template** tab. The default annotations for reagent rows and individual wells on the assay plate will be displayed. The example below is for a Chemiluminescence Immunoassay.

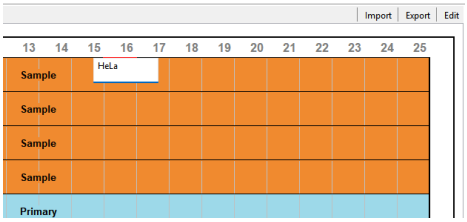


2. Change or add row and well annotations as needed. To do this:

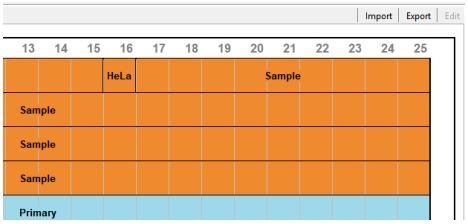
   a. **To change the reagent name for a specific well** – Select the top of the well and then either double-click, right-click and select **Edit**, click **Edit** in the upper right corner of the pane, or press **Ctrl+E**.



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

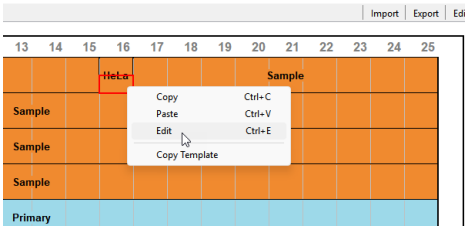


The new information will display in the selected well.

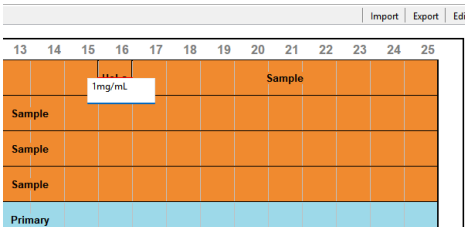


To undo changes, press **Ctrl+Z** or select **Undo** from the **Edit** menu.

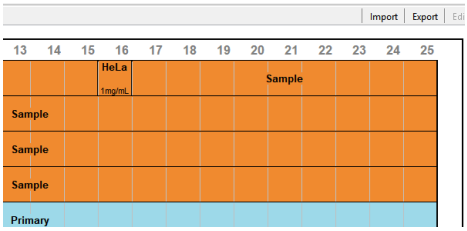
b. **To add an attribute to a specific well** – Select the bottom of the well and then either double-click, right-click and select **Edit**, click **Edit** in the upper right corner of the pane, or press **Ctrl+E**.



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

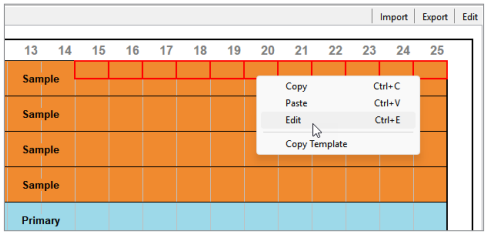


The new information will display in the selected well.

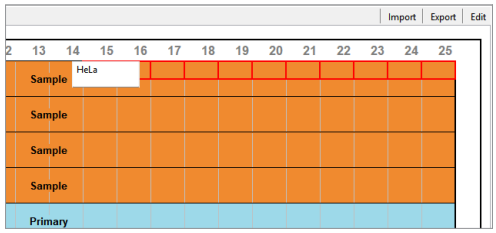


To undo changes, press **Ctrl+Z** or select **Undo** from the **Edit** menu.

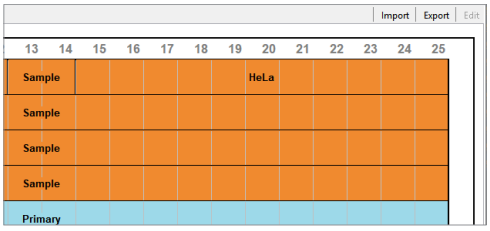
c. **To change the reagent name for multiple wells** – To select individual wells, press and hold the **Ctrl** key and then select the top of individual wells. To select a sequential set of wells or a full row, press and hold the **Shift** key, then select the top of the first well and last well, or hold down the left mouse button and drag the mouse across the top of the sequential wells you want to select. Next, right-click and select **Edit,** click **Edit** in the upper right corner of the pane, press **Ctrl+E**, or double-click with the mouse while still holding down the **Ctrl** or **Shift** key.



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

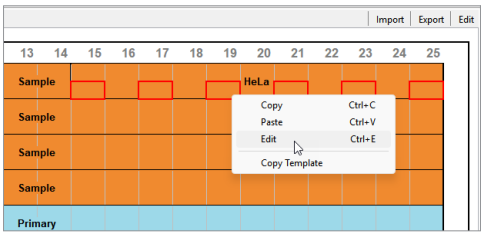


The new information will be displayed in the selected wells.

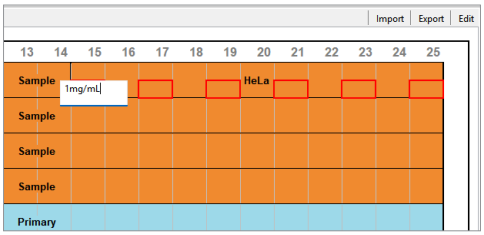


To undo changes, press **Ctrl+Z** or select **Undo** from the **Edit** menu.

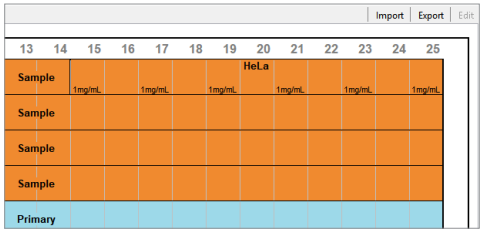
d. **To add an attribute to multiple wells** – To select individual wells, press and hold the **Ctrl** key and then select the bottom of individual wells. To select a sequential set of wells or a full row, press and hold the **Shift** key, then select the bottom of the first well and last well, or hold down the left mouse button and drag the mouse across the bottom of the sequential wells you want to select. Next, right-click and select **Edit**, click **Edit** in the upper right corner of the pane, press **Ctrl+E**, or double-click with the mouse while still holding 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.

Custom reagent names and attributes can also be copied and pasted within Compass for Simple Western, between two copies of Compass, and between Compass and a spreadsheet like Microsoft® Excel®.

To copy/paste a reagent name or attribute from the Sample Template:

1. Select the reagent name or attribute from the Sample Template. You can also select the reagent name and attribute for one or multiple cells by pressing **Ctrl** to select individual annotations or **Shift** to select sequential annotations.
2. Click **Ctrl+C** or select **Copy** in the **Edit** menu.
3. Select the well(s) to paste the information into.

a. If one well is selected, the information will be pasted sequentially.

b. If individual wells are selected by pressing **Shift** while clicking on the wells, the information will be pasted into the selected cells.

4. Press **Ctrl+V** or select **Paste** in the **Edit** menu.

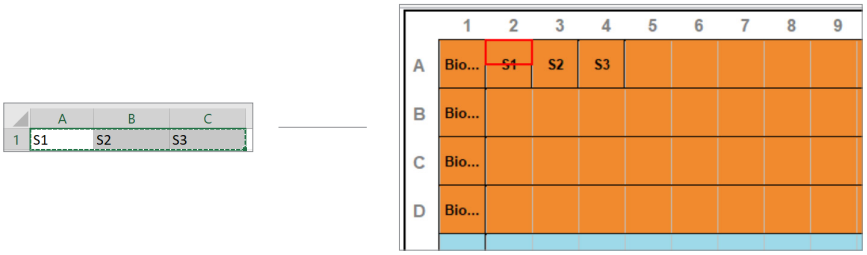
> **NOTE:** Annotations pasted into the Sample Template cannot be undone by pressing **Ctrl+Z** or selecting **Undo** in the **Edit** menu.

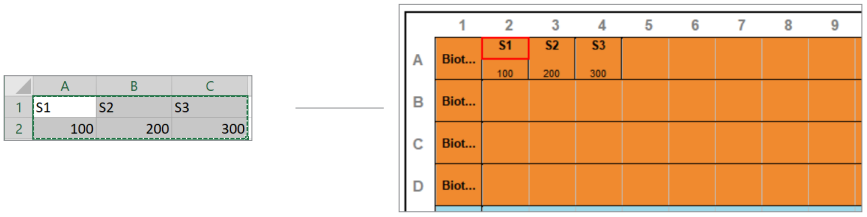
To copy/paste a reagent name or attribute from Microsoft® Excel®:

1. Enter names in a spreadsheet row. To copy/paste names and attributes, enter names in one row and the attributes in the next row.

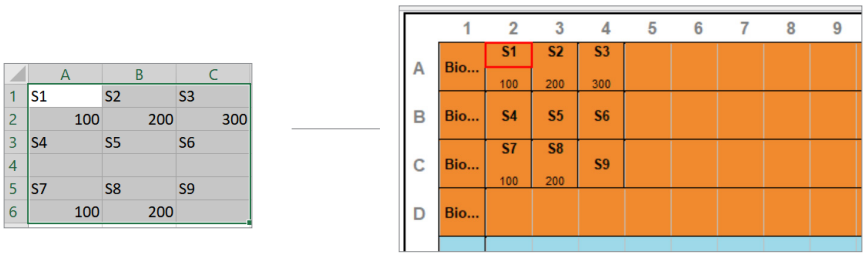
2. Copy the names into the clipboard.

3. Select a well in the Sample Template and paste the names from the clipboard by clicking **Ctrl+V** or selecting **Paste** in the **Edit** menu. Make sure not to select any of the ladder wells (A1, B1, C1, etc.).





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



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

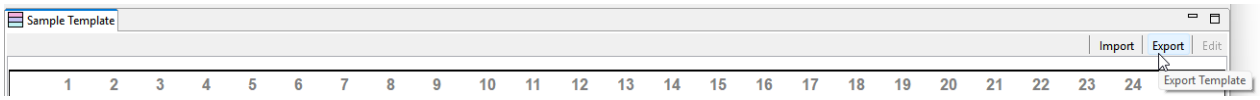
Sample Template information can be exported to a file. There are three file format options:

- 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.

A template can be exported from Compass for Simple Western, populated with reagent names (sample, antibody, etc.) and their corresponding attributes (concentration, dilution factor, etc.), in a spreadsheet like Microsoft® Excel®.

To export and populate a Sample Template:

1. Click **Export** in the upper right corner of the Sample Template pane or select **File** in the main menu and click **Export Template...**.



2. 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 Sample 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.

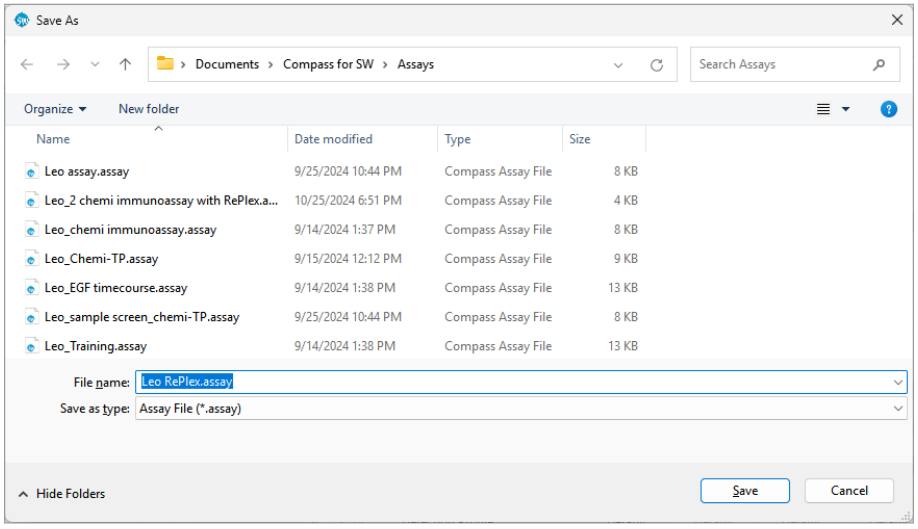
NOTE: Make sure not to edit the first column of the spreadsheet; this corresponds to the ladder wells.

To import the edited CSV file into Compass for Simple Western, select **File** in the main menu, click **Import Template...**, or click the **Import** button in top right corner of the Sample Template pane and then browse to the saved .csv file. Once imported, the edited CSV file displays the edited reagent names and attributes in the Sample Template.

Step 6: Save the Assay

1. Select **File** from the main menu and click **Save As...**.
2. Add an assay comment (optional).
3. Enter the assay name and click **Save**.

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



Creating a New Assay: RePlex

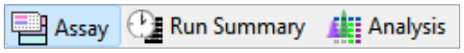
Create an assay using Compass for Simple Western Software. To connect your Leo Instrument to Compass, select **Instrument** in the main menu and click **Connect...**. Then select your instrument and click **Connect**.

IMPORTANT:
If you plan on running a RePlex assay with a Fluorescence Immunoassay on a Leo Instrument initially configured for chemiluminescence only, contact ProteinSimple Technical Support to enable fluorescence functionality. A warning will appear if you try to run a Fluorescence Assay before your Leo Instrument is updated.

NOTES:
If you are using a Wes System, Peggy Sue System, Sally Sue System, or NanoPro 1000 System, 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 a Peggy Sue, Sally Sue, or NanoPro 1000 Systems and software versions 6.x and lower for Wes Systems to create, load, and run assays on these systems, and to analyze data.

To learn more about Compass for Simple Western, refer to the **Compass for Simple Western User Guide**.

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



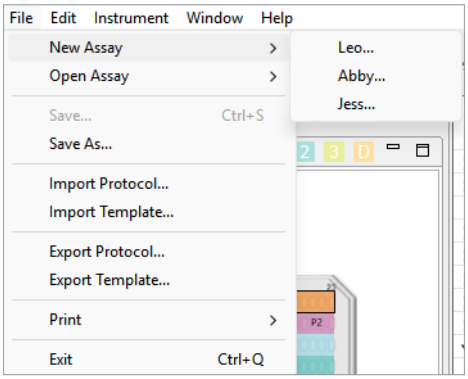
You can run an existing assay or create a new assay. See “Opening and Modifying an Existing Assay” on page 106 and “Leo Assay Combinations” on page 36 for more information.

To create a new assay, we recommend using one of the default template assays and making any necessary modifications from there. See “Creating a Custom Template Assay” on page 107 for more information.

Step 1: Open a Template Assay

1. Select the **Assay** screen.

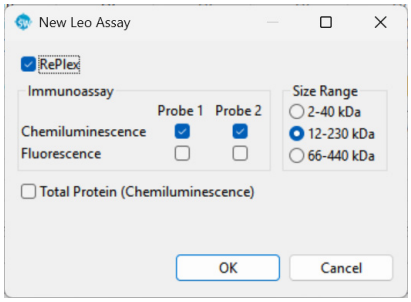
2. If your Leo Instrument is not connected to Compass for Simple Western, select **File** in the main menu, click **New Assay**, and select **Leo**.



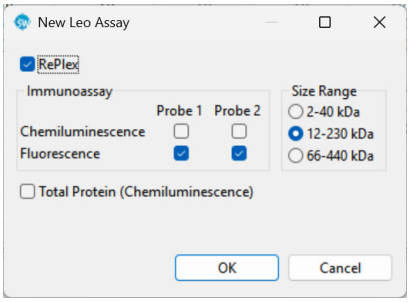
If your Leo Instrument is connected to Compass for Simple Western, select **File** in the main menu and click **New Assay**.

3. Select **RePlex**.
4. Select the appropriate **Assay Type** or **Immunoassay** and **Size Range**. Some examples are shown below.

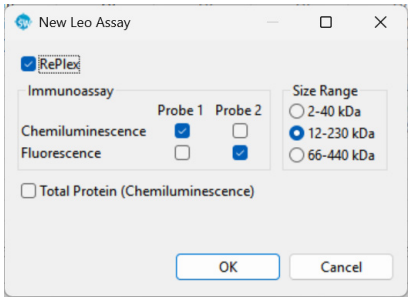
CHEMILUMINESCENCE IMMUNOASSAY (PROBE 1) + CHEMILUMINESCENCE IMMUNOASSAY (PROBE 2) USING REPLEX:



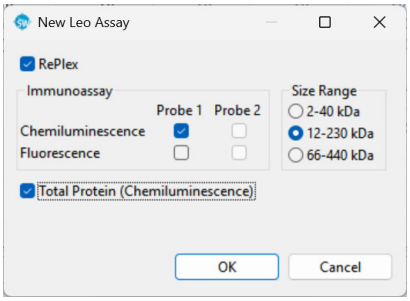
FLUORESCENCE IMMUNOASSAY (PROBE 1) + FLUORESCENCE IMMUNOASSAY (PROBE 2) USING REPLEX:



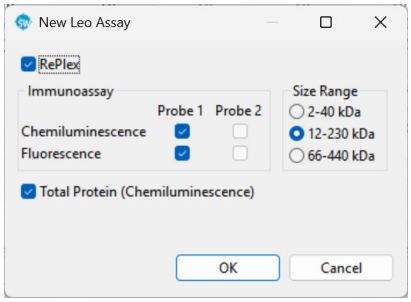
FLUORESCENCE IMMUNOASSAY (PROBE 1) + CHEMILUMINESCENCE IMMUNOASSAY (PROBE 2) USING REPLEX:



CHEMILUMINESCENCE IMMUNOASSAY (PROBE 1) + TOTAL PROTEIN NORMALIZATION (PROBE 2) USING REPLEX AND CHEMILUMINESCENCE DETECTION:



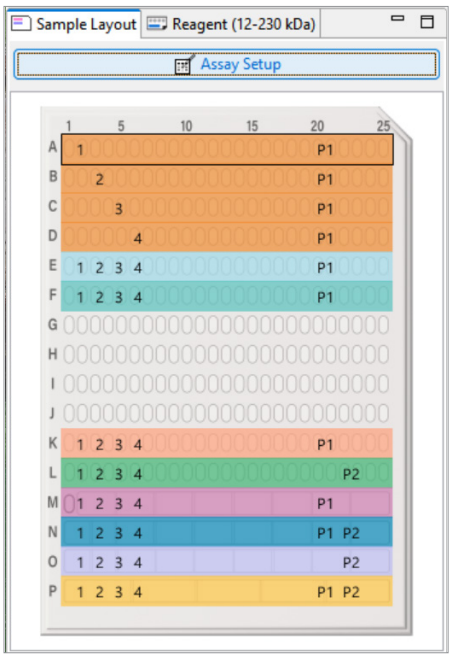
CHEMILUMINESCENCE AND FLUORESCENCE IMMUNOASSAY (PROBE 1) + TOTAL PROTEIN NORMALIZATION (PROBE 2) USING REPLEX AND CHEMILUMINESCENCE DETECTION:

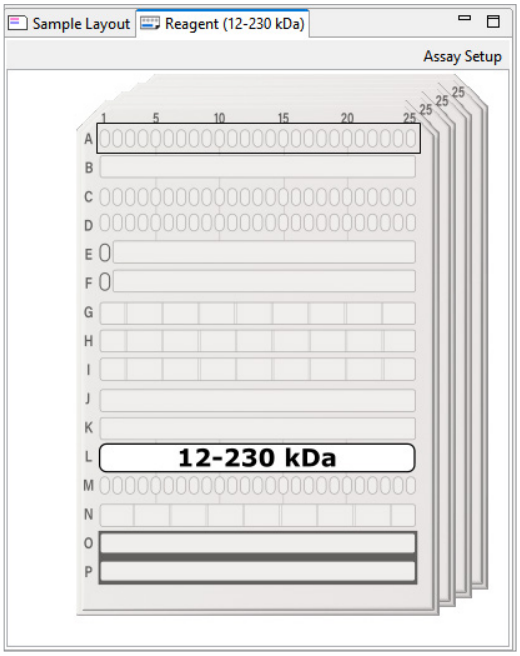


NOTES:
The default detection profile for Fluorescence Immunoassays is the NIR channel. Refer to “To add a Detection Profile to the IR channel:” on page 64 for info on adding an IR detection profile to your assay.

When performing Total Protein Normalization assay using RePlex, Probe 1 will be used for the Chemiluminescence or Fluorescence Immunoassay and Probe 2 will be used for the Total Protein Assay.

5. Click **OK**.
6. A template assay with default reagent locations will display in the Sample Layout pane of the Assay screen. The Reagent pane will display the size range selected. The Sample Layout pane example shown below is for a Fluorescence Immunoassay + Total Protein Normalization Using RePlex and Chemiluminescence Detection, and the Reagent pane is for an assay using the 12–230 kDa Size Range.



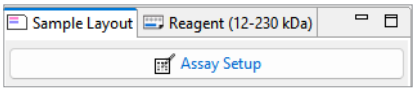


The numbers on the Sample Plate indicate the row each cartridge will load reagents from. Reagents used in Probe 1 are labeled “P1” on the plate, those used in Probe 2 are labeled ‘P2’.

NOTE: If you make changes to the assay, such as adding rows or changing the reagent assigned to a row, be sure to select **File** and **Save** before proceeding.

Step 2: Assign Sample Plate Reagents

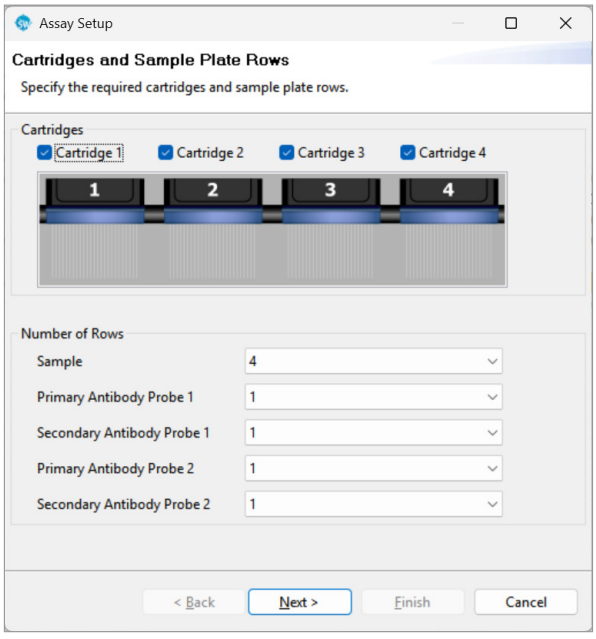
1. Click on the **Assay Setup** button under the Sample Layout or Reagent tab.



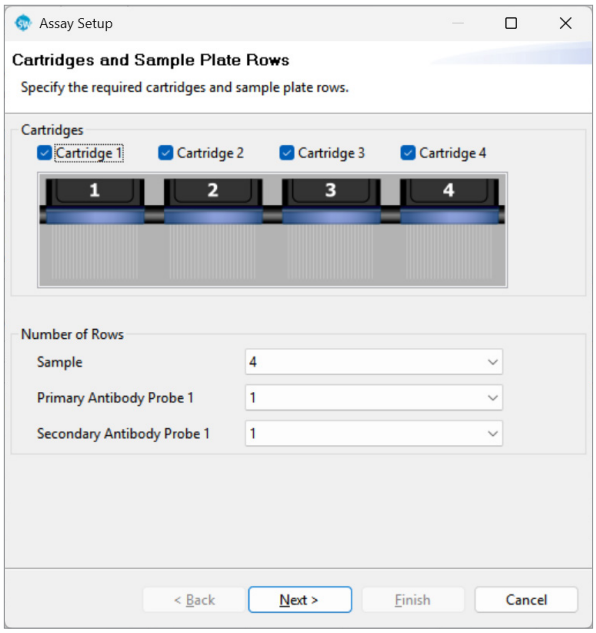
2. Define the assay parameters.
   a. Deselect the boxes next to the cartridge holder as needed to indicate which holders will be loaded with a cartridge.
   b. Select the number of sample, primary antibody, and secondary antibody rows pipetted on the Sample Plate using the drop-downs.

      Default parameters with four rows of samples are shown. The number of Samples rows will automatically adjust if less than four cartridges are selected.

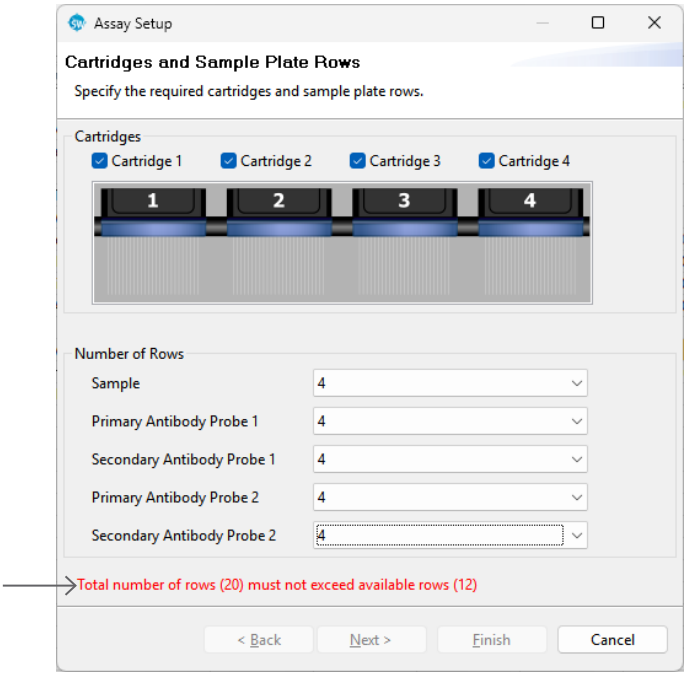
CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY + CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY USING REPLEX



CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY + TOTAL PROTEIN NORMALIZATION USING REPLEX AND CHEMILUMINESCENCE DETECTION



A warning will appear if you try to add more rows than are allowed.

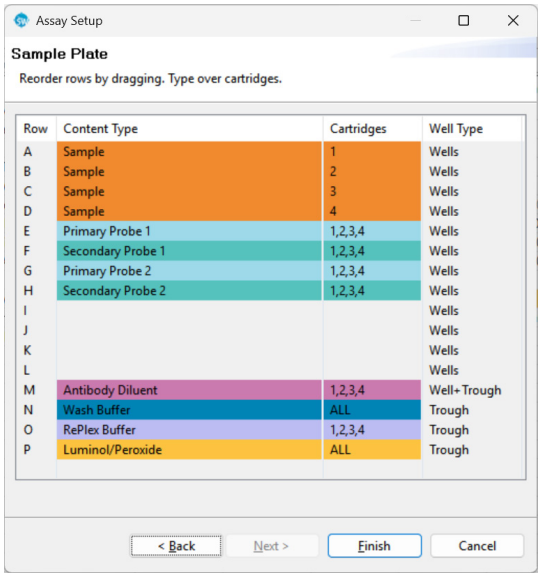


3. Click **Next**.

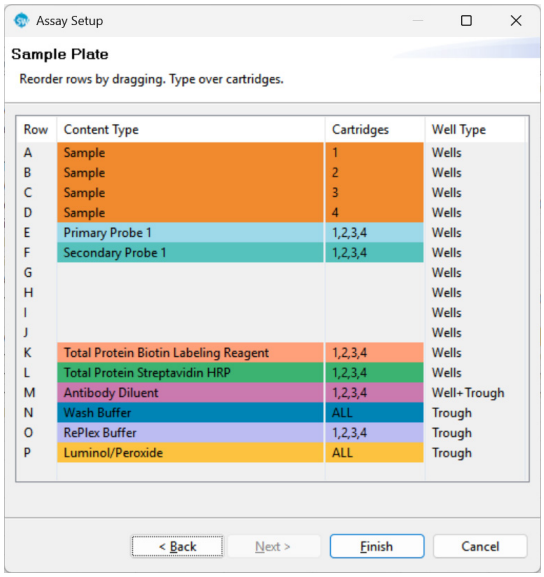
4. An ‘Assay Setup - Sample Plate’ prompt will appear. Color coding is used to identify various assay reagents.

    - **Orange** – Samples and Biotinylated ladder
    - **Light Teal** – Primary antibody
    - **Teal** – Secondary conjugate
    - **Magenta** – Antibody Diluent (2 or Milk-free)
    - **Blue** – Wash Buffer
    - **Gold (if applicable)** – Luminol-S/Peroxide mix
    - **Peach (if applicable)** – Total Protein Biotin Labeling Reagent
    - **Green (if applicable)** – Total Protein Streptavidin-HRP
    - **Purple (if applicable)** – RePlex Reagent

CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY + CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY USING REPLEX



CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY + TOTAL PROTEIN NORMALIZATION USING REPLEX AND CHEMILUMINESCENCE DETECTION



NOTE: Luminol-S/Peroxide will only appear in Row P if a Chemiluminescence Immunoassay is being performed.

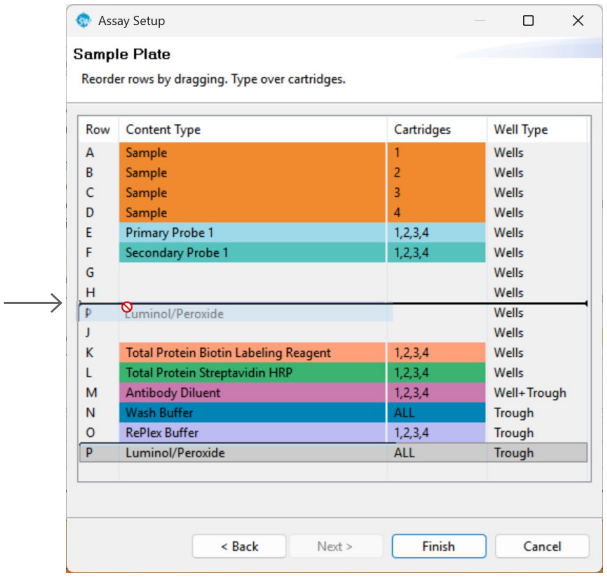
5. The default protocol automatically assigns all reagent locations for the assay.

   Reagent assignments to rows M–P should not be modified.

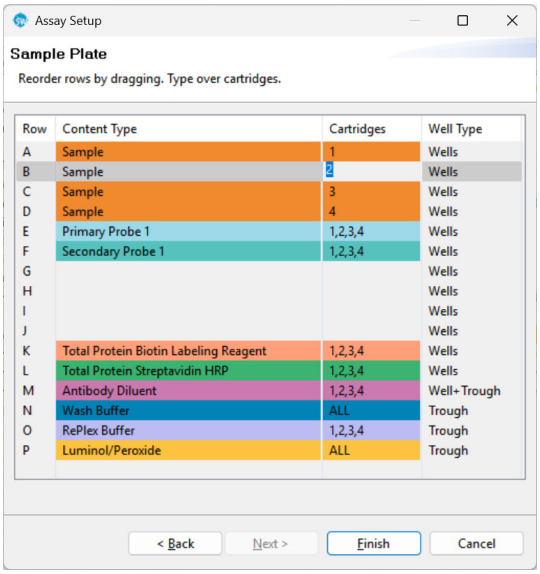
6. Modify reagent assignments as needed for rows A–L.

   a. Reorder row locations by clicking on the row and dragging it to the desired location. The examples below are from setting up a Chemiluminescence Immunoassay + Total Protein Normalization using RePlex and Chemiluminescence Detection.

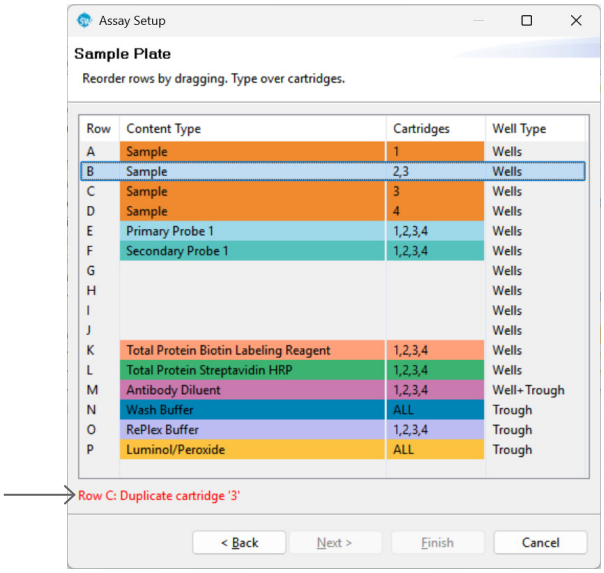
      A warning will appear if you try to move the reagent to a row that is not allowed.

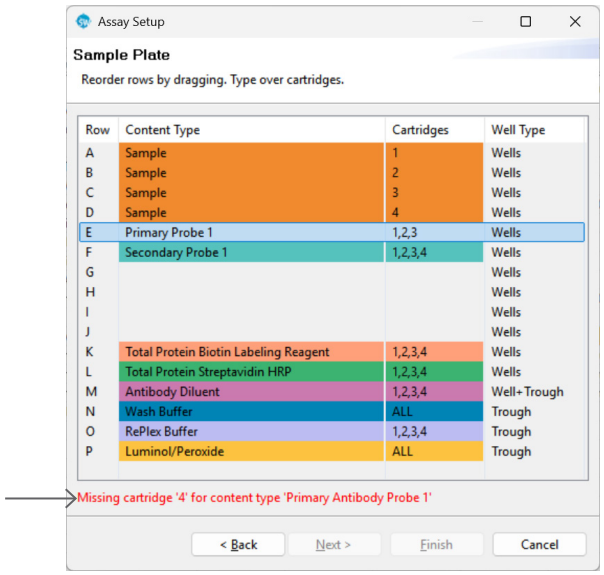


   b. Change the row a cartridge will load a sample, primary antibody, or secondary antibody from by clicking on the cell in the Cartridges column and typing in the cartridge number.



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

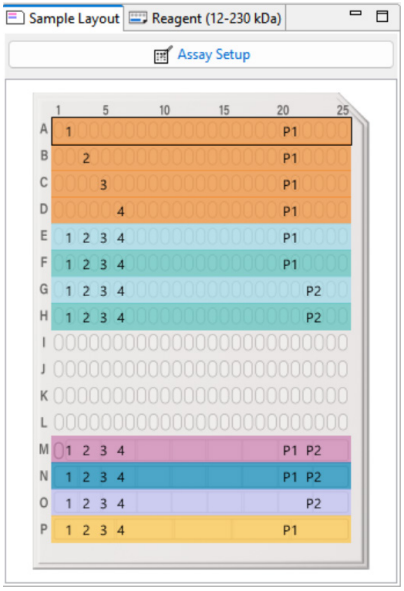




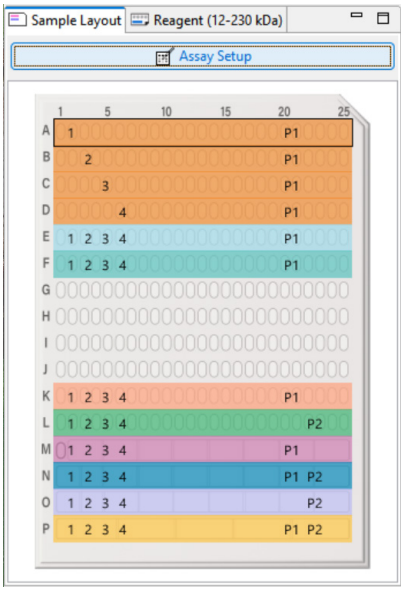
7. To add or remove a row, click the **Back** button and adjust the number of rows using the drop-down menus.
8. Click **Finish**. You can also adjust the row a cartridge will load from later in the Protocol pane.

9. A Sample Plate with updated reagent locations will display in the Assay screen. Numbers on the Sample Plate indicate the row each cartridge will be loaded from. Reagents used in Probe 1 are labeled ‘P1’ on the plate, those used in Probe 2 are labeled ‘P2’.

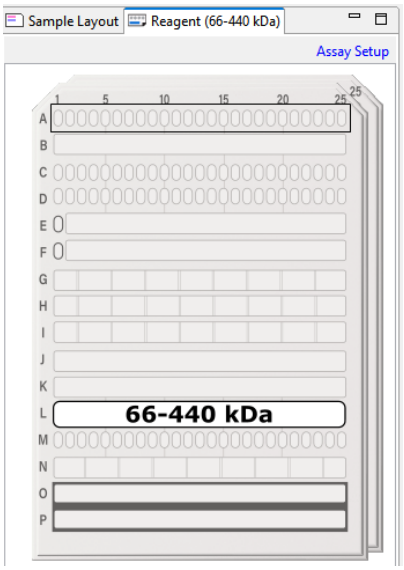
CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY + CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY USING REPLEX



CHEMILUMINESCENCE AND/OR FLUORESCENCE IMMUNOASSAY + TOTAL PROTEIN NORMALIZATION USING REPLEX AND CHEMILUMINESCENCE DETECTION



10. The Reagent pane will display the size range selected and the number of Pre-filled Reagent Plates required to perform your run. The Reagent pane example below is for a run using 66–440 kDa plates that is running two capillary cartridges.



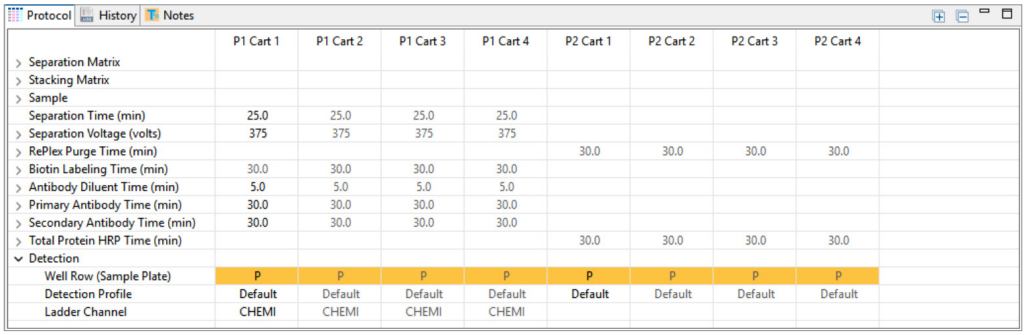
Step 3: Modifying the Assay Protocol (Optional)

NOTES:
The capillary cartridges are all run in parallel, so voltage- and time-based parameters for all the cartridges will 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.

When performing a Total Protein Normalization Assay using RePlex, probe 1 will be used for the Chemiluminescence Immunoassay and/or Fluorescence Immunoassay and Probe 2 will be used for the Total Protein Assay.

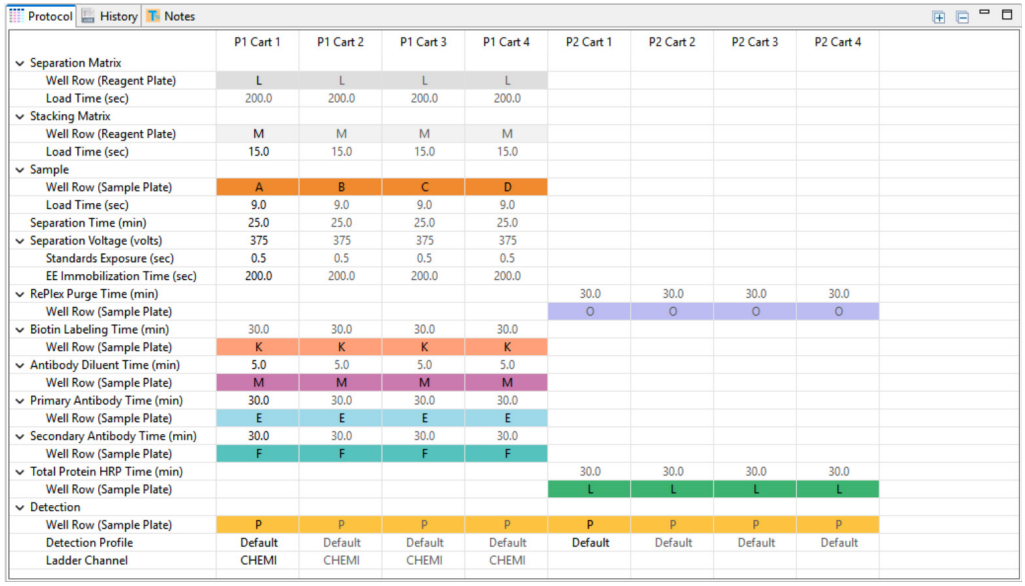
To Modify an Assay Protocol:

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 + Total Protein Normalization using RePlex and Chemiluminescence Detection using 4 cartridges.



	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
Biotin Labeling Time (min)	30.0	30.0	30.0	30.0				
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				
Total Protein HRP Time (min)					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
Biotin Labeling Time (min)	30.0	30.0	30.0	30.0				
Well Row (Sample Plate)	K	K	K	K				
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				
Total Protein HRP Time (min)					30.0	30.0	30.0	30.0
Well Row (Sample Plate)					L	L	L	L
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.

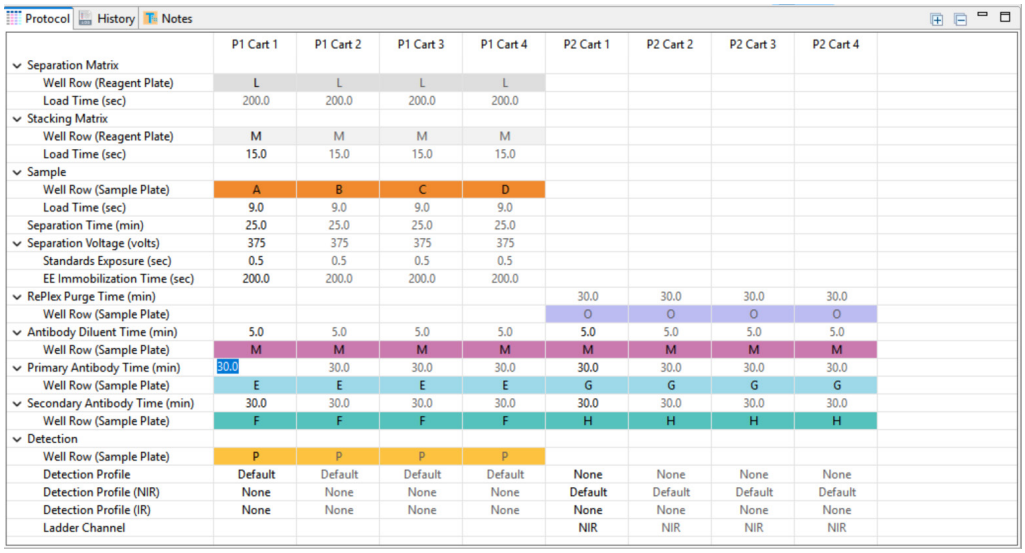
2. You can change the primary or secondary antibody incubation time.

To change the primary antibody incubation time:

a. Click the cell with the numerical value next to Primary Antibody Time (min) in the following column:

- For Probe 1: P1 Cart 1
- For Probe 2 (if applicable): P2 Cart 1

b. Enter a new value in minutes. The example below is for a Chemiluminescence Immunoassay + Fluorescence Immunoassay Using RePlex. The Chemiluminescence Immunoassay is performed in Probe 1 and the Fluorescence Immunoassay is performed in Probe 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)	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				
Detection Profile	Default	Default	Default	Default	None	None	None	None
Detection Profile (NIR)	None	None	None	None	Default	Default	Default	Default
Detection Profile (IR)	None	None	None	None	None	None	None	None
Ladder Channel					NIR	NIR	NIR	NIR

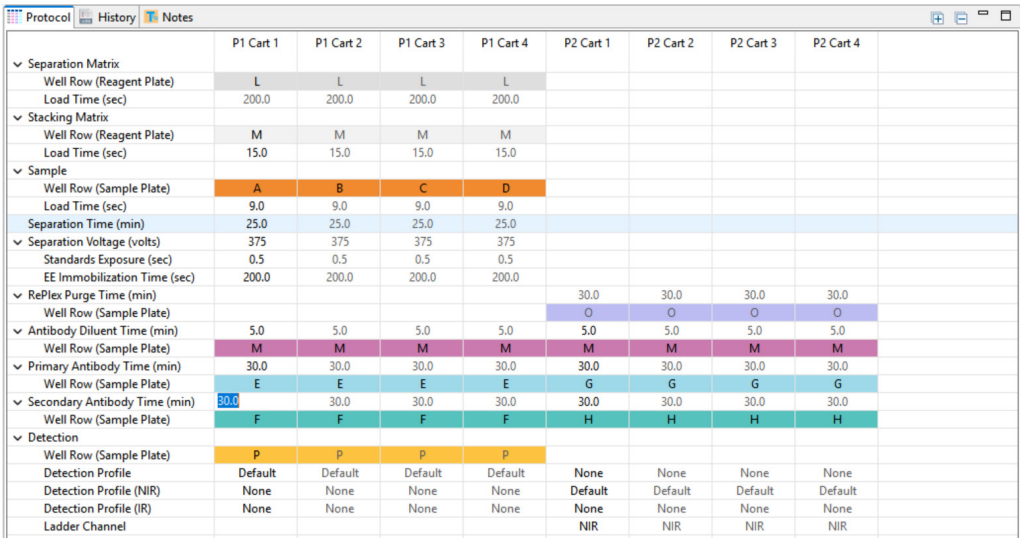
c. The values for other cartridges will automatically update.

To change the secondary antibody incubation time:

a. Click the cell with the numerical value next to Secondary Antibody Time (min) in the following column:

- For Probe 1: P1 Cart 1
- For Probe 2 (if applicable): P2 Cart 1

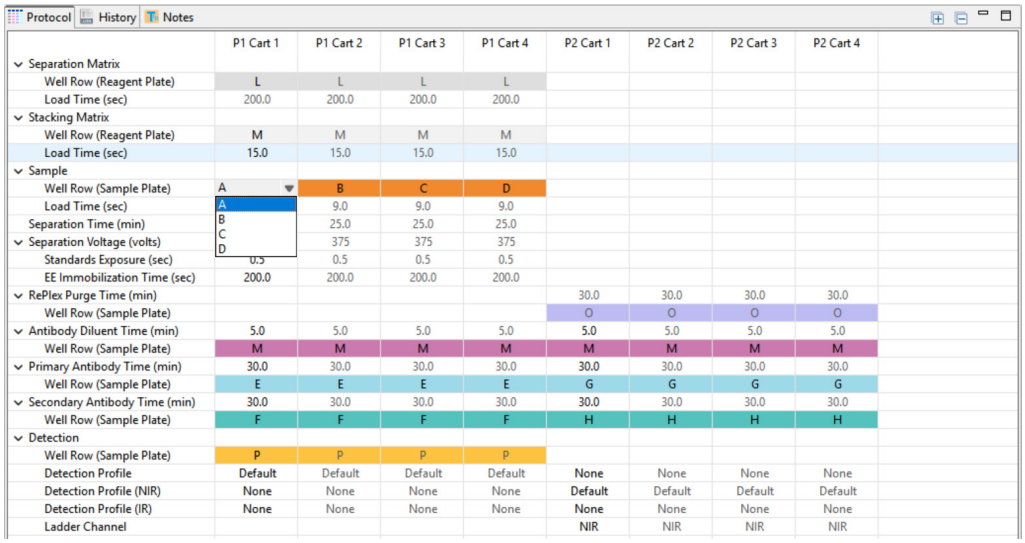
b. Enter a new value in minutes. The example below is for a Chemiluminescence Immunoassay + Fluorescence Immunoassay Using RePlex. The Chemiluminescence Immunoassay is performed in Probe 1 and the Fluorescence Immunoassay is performed in Probe 2.



	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)	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				
Detection Profile	Default	Default	Default	Default	None	None	None	None
Detection Profile (NIR)	None	None	None	None	Default	Default	Default	Default
Detection Profile (IR)	None	None	None	None	None	None	None	None
Ladder Channel					NIR	NIR	NIR	NIR

c. The values for the other cartridges will automatically update.

3. **For reagents loaded in rows A–L:** You can change the row in the Sample Plate that a cartridge will load a reagent from. Click the cell in the column next to the Well Row and select a different row:



	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				
Detection Profile	Default	Default	Default	Default	None	None	None	None
Detection Profile (NIR)	None	None	None	None	Default	Default	Default	Default
Detection Profile (IR)	None	None	None	None	None	None	None	None
Ladder Channel					NIR	NIR	NIR	NIR

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

4. Leo RePlex protocols use default detection profiles.

CHEMILUMINESCENCE DETECTION PROFILE

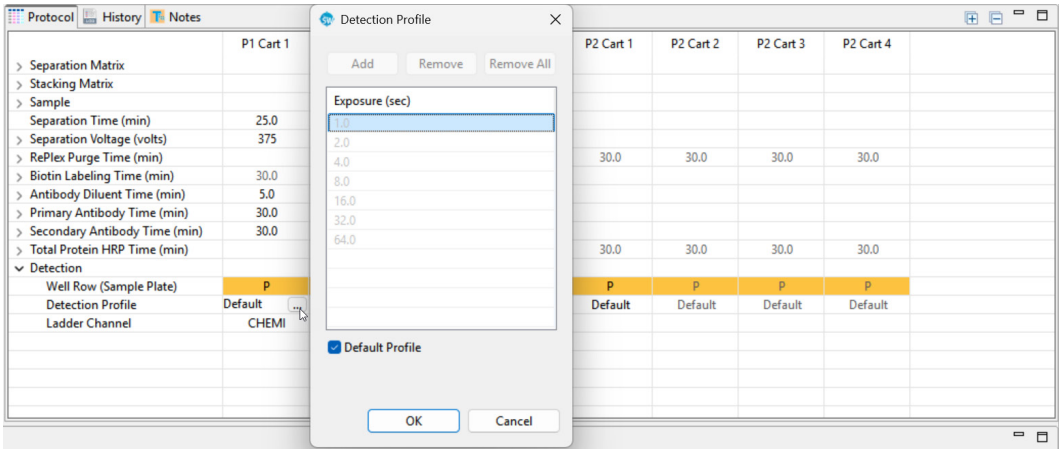
The default detection profile is similar to the RePlex Dynamic Range (RDR) exposure series used for Jess and Abby RePlex assays, but removes the 128 and 512 second exposures. See the **Compass for Simple Western User Guide** for more information about RDR detection profiles.

To adjust exposures in the Detection Profile:

a. If needed, click the gray arrow next to Detection to expand the row.

b. Depending on the probe being modified:

- For a Chemiluminescence Immunoassay: Select the Default cell in the 'P1 Cart 1' (for Probe 1) or 'P2 Cart 1 (for Probe 2) column next to Detection Profile. Click the **...** button that appears to open the Detection Profile window.
- For a Total Protein Normalization assay using Chemiluminescence Detection: Select the Default cell in the 'P2 Cart 1' column next to Detection Profile. Click the ... button that appears to open the Detection Profile window.



NOTE: When performing Total Protein Normalization using RePlex, Probe 1 will be used for the Chemiluminescence and/or Fluorescence Immunoassay and Probe 2 will be used for the Total Protein assay.

c. Deselect the Default Profile checkbox.

d. To remove times, click the **Remove** or **Remove All** button.

NOTE: You can only use the **Remove All** button if the Ladder Channel is different from the Detection Profile channel you are modifying.

e. To add additional times to the protocol, click the **Add** button and enter the values.

f. Select **OK**.

g. The number of exposures in the modified Detection Profile will appear.

A total of seven exposures are allowed, but no more than one exposure can be longer than 32 seconds in Chemiluminescence Immunoassays in a RePlex assay.

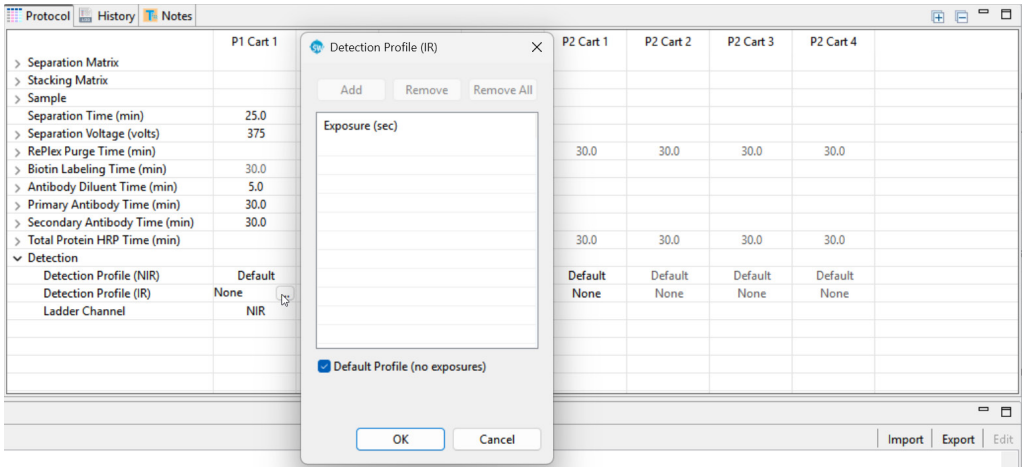
FLUORESCENCE DETECTION PROFILES

The default detection profile is in the NIR channel with 6 standard exposures.

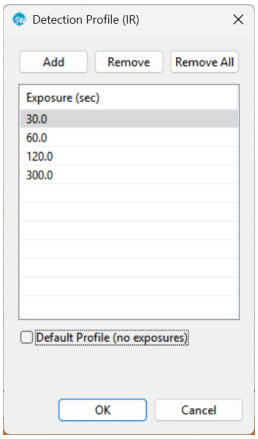
To add a Detection Profile to the IR channel:

a. If needed, click the gray arrow next to Detection to expand the row.

b. 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.



d. To remove times, click the **Remove** button.

e. To add additional times to the protocol, click the **Add** button and enter the values.

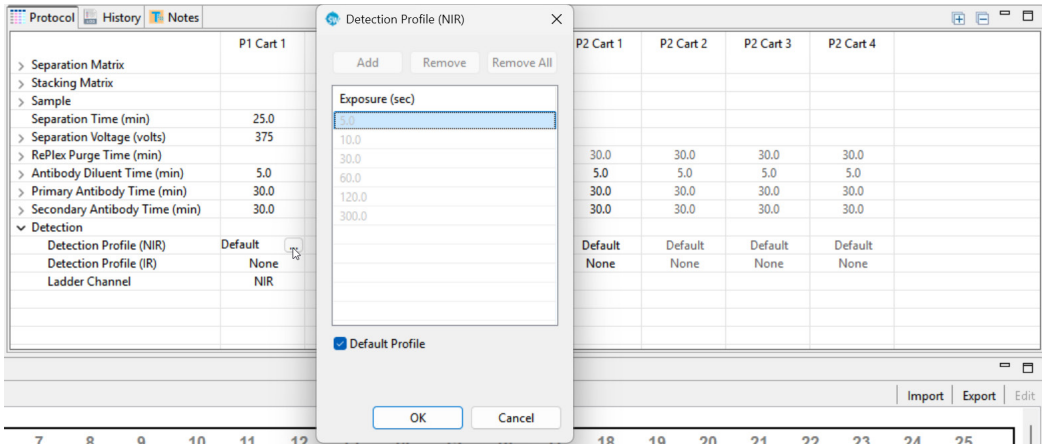
f. Select **OK**.

g. The number of exposures in the IR channel Detection Profile will appear.

To remove the Detection Profile from the NIR channel:

a. If needed, click the gray arrow next to Detection to expand the row.

b. Select the Default cell in the 'P1 Cart 1' (for Probe 1) or 'P2 Cart 1 (for Probe 2) column next to Detection Profile (NIR). Click the **...** button that appears to open the Detection Profile window.



c. Deselect the Default Profile checkbox.

d. Click **Remove All**.

e. Select **OK**.

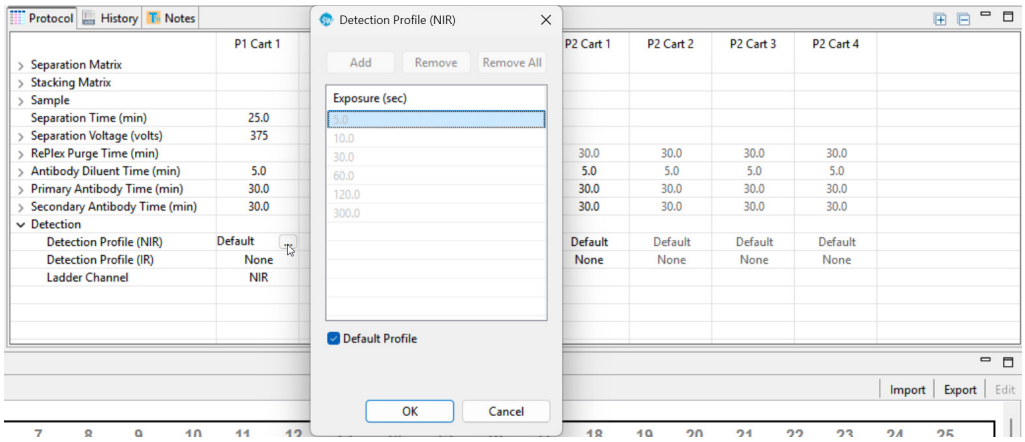
f. 'None' will appear in the column next to Detection Profile (NIR) in the modified protocol.

NOTE: You can only use the **Remove All** button to remove all exposure times if the Ladder Channel is different from the Detection Profile channel you are modifying.

To adjust exposures in the NIR or IR channel:

a. If needed, click the gray arrow next to Detection to expand the row.

b. Select the Default cell or None cell in the 'P1 Cart 1' (for Probe 1) or 'P2 Cart 2' (for Probe 2) column next to Detection Profile (NIR) or Detection Profile (IR). Click the **...** button that appears to open the Detection Profile window.



c. Deselect the Default Profile or Default Profile (no exposures) checkbox.

d. To remove times, click the **Remove** button or **Remove All**.

NOTE: You can only use the **Remove All** button to remove all exposure times if the Ladder Channel is different from the Detection Profile channel you are modifying.

e. To add additional times to the protocol, click the **Add** button and enter the values.

f. Select **OK**.

g. The number of exposures in the modified Detection Profile(s) will appear.

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

Exporting and Importing Assay Protocols

A modified assay protocol can be exported for later use. Exporting an assay protocol exports information from the Protocol pane only.

To export an assay protocol:

1. Select **File** in the main menu and click **Export Protocol...**.
2. The default directory is Compass for SW/Assays. Change the directory if needed.
3. Enter a protocol name and click **Save**. The protocol will be saved as a *.protocol file. The protocol file will include the information in the Protocol pane (steps and row locations), but will not include information in the Sample Template pane.

To import a previously saved assay protocol:

1. Select **File** in the main menu and click **Import Protocol...**.
2. Select the *.protocol file.
3. Click **Open**.

Steps 4–6

Steps 4 through 6 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 66 to continue.

Creating a New Assay: Protein Normalization

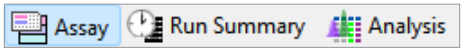
Create an assay using Compass for Simple Western Software. To connect your Leo Instrument to Compass, select **Instrument** in the main menu and click **Connect...**. Then select your instrument and click **Connect**.

IMPORTANT: If you plan on running a Protein Normalization assay on a Leo Instrument initially configured for chemiluminescence only, contact ProteinSimple Technical Support to enable fluorescence functionality. A warning will appear if you try to run a Fluorescence Assay before your Leo Instrument is updated.

NOTES:
If you are using a Wes System, Peggy Sue System, Sally Sue System, or NanoPro 1000 System, 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 a Peggy Sue, Sally Sue, or NanoPro 1000 Systems and software versions 6.x and lower for Wes Systems to create, load, and run assays on these systems, and to analyze data.

To learn more about Compass for Simple Western, refer to the **Compass for Simple Western User Guide**.

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

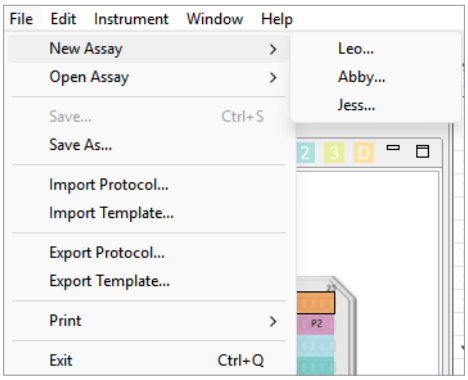


You can run an existing assay or create a new assay. See “Opening and Modifying an Existing Assay” on page 106.

To create a new assay, we recommend using one of the default template assays and making any necessary modifications from there. See “Creating a Custom Template Assay” on page 107 for more information.

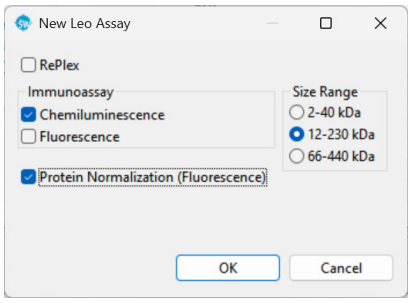
Step 1: Open a Template Assay

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



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

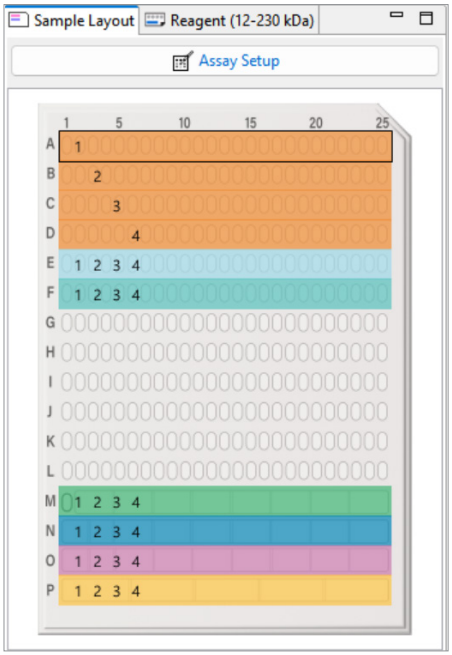
3. Select **Protein Normalization (Fluorescence)** along with the appropriate **Immunoassay** and **Size Range**.

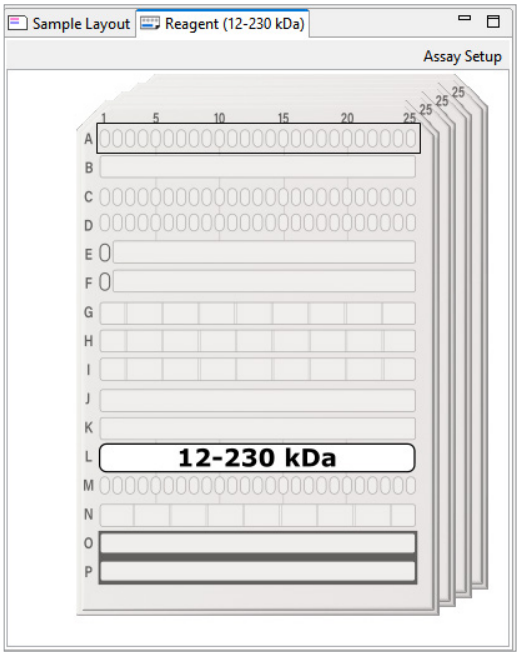


NOTE: Protein Normalization is not available for a RePlex assay. For a RePlex assay, use a Total Protein (Chemiluminescence) assay to normalize your protein concentration.

4. Click **OK**.

5. A template assay with default reagent locations will display in the Sample Layout pane of the Assay screen. The Reagent pane will display the size range selected. The Sample Layout pane example shown below is for a Chemiluminescence Immunoassay + Total Protein Normalization using the PN Channel, and the Reagent pane is for an assay using the 12–230 kDa Size Range.



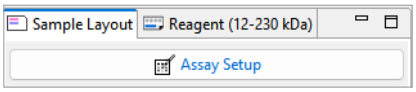


The numbers on the Sample Plate indicate the row each cartridge will load reagents from.

NOTE: If you make changes to the assay, such as adding rows or changing the reagent assigned to a row, be sure to select **File** and **Save** before proceeding.

Step 2: Assign Sample Plate Reagents

1. Click on the **Assay Setup** button under the Sample Layout or Reagent tab.

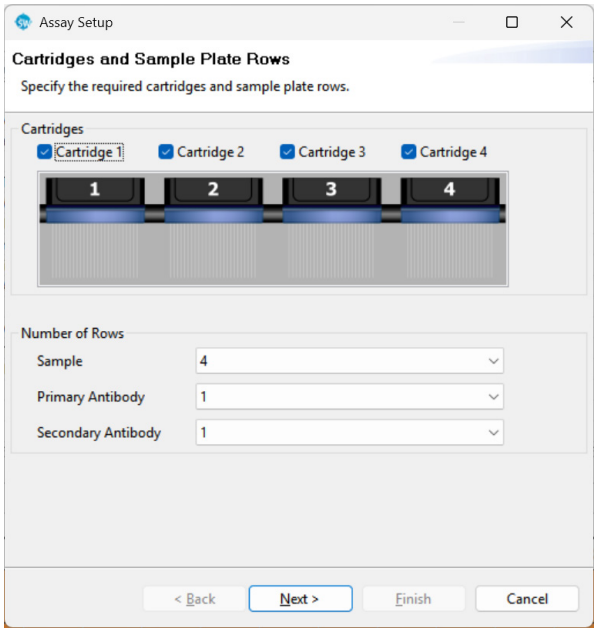


2. Define the assay parameters.

   a. Deselect the boxes next to the cartridge holder as needed to indicate which holders will be loaded with a cartridge.

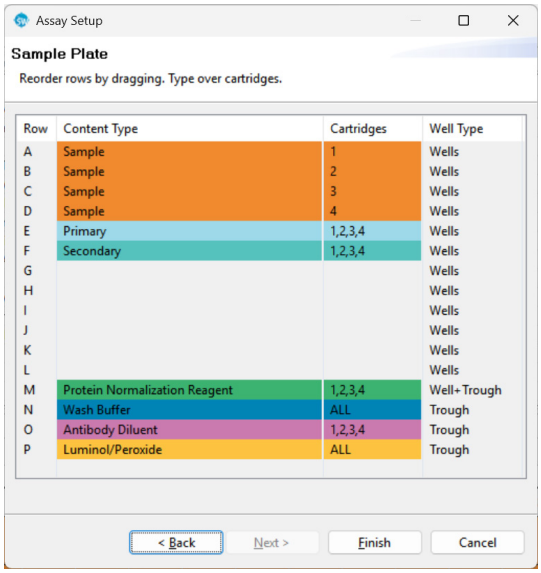
b. Select the number of sample, primary antibody, and secondary antibody rows pipetted on the Sample Plate using the drop-downs.

   Default parameters with four rows of samples are shown. The number of Samples rows will automatically adjust if less than four cartridges are selected.



3. Click **Next**.
4. An 'Assay Setup - Sample Plate' prompt will appear. Color coding is used to identify various assay reagents.
   - **Orange** – Samples and Biotinylated ladder
   - **Light Teal** – Primary antibody
   - **Teal** – Secondary conjugate
   - **Magenta** – Antibody Diluent (2 or Milk-free)
   - **Green** – Protein Normalization Reagent
   - **Blue** – Wash Buffer
   - **Gold (if applicable)** – Luminol-S/Peroxide mix

CHEMILUMINESCENCE OR FLUORESCENCE IMMUNOASSAY + TOTAL PROTEIN NORMALIZATION USING THE PN CHANNEL



NOTE: Luminol-S/Peroxide will only appear in Row P if a Chemiluminescence Immunoassay is being performed.

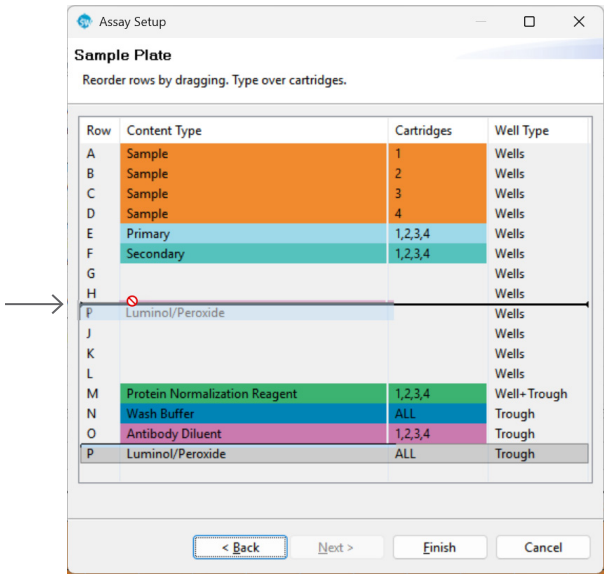
5. The default protocol automatically assigns all reagent locations for the assay.

   Reagent assignments to rows M–P should not be modified.

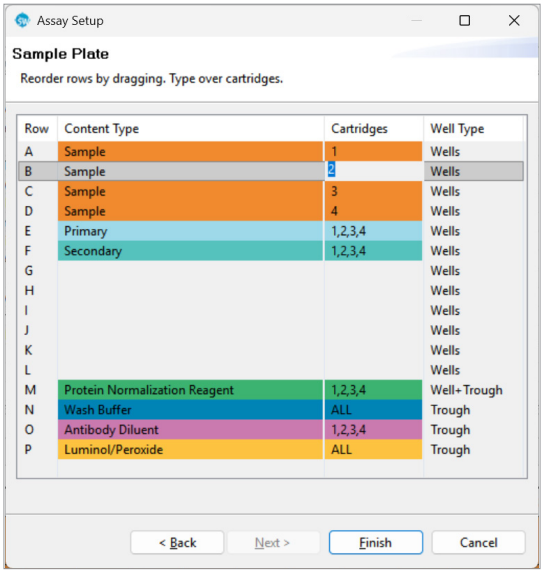
6. Modify reagent assignments as needed for rows A–L.

   a. Reorder row locations by clicking on the row and dragging it to the desired location. The examples below are from setting up a Chemiluminescence Immunoassay + Total Protein Normalization using the PN Channel.

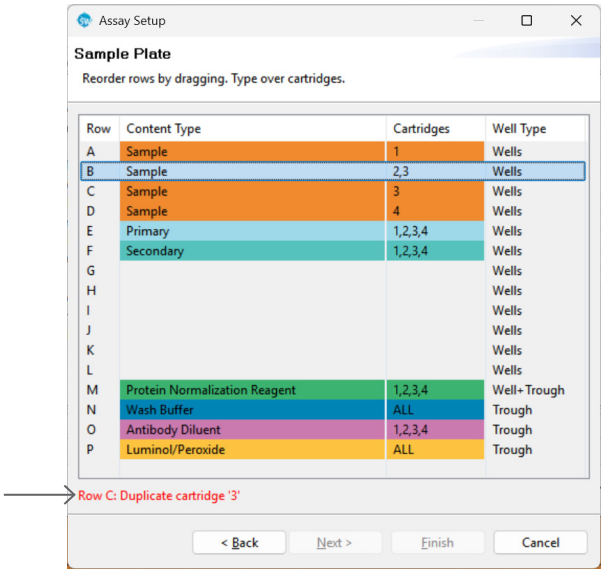
      A warning will appear if you try to move the reagent to a row that is not allowed.

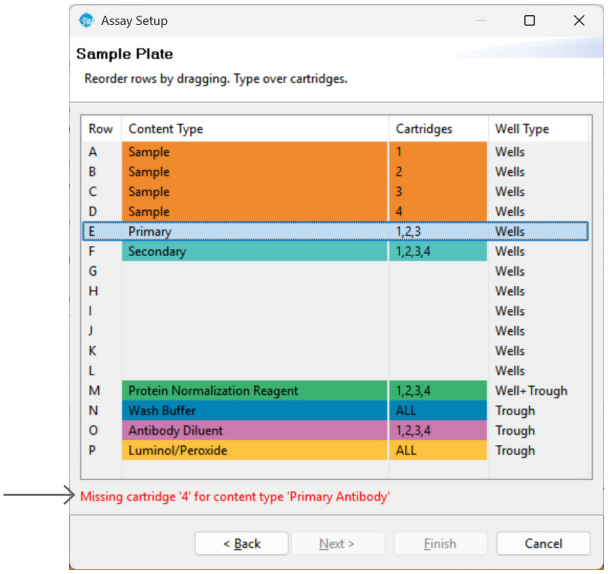


   b. Change the row a cartridge will load a sample, primary antibody, or secondary antibody from by clicking on the cell in the Cartridges column and typing in the cartridge number.



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

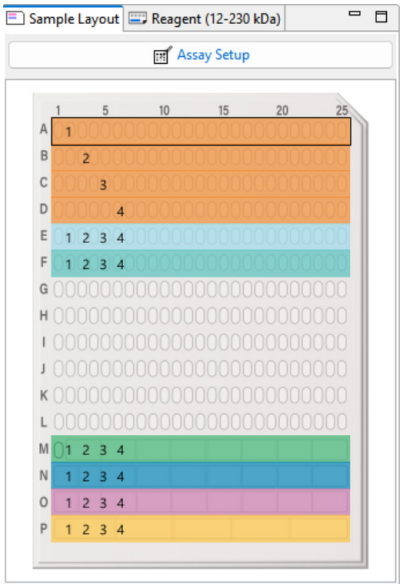




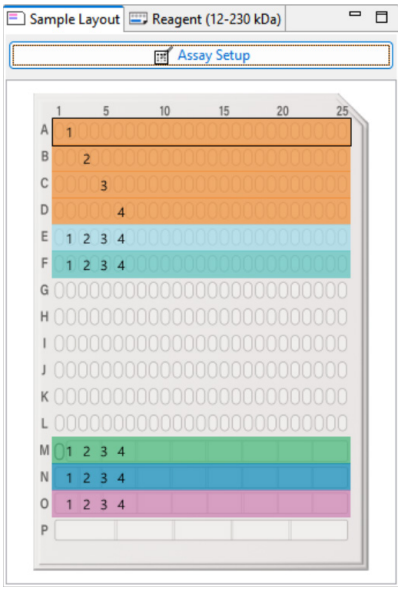
7. To add or remove a row, click the **Back** button and adjust the number of rows using the drop-down menus.
8. Click **Finish**. You can also adjust the row a cartridge will load from later in the Protocol pane.

9. A Sample Plate with updated reagent locations will display in the Assay screen. Numbers on the Sample Plate indicate the row each cartridge will be loaded from. Some examples are shown below:

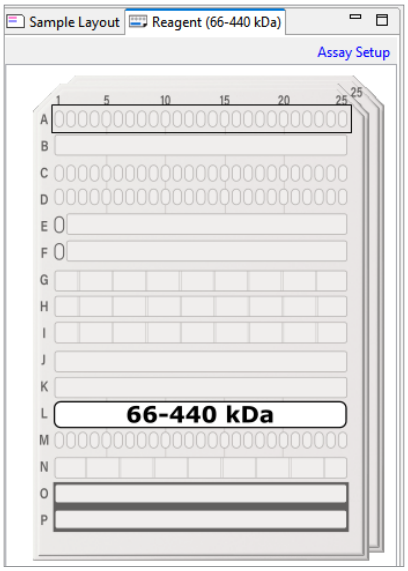
CHEMILUMINESCENCE IMMUNOASSAY + TOTAL PROTEIN NORMALIZATION USING THE PN CHANNEL



FLUORESCENCE IMMUNOASSAY + TOTAL PROTEIN NORMALIZATION USING THE PN CHANNEL



10. The Reagent pane will display the size range selected and the number of Pre-filled Reagent Plates required to perform your run. The Reagent pane example below is for a run using 66–440 kDa plates that is running two capillary cartridges.

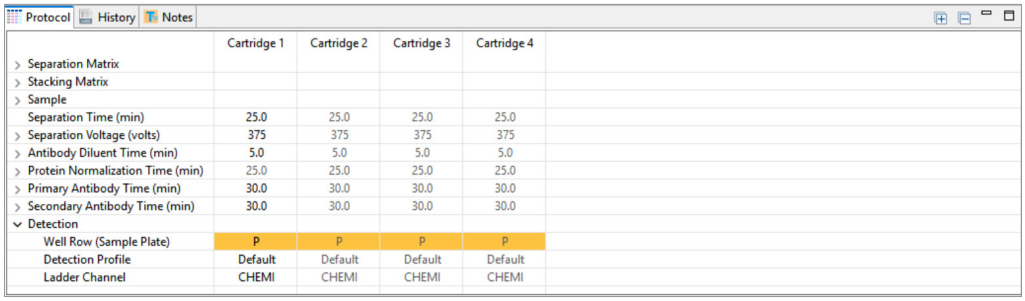


Step 3: Modifying the Assay Protocol (Optional)

NOTE: The capillary cartridges are all run in parallel, so voltage- and time-based parameters for all the cartridges will 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.

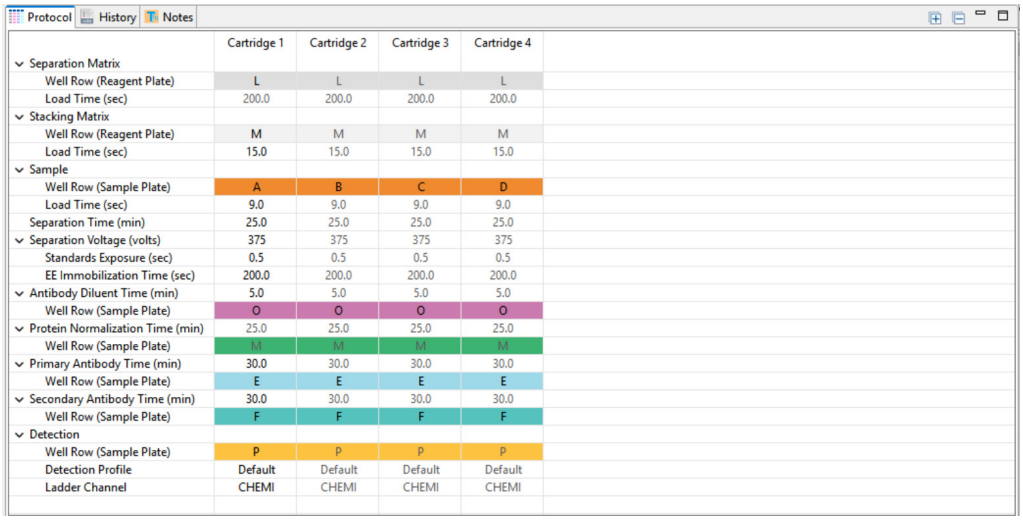
To Modify an Assay Protocol:

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 + Total Protein Normalization using the PN Channel using 4 cartridges.



	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
Protein Normalization Time (min)	25.0	25.0	25.0	25.0
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)	O	O	O	O
Protein Normalization Time (min)	25.0	25.0	25.0	25.0
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

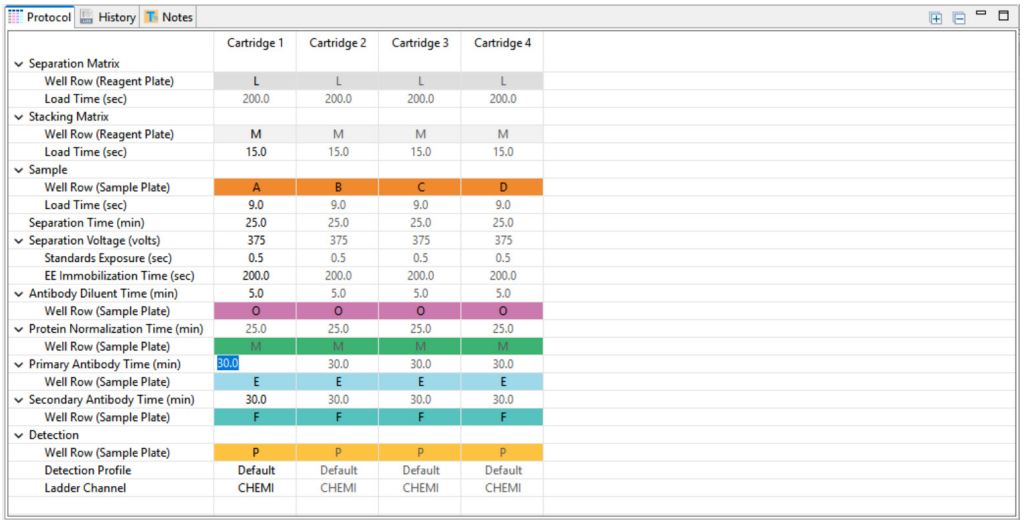
NOTE: To expand all parameters, click the ⊞ button at the upper right of the pane. To collapse all parameters, click the ⊟ button at the upper right of the pane.

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

To change the primary antibody incubation time:

a. Click the cell with the numerical value next to Primary Antibody Time (min) in the 'Cartridge 1' column:

b. Enter a new value in minutes.



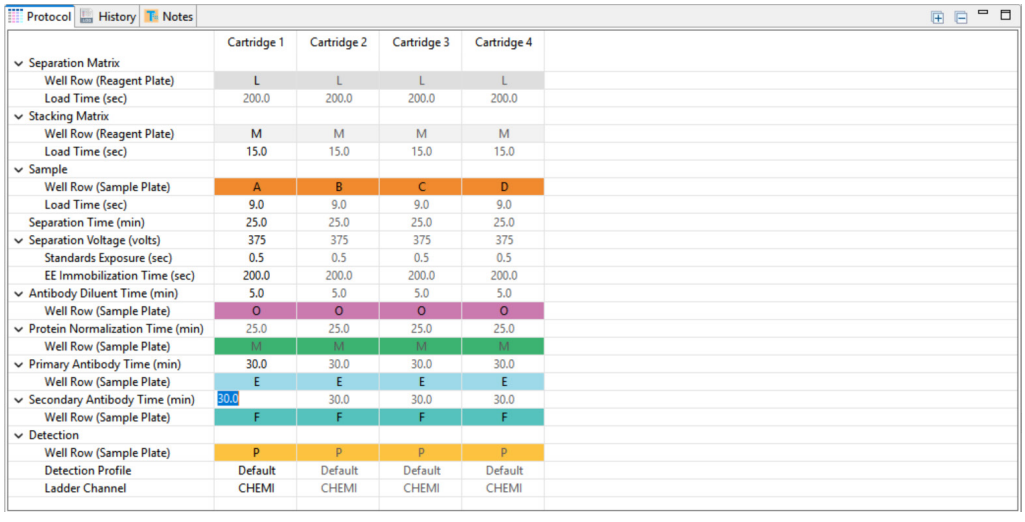
	Cartridge 1	Cartridge 2	Cartridge 3	Cartridge 4
Separation Matrix				
Well Row (Reagent Plate)	L	L	L	L
Load Time (sec)	200.0	200.0	200.0	200.0
Stacking Matrix				
Well Row (Reagent Plate)	M	M	M	M
Load Time (sec)	15.0	15.0	15.0	15.0
Sample				
Well Row (Sample Plate)	A	B	C	D
Load Time (sec)	9.0	9.0	9.0	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)	O	O	O	O
Protein Normalization Time (min)	25.0	25.0	25.0	25.0
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

c. The values for other cartridges will automatically update.

To change the secondary antibody incubation time:

a. Click the cell with the numerical value next to Secondary Antibody Time (min) in the 'Cartridge 1' column:

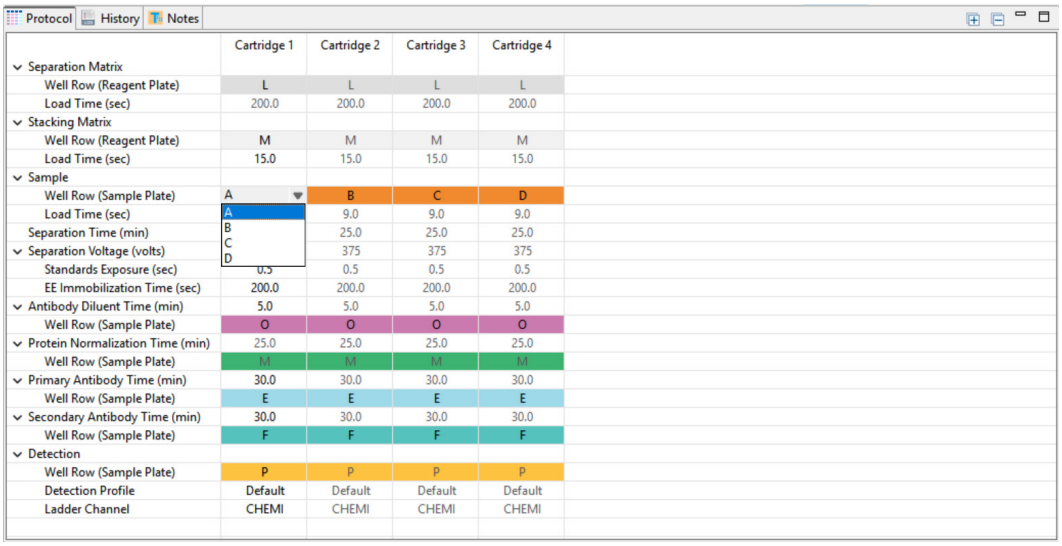
b. Enter a new value in minutes.



	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)	O	O	O	O
Protein Normalization Time (min)	25.0	25.0	25.0	25.0
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

c. The values for the other cartridges will automatically update.

3. **For reagents loaded in rows A–L:** You can change the row in the Sample Plate that a cartridge will load a reagent from. Click the cell in the column next to the Well Row and select a different row:



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

4. Leo immunoassay protocols use default detection profiles.

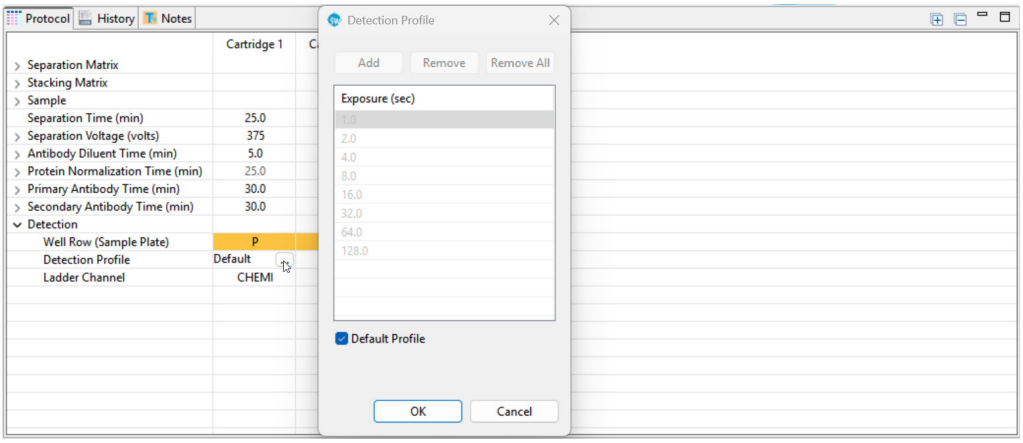
CHEMILUMINESCENCE DETECTION PROFILE

The default detection profile is similar to the High Dynamic Range (HDR) exposure series used for Jess and Abby assays, but removes the 512 second exposure. See the **Compass for Simple Western User Guide** for more information about HDR detection profiles.

To adjust exposures in the Detection Profile:

a. If needed, click the gray arrow next to Detection to expand the row.

b. Select the Default cell in the 'Cartridge 1' column next to Detection Profile and click the **...** button that appears to open the Detection Profile window.



c. Deselect the **Default Profile** checkbox.

d. To remove times, click the **Remove** button.

e. To add additional times to the protocol, click the **Add** button and enter the values.

f. Select **OK**.

g. The number of exposures in the modified chemiluminescence Detection Profile will appear.

NOTES:
You can only use the **Remove All** button to remove all exposure times if the Ladder Channel is different from the Detection Profile channel you are modifying.

No more than two exposures can be longer than 32 seconds in Chemiluminescence Immunoassays.

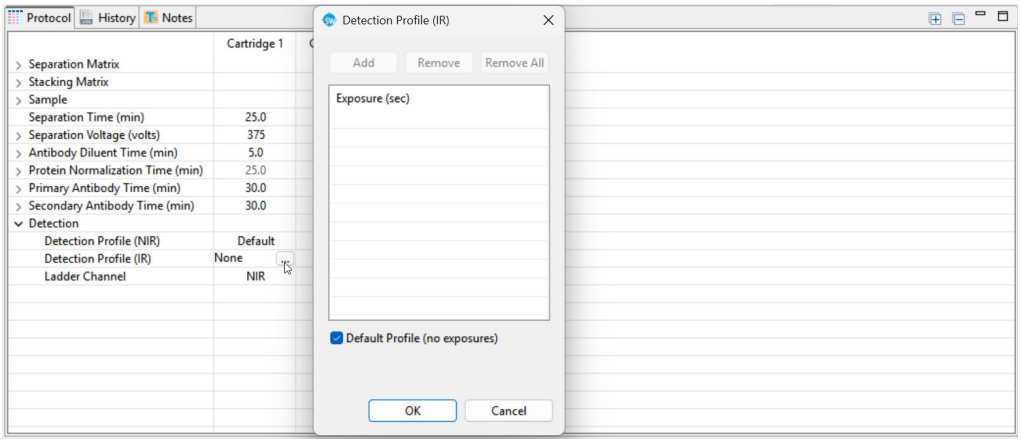
FLUORESCENCE DETECTION PROFILES

The default detection profile is in the NIR channel with 6 standard exposures.

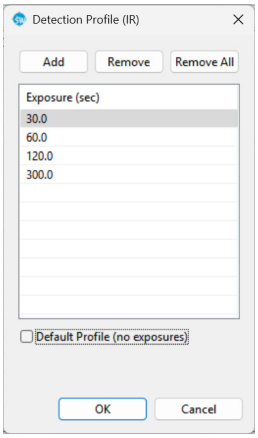
To add a Detection Profile to the IR channel:

a. If needed, click the gray arrow next to Detection to expand the row.

b. 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.



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



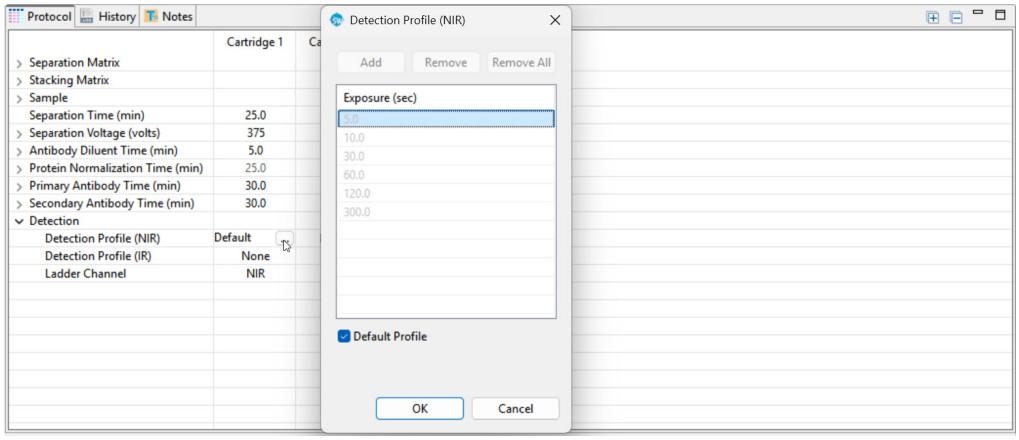
d. Select **OK**.

e. The number of exposures in the IR channel Detection Profile will appear.

To remove the Detection Profile from the NIR channel:

a. If needed, click the gray arrow next to Detection to expand the row.

b. Select the Default cell in the 'Cartridge 1' column next to Detection Profile (NIR) and click the ... button that appears to open the Detection Profile window.



c. Deselect the Default Profile checkbox.

d. Click **Remove All**.

e. Select **OK**.

f. 'None' will appear in the column next to Detection Profile (NIR) in the modified protocol.

NOTE: You can only use the **Remove All** button to remove all exposure times if the Ladder Channel is different from the Detection Profile channel you are modifying.

To adjust exposures in the NIR or IR channel:

a. If needed, click the gray arrow next to Detection to expand the row.

b. Select the Default cell or None cell in the 'Cartridge 1' column next to Detection Profile (NIR) or Detection Profile (IR), respectively. Click the **...** button that appears to open the Detection Profile window.

c. Deselect the Default Profile or Default Profile (no exposures) checkbox.

d. To remove times, click the **Remove** button or **Remove All**.

NOTE: You can only use the **Remove All** button to remove all exposure times if the Ladder Channel is different from the Detection Profile channel you are modifying.

e. To add additional times to the protocol, click the **Add** button and enter the values.

f. Select **OK**.

g. The number of exposures in the modified Detection Profile(s) will appear.

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

Exporting and Importing Assay Protocols

A modified assay protocol can be exported for later use. Exporting an assay protocol exports information from the Protocol pane only.

To export an assay protocol:

1. Select **File** in the main menu and click **Export Protocol...**.
2. The default directory is Compass for SW/Assays. Change the directory if needed.
3. Enter a protocol name and click **Save**. The protocol will be saved as a *.protocol file. The protocol file will include the information in the Protocol pane (steps and row locations), but will not include information in the Sample Template pane.

To import a previously saved assay protocol:

1. Select **File** in the main menu and click **Import Protocol...**.
2. Select the *.protocol file.
3. Click **Open**.

Steps 4–6

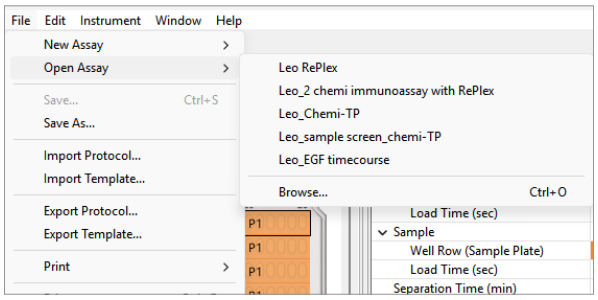
Steps 4 through 6 for creating a Chemiluminescence and/or Fluorescence Immunoassay with Total Protein Normalization using the PN channel are the same as when you're creating an Immunoassay. Please go to "Step 4: Add Assay Notes (Optional)" on page 66 to continue.

Opening and Modifying an Existing Assay

To open an existing assay.

1. Select the **Assay** screen.

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

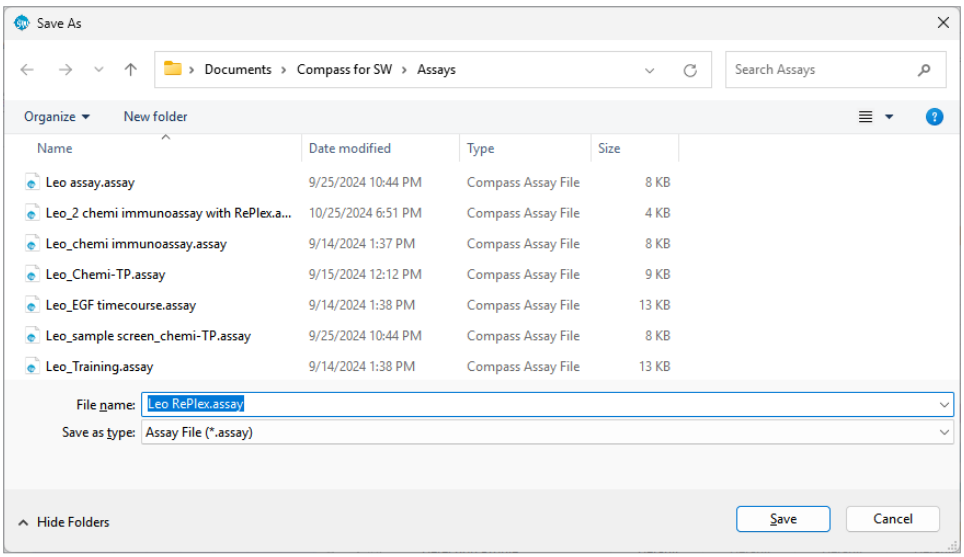


3. A list of the last five assays opened will display. Select one of these assays or click **Browse...** to open the Assay folder and select a different assay.
4. To make changes to the assay, follow the instructions under “Step 3: Modifying the Assay Protocol (Optional)” on page 60.
5. Select **File** from the main menu and click **Save**.

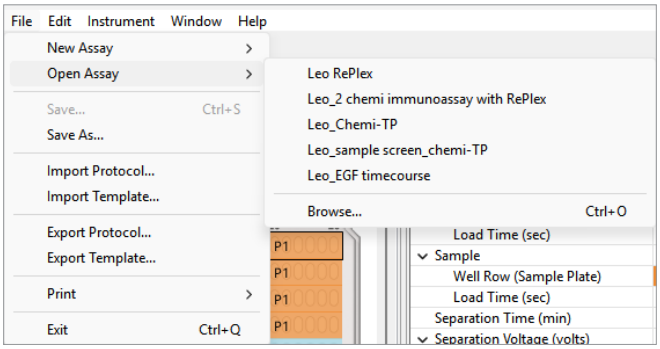
Creating a Custom Template Assay

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 in “Step 3: Modifying the Assay Protocol (Optional)” on page 60 to make changes to the assay.
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:

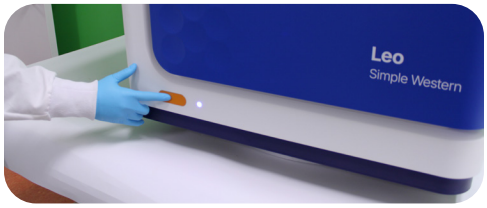


Installing the Cartridges and Plates

NOTES:
Capillaries are moisture- and light-sensitive. Keep the cartridge in the sealed package until you are ready to insert the capillary cartridge into your Leo Instrument.

To prevent well evaporation and to get the best results, keep a cover on the Sample Plate until you are ready to use it. Once you've pipetted your sample and reagents into the plate, it can't be stored for later use.

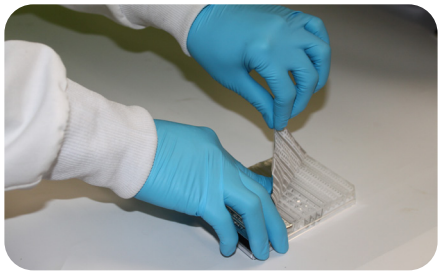
1. Open the Leo Instrument door by pushing the orange button below the door.



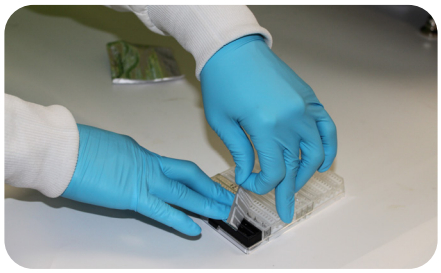
NOTE: The status light on the Leo Instrument front panel will blink rapidly as the door disengages.

2. Swing the door open to access the capillary cartridge holders, Pre-filled Reagent Plate tray, and Sample Plate tray. The lights above the capillary cartridge holder(s) will be **orange**.

3. Remove the plate seals from the Pre-filled Reagent Plate(s).
   a. Hold the plate firmly on the bench.
   b. Carefully peel off the upper seal from the top corner.



   c. Carefully peel off the bottom seal from the side.

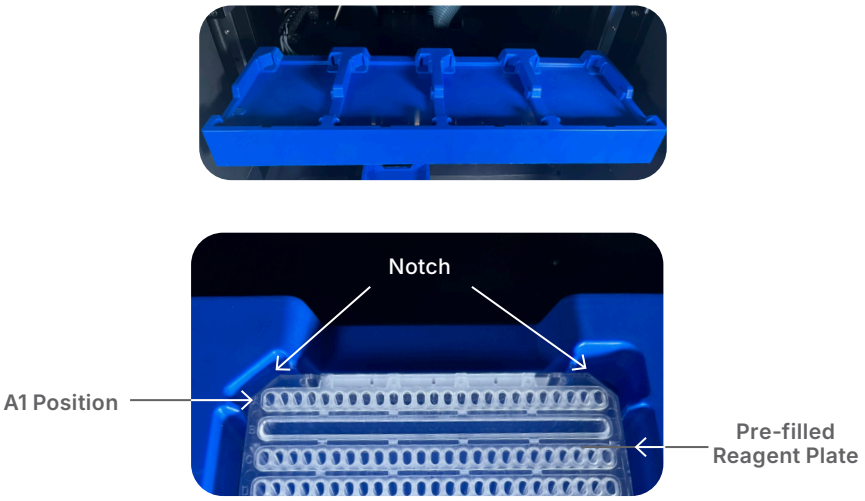


IMPORTANT:
When removing the bottom seal, slowly remove the seals to avoid jostling liquid out of the plate or into adjacent wells.

Pop any large bubbles with a dry pipette tip.

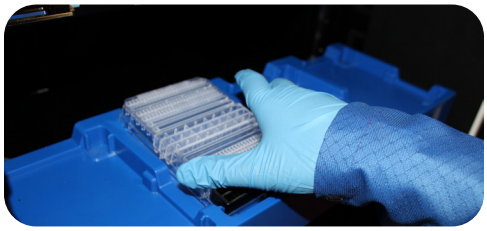
4. Place the Pre-filled Reagent Plate(s) in the Pre-filled Reagent Plate tray holders.

   a. A plate holder has a notch at the upper right corner and the upper left corner to ensure that the plates are placed in the correct orientation.

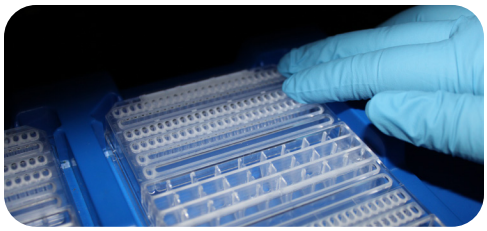


NOTE: Keep the Pre-filled Reagent Plate flat during transfer to avoid spills and shifting of reagents.

   b. Insert the bottom of the plate first.

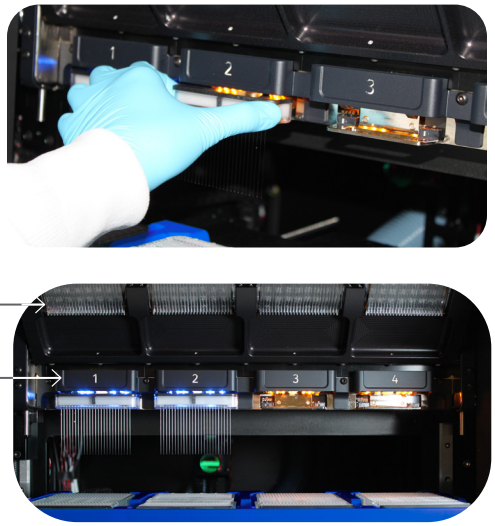


   c. Push the top of the plate down.



IMPORTANT: Make sure the Pre-filled Reagent Plates are firmly seated and level in the holders.

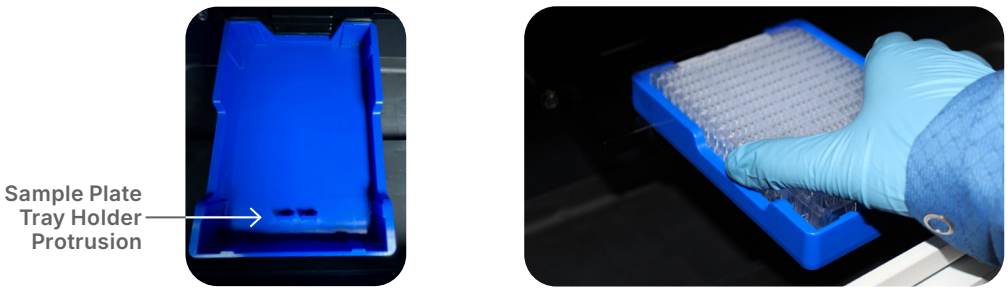
5. Remove the capillary cartridge(s) from the packaging and insert them into the capillary cartridge holder(s). The interior light(s) will turn from **orange** to **blue**.



NOTE: Take care not to touch the cartridge shields when installing the cartridges.

6. Remove the Sample Plate cover and place the plate in the Sample Plate tray holder. The holder has a protrusion at the bottom center to ensure a Sample Plate, and not a Pre-filled Reagent Plate, is placed in the 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.



IMPORTANT:
Make sure the Sample Plate is firmly seated and level in the holder.

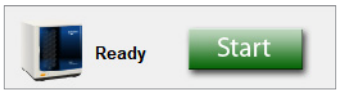
7. Close the Leo Instrument door.

> **NOTE:** Do not attempt to open the door again until the status light turns solid **blue**.

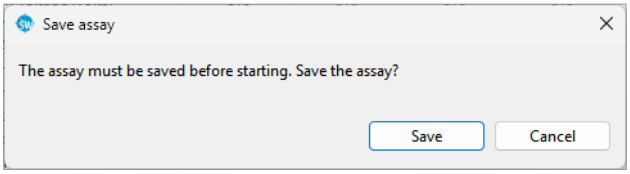
8. After the door closes, Compass will scan the capillary cartridge(s), Pre-filled Reagent Plate(s), and Sample Plate barcodes to ensure the correct number are loaded and that the correct size range plates match the assay.
   a. The status light will quickly blink **blue** while the instrument homes and initializes.
   b. The status light will slowly blink **blue** while the barcodes are being read.
   c. The status light will be solid **blue** when the Leo System is done scanning the barcodes. You can now start your run.

Starting the Assay

1. Click **Start** to start your run. You can also start a run by selecting **Instrument** in the main menu and clicking **Start...**.



2. If you have modified your assay, you will be asked to save the assay before starting the run. Click **Save**.



3. The Start Run dialog will appear. An icon will indicate if the instrument is not ready to start the run.

- **Door open** – Close the door to start the run.
- **Door closed, instrument scanning cartridge and plate barcodes** – Wait until the scan is complete. The status light will be solid **blue**.

4. After the door is closed and the barcode scan is complete, the Start Run dialog will display the scanned barcodes or warn you if:
   - **Plate is expired** – You have loaded a Pre-filled Reagent Plate that is past its expiration date. You will be able to start the run.
   - **Cartridge is expired** – You have loaded a capillary cartridge that is past its expiration date. You will be able to start the run.

- **Assay mismatch:** MW – The Pre-filled Reagent Plate size range does not match the assay size range. You will be able to start the run.
- **Assay mismatch:** Capillaries – You have loaded a 13-capillary cartridge that is not compatible with the Leo Instrument. You will not be able to start the run.
- **Cartridge missing** – No capillary cartridge is detected in a holder that should contain a cartridge based on the assay setup. You will not be able to start the run.

Hover over the warning icon to learn more about the warning details.

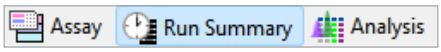
NOTE: If a barcode cannot be scanned, a note reminding you to confirm the cartridge or Pre-filled Reagent Plate matches the assay parameters will appear.

5. Click in the **Results file name** box if you want to change the default run file name. Otherwise, just leave it as is.
6. If you don't want to save the file to the default Runs folder, click **Browse...** to select a different location.
7. Enter any run details you'd like in the Comments box (optional). The comment will be saved in the Run Summary History pane.
8. Click **Start**.
9. The Status Mode next to the instrument icon will change to Running, with a progress bar indicating when the run will be completed, and the status light will change from steady blue to pulsing blue to let you know your Leo Instrument is running.

Monitoring a Run

Run Summary Screen Overview

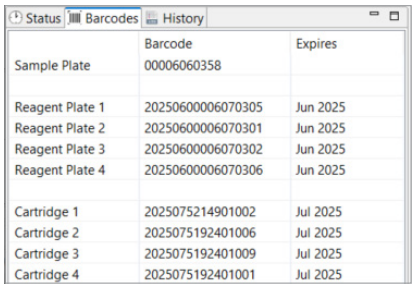
The Run Summary screen monitors run progress, displays movies of the fluorescent standards separation, and displays current and voltage plots for a run. Information collected is available during and after the run is completed. To access this screen, click **Run Summary** in the screen tab:



NOTE: Refer to the **Compass for Simple Western User Guide** to learn more about Compass Software features.

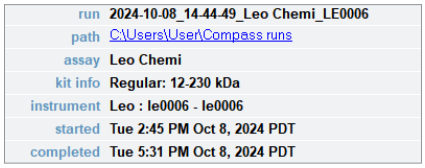
Viewing File and Run Status Information

The Barcode pane records the serial number of the Sample Plate, and serial numbers and expiration dates of the Pre-filled Reagent Plate(s) and capillary cartridge(s).



	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

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



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

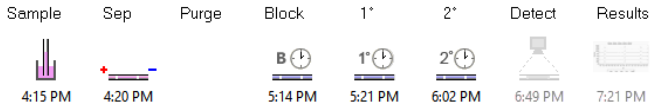
- **To go to the run file directory location** - Double click the path hyperlink, or right-click and select **Open Directory**.
- **To copy the path** - Right-click on the path hyperlink and click **Copy Path**. The path can then be copied into documents. The path can also be copied into the Windows Explorer address bar to launch Compass for Simple Western and open the run file automatically.
- **Kit info** - Displays the type of kit used to run the assay (Regular for immunoassays and RePlex assays) and the molecular range.

Each assay step that is executed during the run is represented by an icon in the Run Summary pane. The time the individual step is scheduled to start is shown under each icon.

Details on what happens during each step are as follows:

Step	Description
Sample  12:54 PM	**Sample Loading Step** - Capillary cartridge(s) are moved to the Pre-filled Reagent Plate(s) where Separation Matrix and Stacking Matrix are aspirated first before moving to the Sample Plate where the biotinylated ladder and samples are aspirated. Low vacuum is applied to filled cartridges to keep the reagents in the capillaries when other cartridges are being loaded. Capillary cartridges are then transferred to the running buffer trough in the Pre-filled Reagent Plate(s).
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 from the Pre-filled Reagent Plate(s).
PN  4:38 PM	**Protein Normalization Step (Protein Normalization Assays only)** - Capillary cartridge(s) are moved to the Sample Plate and Protein Normalization Reagent is aspirated. When the incubation is complete, Wash Buffer is aspirated from the Pre-filled Reagent Plate(s).
Label  5:08 PM	**Label Step (Total Protein Assays only)** - Capillary cartridge(s) aspirate Biotin Labeling Reagent from the Sample Plate. When incubation is complete, Wash Buffer is aspirated from the Pre-filled Reagent Plate(s).
Block  2:00 PM	**Blocking Step** - Capillary cartridge(s) are moved to the Sample Plate and blocking reagent (Antibody Diluent) is aspirated. When incubation is complete, Wash Buffer is aspirated from the Pre-filled Reagent Plate(s).
1°  2:16 PM	**Primary Antibody (1°) Step** - Capillary cartridge(s) are moved to the Sample Plate and primary antibody is aspirated. When incubation is complete, Wash Buffer is aspirated from the Pre-filled Reagent Plate(s).
2°  4:22 PM	**Secondary Antibody (2°) Step** - Capillary cartridge(s) are moved to the Sample Plate and secondary HRP/NIR/IR antibody conjugate and Streptavidin-HRP/NIR is aspirated. When incubation is complete, Wash Buffer is aspirated from the Pre-filled Reagent Plate(s).
HRP  8:43 PM	**HRP Step (Total Protein Assays only) -**Capillary cartridge(s) are moved to the Sample Plate and Total Protein Streptavidin-HRP is aspirated. When incubation is complete, Wash Buffer is aspirated from the Pre-filled Reagent Plate(s).
Detect  5:28 PM	**Detect Step** - Capillary cartridge(s) are moved to the Sample Plate and Luminol-S/Peroxide solution is aspirated (chemiluminescence only). Emitted chemiluminescent light is detected with the CCD camera. For fluorescence detection, emitted fluorescent signal is detected.
Purge  7:57 PM	**Purge Step (RePlex Assay only)** - Capillary cartridge(s) are moved to the Pre-filled Reagent Plate(s) and Wash Buffer is aspirated. Cartridges are moved to the Sample Plate and RePlex Reagent mix is then aspirated to remove the Probe 1 primary and secondary antibodies from the immobilized sample proteins. The cartridges are moved back to the Pre-filled Reagent Plate(s) for another round of Wash Buffer.
Results  6:00 PM	**Results Step** - Results are available in the Analysis screen.

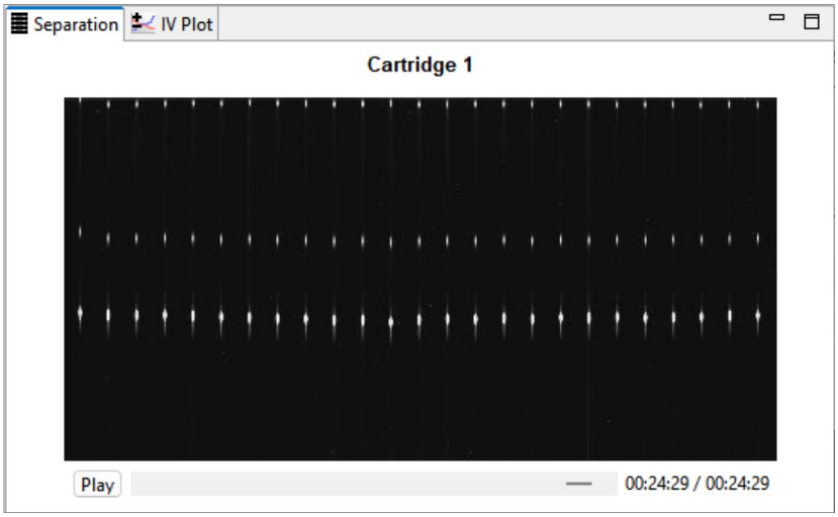
When a run is in progress, icons for steps that have not been executed yet will be gray (inactive). In the following example, the Secondary Antibody (2°) step is executing and the Detect and Results steps have not started.



Watching Standards Separation Movies

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

1. Click the **Separation** tab.

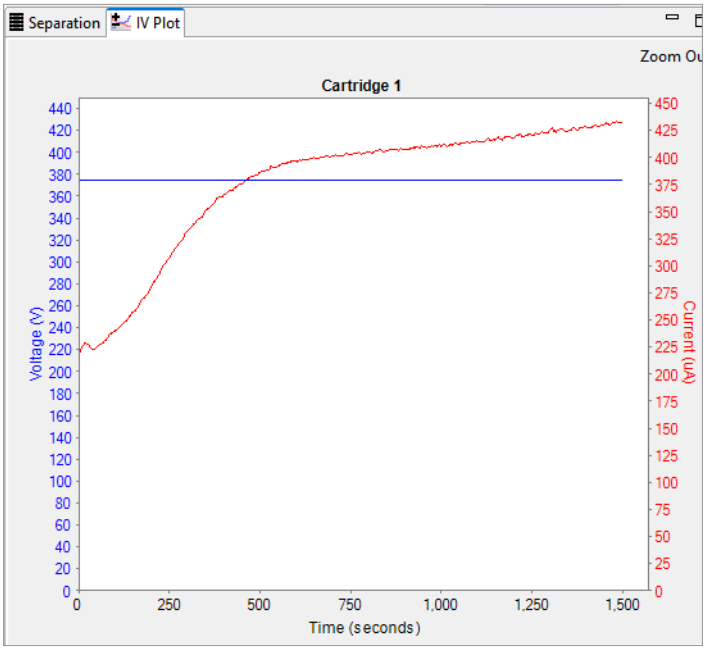


2. The player control panel has play/pause, rewind, and fast forward buttons, and a slider bar that allows you to scroll through the movie manually:
3. Click **Play** (button on the far left) to view the movie.

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

Viewing Current and Voltage Plots

You can view plots of the total current and voltages measured during separation. To do this, click the **IV Plot** tab.



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

- **To zoom in on an area of the plot** - Hold the left 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.

Post-Run Procedures

1. Remove the capillary cartridge(s).

IMPORTANT: If you turn the Leo Instrument off after the run, liquid may leak out of the cartridge if left in the instrument after Leo is powered down. See “Shutdown” on page 34 for information on turning the instrument off.

2. Remove the Pre-filled Reagent Plate(s).
3. Remove the Sample Plate.
4. Dispose of the Pre-filled Reagent Plate(s) according to your institution's policy for disposal of reagents containing SDS.
5. Dispose of the Sample Plate and capillary cartridge(s). Sample Plate and capillary cartridge disposal will depend on the samples that have been assayed. If sample origins are unknown, we recommend that used capillary cartridges and Sample Plates be disposed of as biohazard waste.

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

!WARNING! BIOHAZARD
Samples and 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 environ-mental 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.

Size Assay Data Analysis

Refer to the **Compass for Simple Western User Guide** for information on analyzing data generated by your Leo System.

Chapter 6:

Compass Access Control for 21 CFR Part 11 Compliance Features

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 21 CFR 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 (i.e., 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 Software can be run with or without Access Control enabled. When Access Control is disabled, no user login 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 login 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. See “Enabling Access Control” on page 131 for more information.

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.

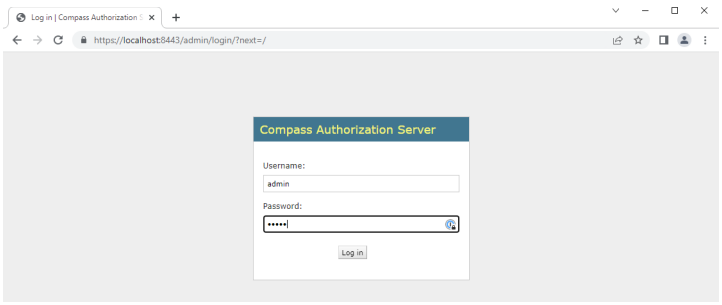
The default server administrator is:

- 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.

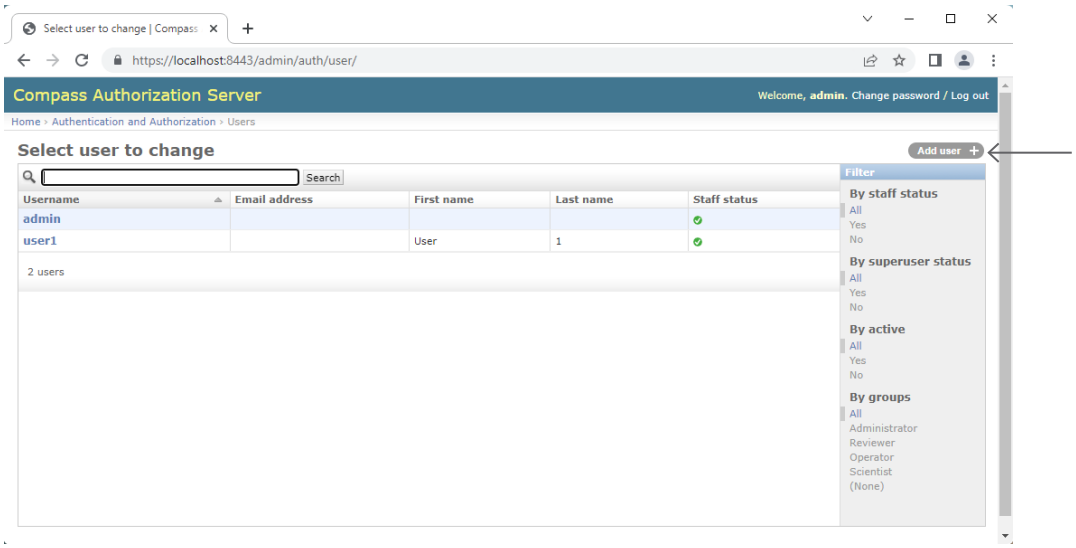


Adding Non-admin Users

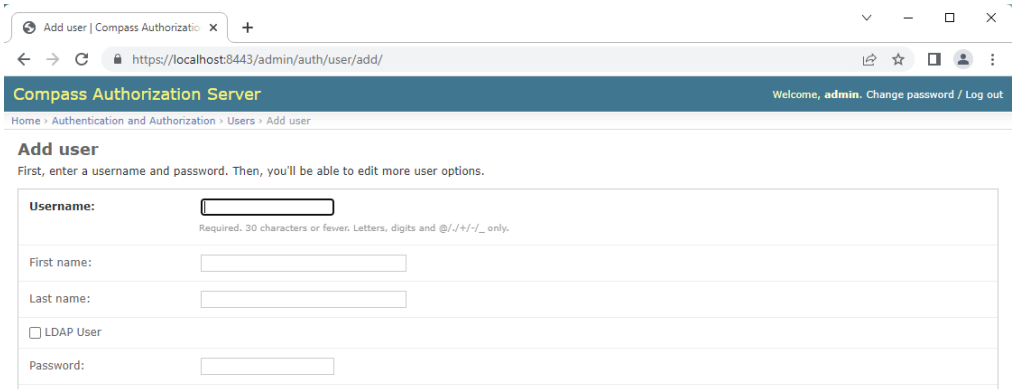
Add a user to the server to allow that user to log in to Compass for Simple Western. To do this:

1. Select **Users** from the Site Administration home page:

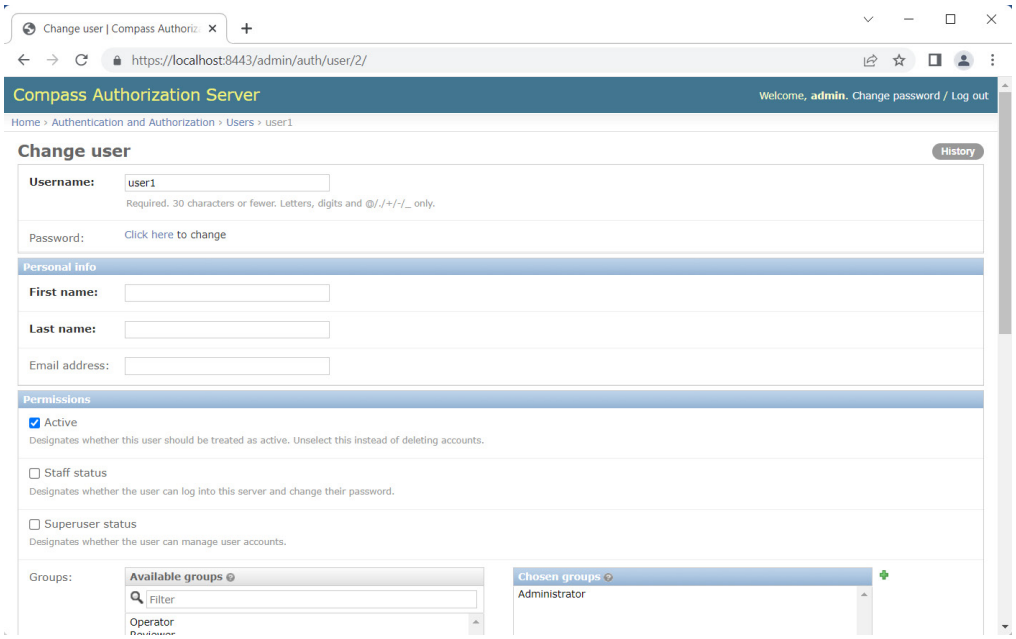
2. From the Users page, select **Add User**:



3. Fill in the fields to create a new user:



After adding a new user more information can be added:



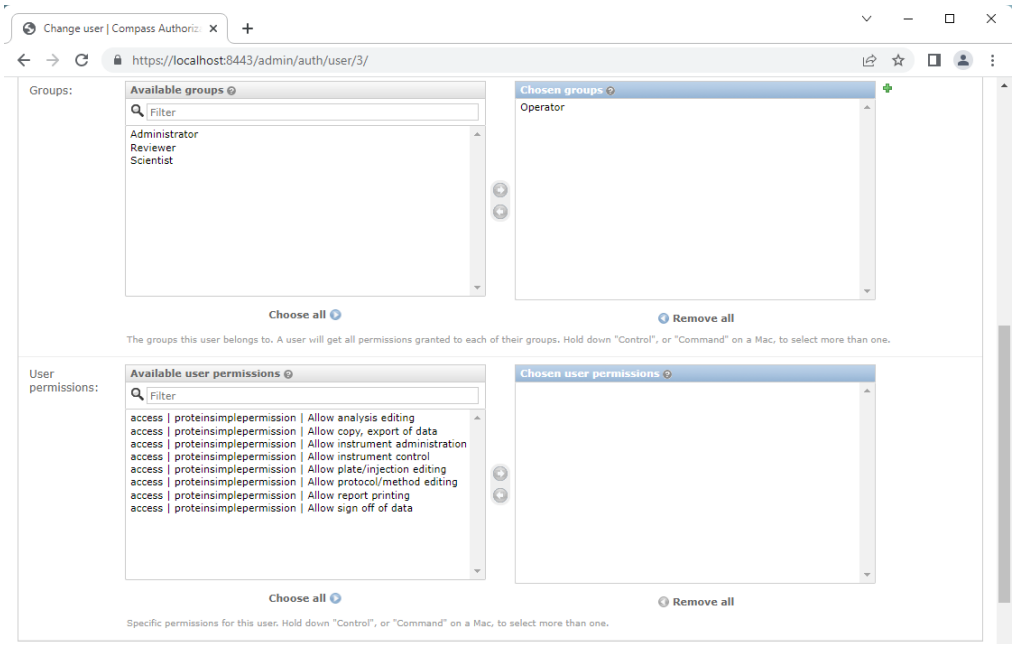
NOTES:
Users are blocked after the number of login failures defined in the Password policy setting. See “Password Policy Settings” on page 130 for more information.

Non-admin users will not be able to disable Access Control when the CAS connection is lost.

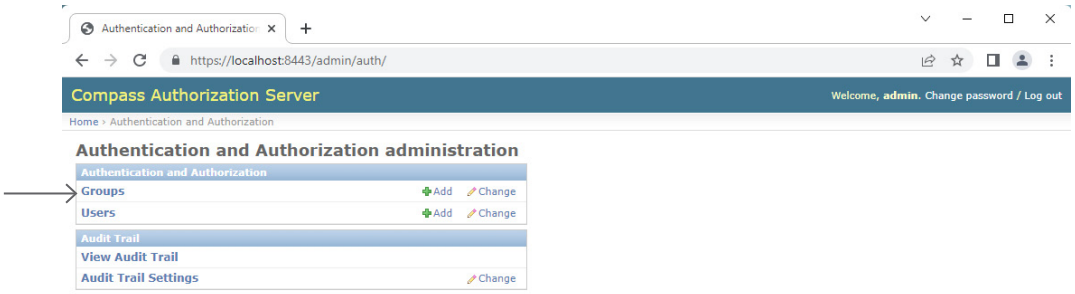
Permissions

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.

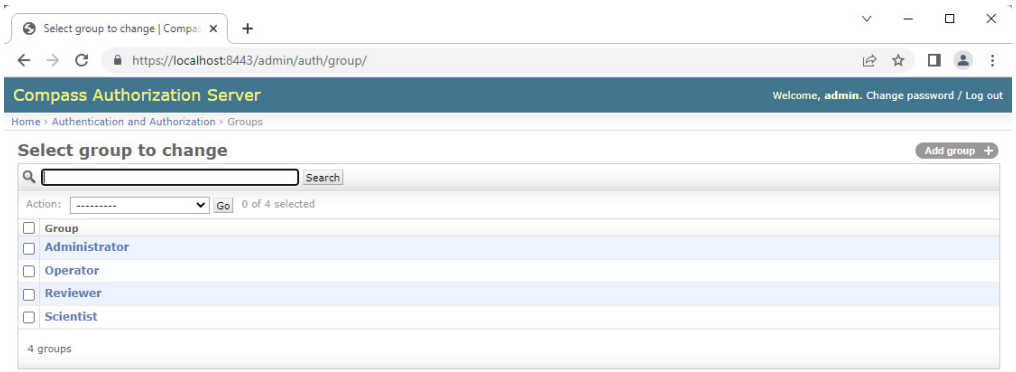
Users can belong to groups that have multiple permissions such as Operator or Scientist:



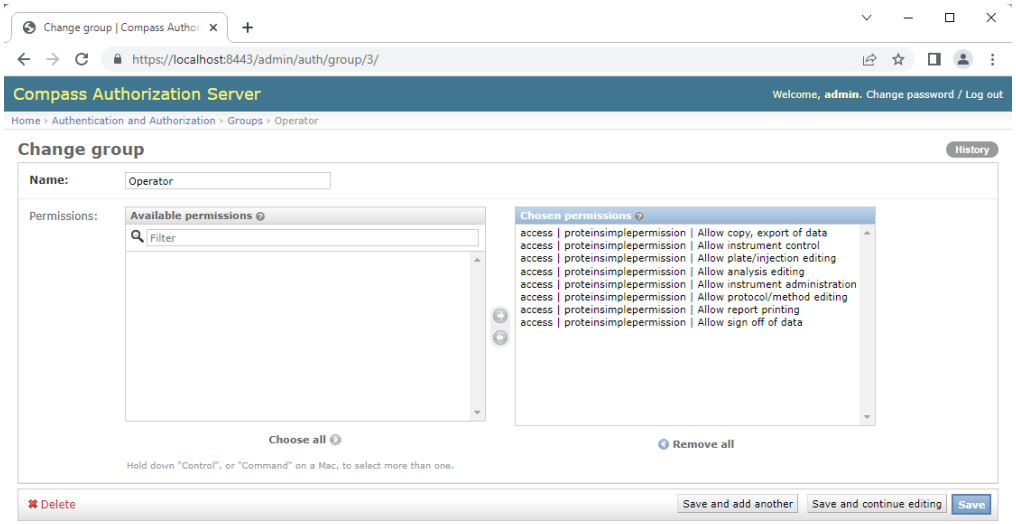
Use the Groups page to change the permissions in a group or create new groups:



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:

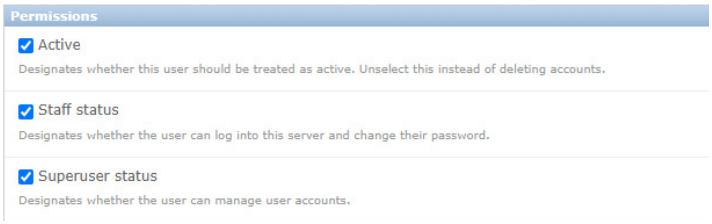
- **Allow analysis editing** - lets users change analysis settings
- **Allow copy, export of data** - lets users copy and export data
- **Allow instrument control** - gives users permission to connect to an instrument
- **Allow plate editing** - lets users update the plate layout and edit sample info
- **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 the instrument, 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 121 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 to in order to have Compass for Simple Western permissions.

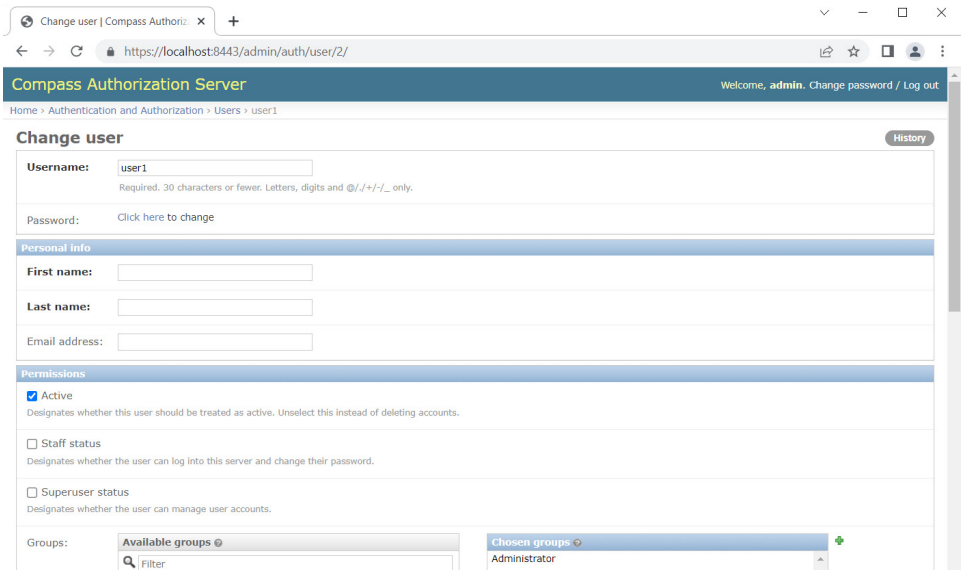
Only Admins will be able to disable Access Control when the CAS connection is lost.

Resetting User Passwords

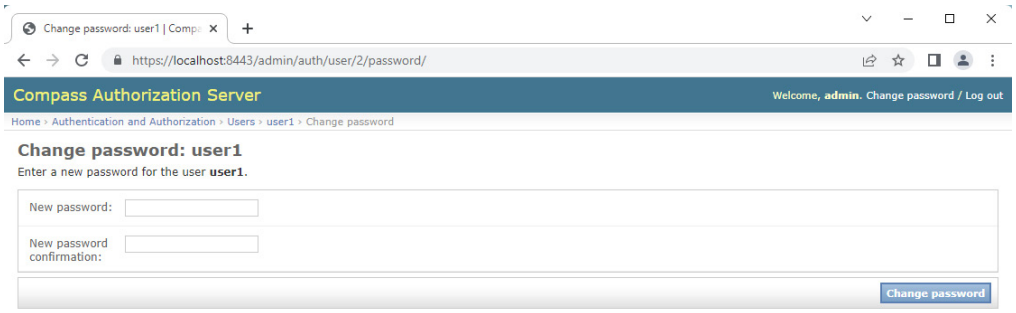
NOTE: Users are blocked after the number of login failures defined in the Password policy setting.

To reset a user password:

1. Select **Users** from the Site Administration home page, then select the user to change. The following screen displays:



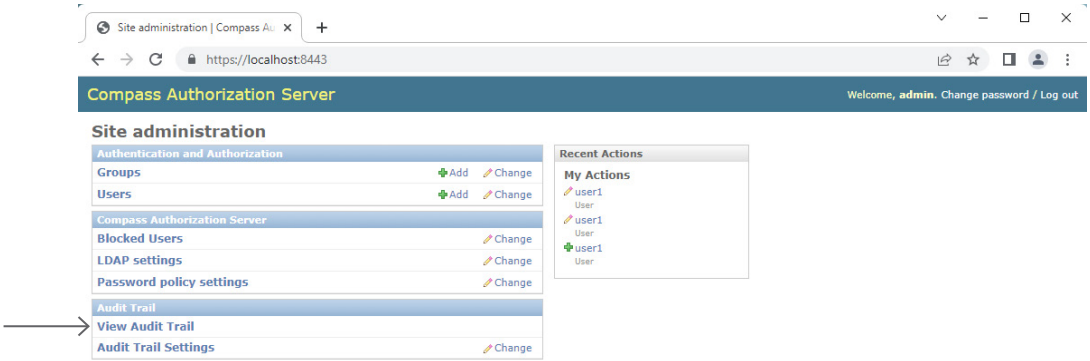
2. Click the text link to access the password change form:

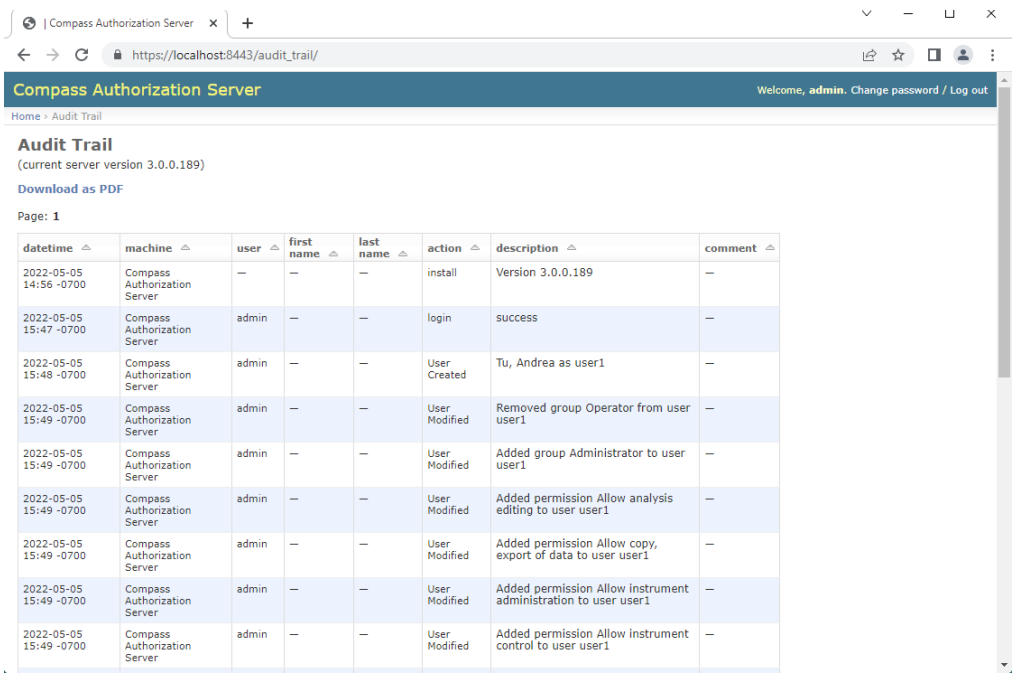


3. Enter the new password, then click **Change password**.

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.





datetime	machine	user	first name	last name	action	description	comment
2022-05-05 14:56 -0700	Compass Authorization Server	–	–	–	install	Version 3.0.0.189	–
2022-05-05 15:47 -0700	Compass Authorization Server	admin	–	–	login	success	–
2022-05-05 15:48 -0700	Compass Authorization Server	admin	–	–	User Created	Tu, Andrea as user1	–
2022-05-05 15:49 -0700	Compass Authorization Server	admin	–	–	User Modified	Removed group Operator from user user1	–
2022-05-05 15:49 -0700	Compass Authorization Server	admin	–	–	User Modified	Added group Administrator to user user1	–
2022-05-05 15:49 -0700	Compass Authorization Server	admin	–	–	User Modified	Added permission Allow analysis editing to user user1	–
2022-05-05 15:49 -0700	Compass Authorization Server	admin	–	–	User Modified	Added permission Allow copy, export of data to user user1	–
2022-05-05 15:49 -0700	Compass Authorization Server	admin	–	–	User Modified	Added permission Allow instrument administration to user user1	–
2022-05-05 15:49 -0700	Compass Authorization Server	admin	–	–	User Modified	Added permission Allow instrument control to user user1	–

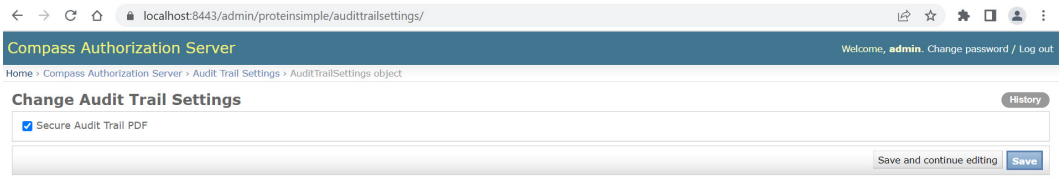
Here are the actions current logged in the Audit Trail:

- **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.

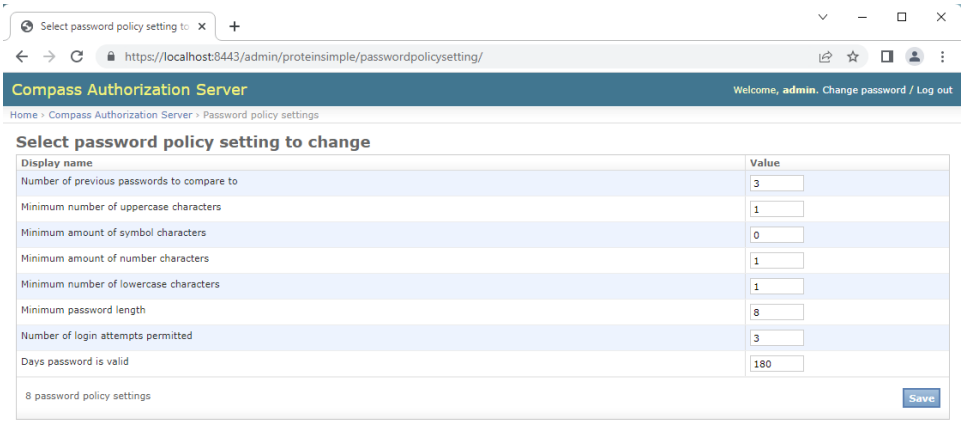
Audit Trail Settings

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

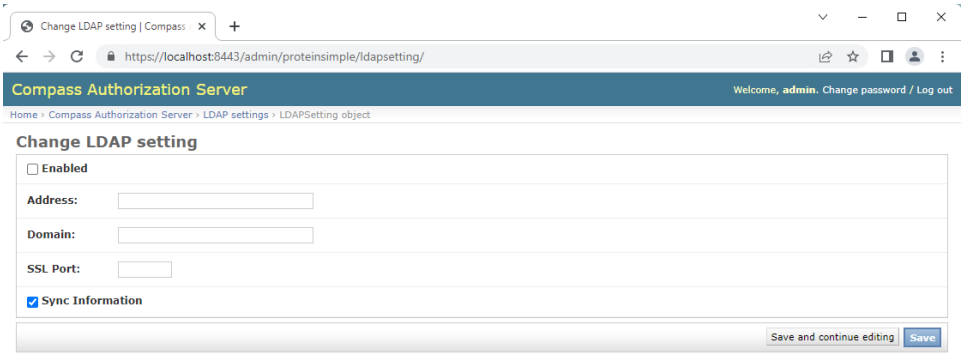
These settings let administrators set password policies. Select **Password policy settings** from the Site Administration home page to make changes.



LDAP Settings

LDAP settings 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 121 and select the LDAP User checkbox. Passwords aren’t required for LDAP users.

NOTE: When enabling LDAP settings, ensure the SSL port number reflects a secure LDAP port (636 recommended). Compass Authorization Server for Simple Western doesn’t support non-secure connections.

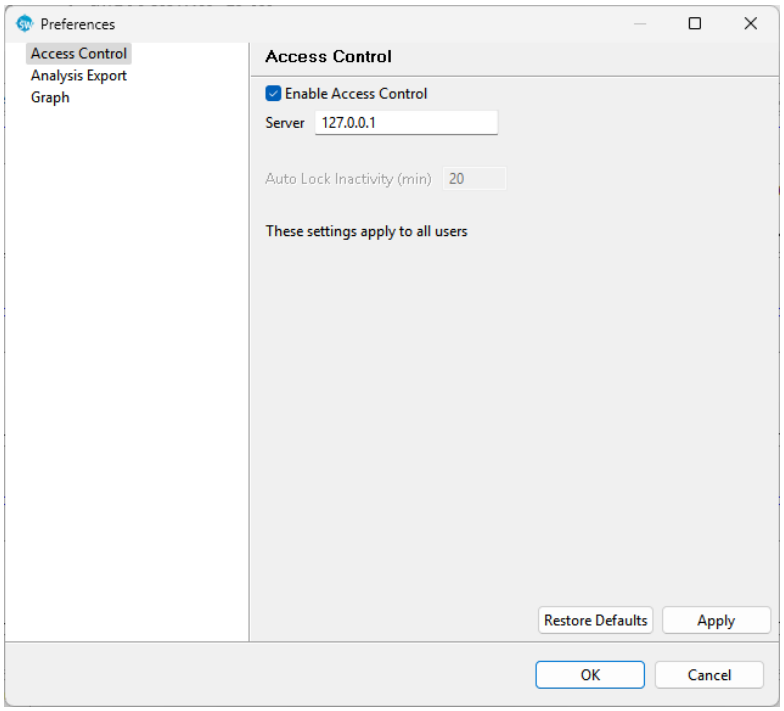
Encryption Details

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:

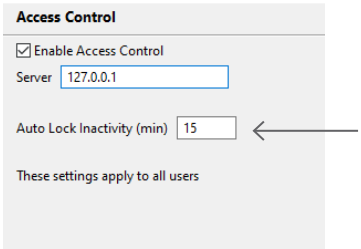
1. Check the **Enable** box.
2. Enter the IP address of the Compass Authorization Server for Simple Western. Use format X.X.X.X or localhost if installing the server on the local machine.
3. Close Compass for Simple Western. The next time the software is launched, a user log in will be required.

NOTES:
Access Control can only be disabled by logging into Compass for Simple Western and deselecting the **Enable** box in the Access Control page of Preferences.

Changing the Software Inactivity Auto Lock

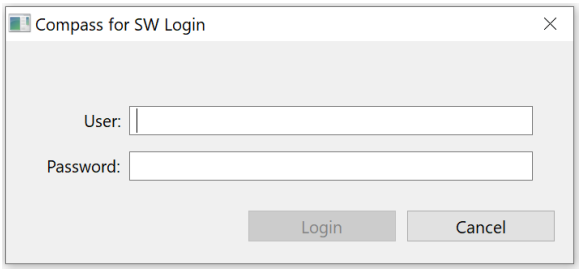
Compass for Simple Western Software automatically locks to prevent user access after a period of inactivity. Once the software is locked, users must log in again. Only users with instrument administrator permission can set the auto lock inactivity time.

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

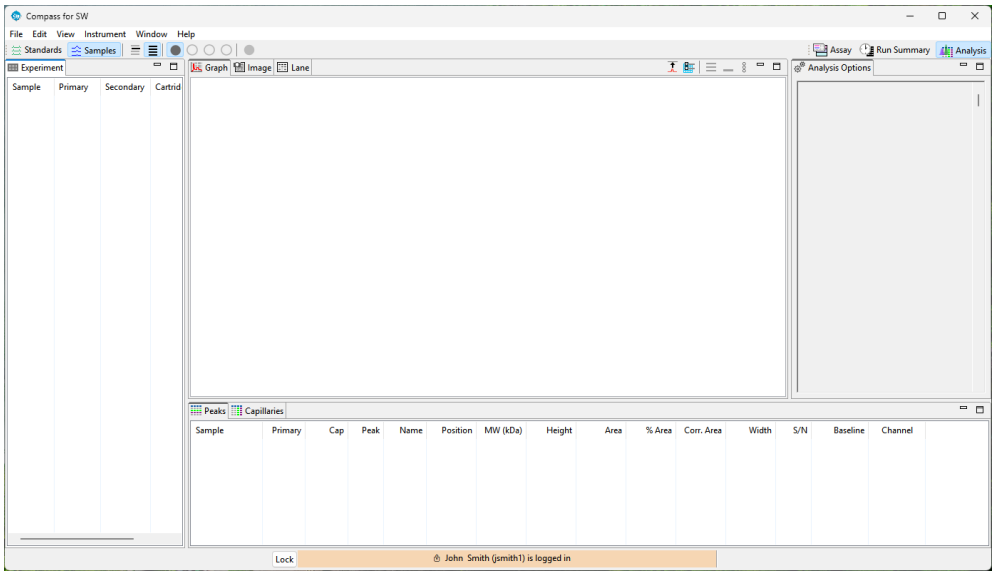
With Access Control enabled, all users must log in to Compass for Simple Western whenever the software is launched.



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.

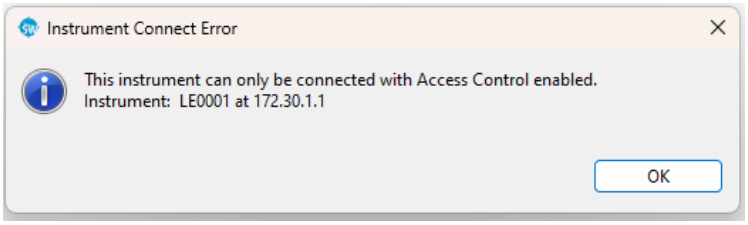
A successful log in will display the Compass for Simple Western main window with the user information in the lower status bar. The full username is displayed with the unique user ID in parenthesis:



Connecting Compass to Leo Systems with Access Control Enabled

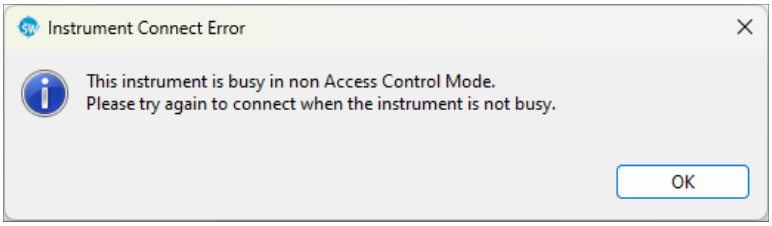
A Leo System will lock into CFR mode after you connect to the system with an instance of Compass for Simple Western Software that has Access Control enabled. See “Enabling Access Control” on page 131 for more information.

Once in CFR mode, Compass will only be able to connect to the Leo Instrument if Access Control is enabled. A warning will appear if you try to connect using an instance of Compass without Access Control enabled. This warning will not impact any current assay or test the instrument is in the middle of performing.



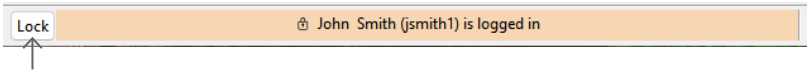
The Leo Instrument will reset so it is not in CFR mode when you turn the system off and then back on.

If you Leo System is currently running an assay or test in non-CFR mode, you will not be able to connect to the instrument with an instance of Compass for Simple Western with Access Control enabled. A warning will appear if this happens:



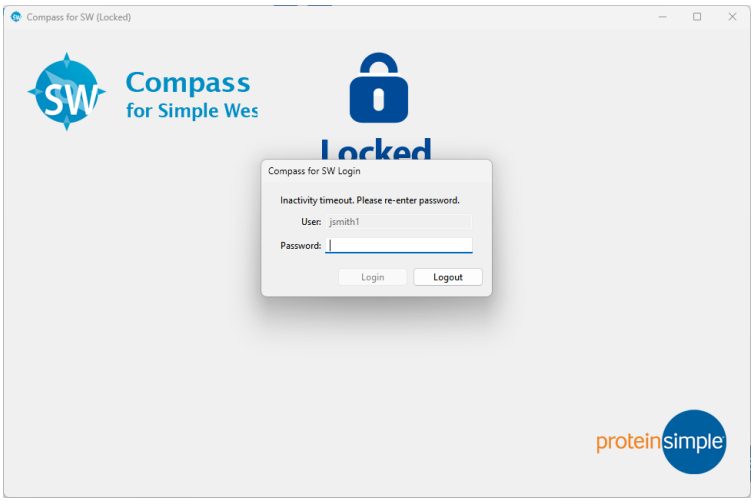
Locking and Unlocking the Application

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 132 for information on how to modify the lock-out timing.



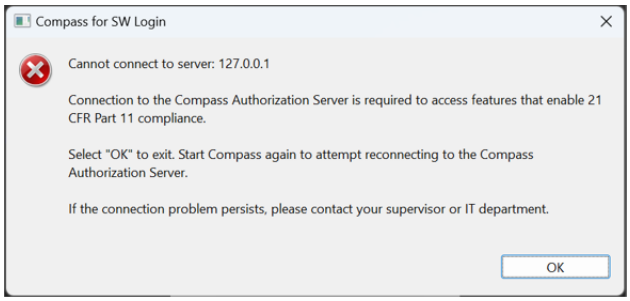
Resolving Log In Issues

Log in failures may occur when:

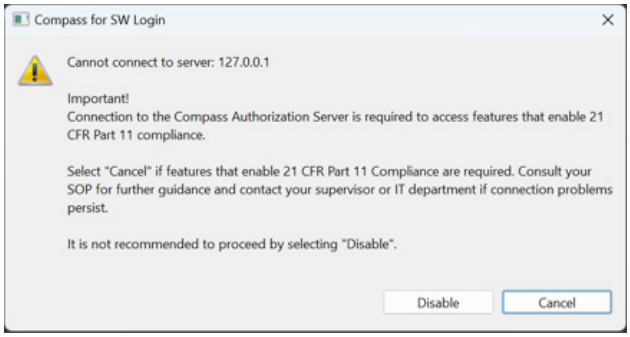
- The server is temporarily unavailable
- Compass for Simple Western is using the wrong IP address

When this happens, the following message displays:

USER:



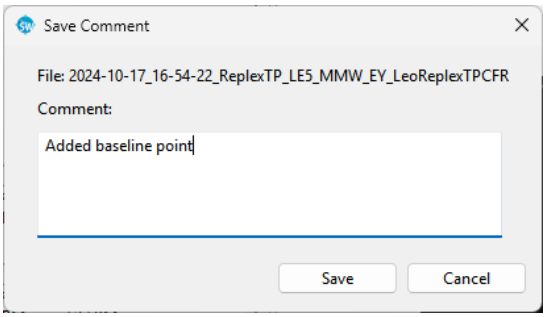
ADMIN USER:



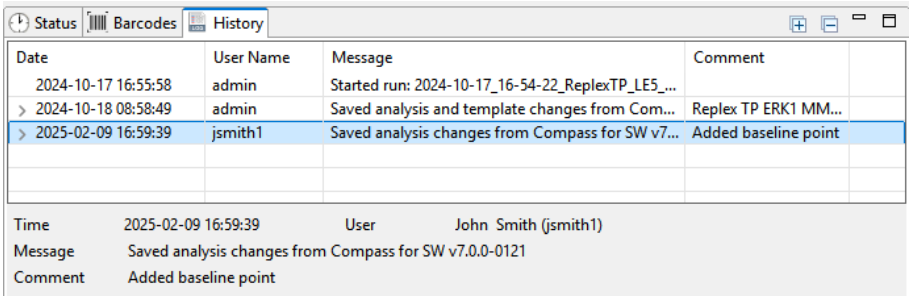
Click **Disable** to restart Compass for Simple Western with Access Control disabled. Verify or correct the server IP address then close and restart the software to log in with Access Control enabled.

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:

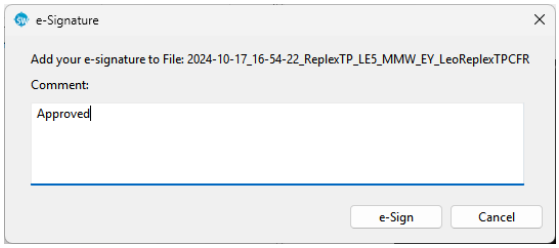


The comment is added to the signature entry in the file History:

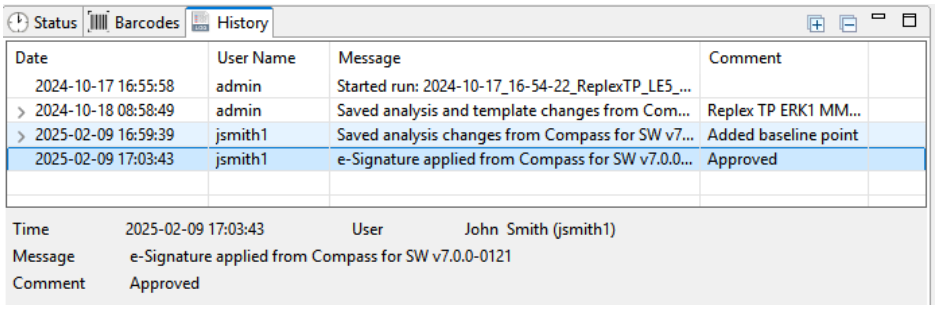


Signing Files

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*.

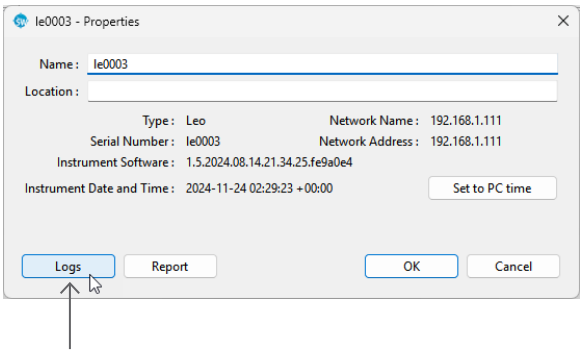


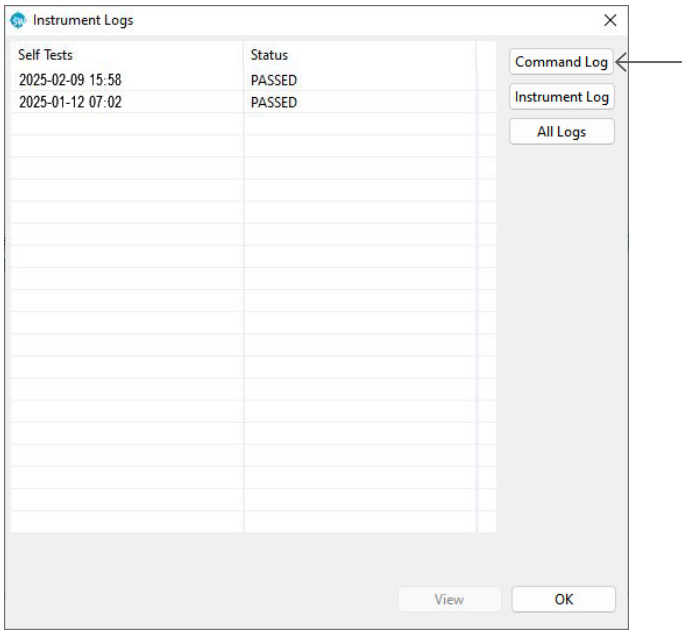
Exporting Uncontrolled Files

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.

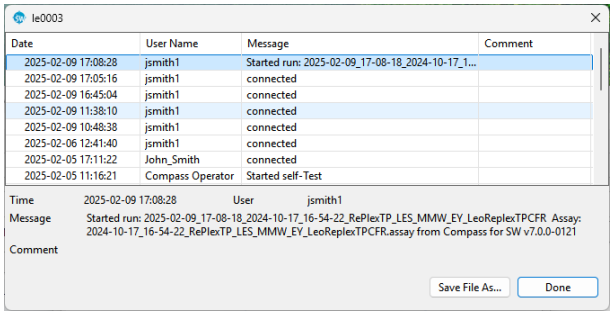
Instrument Command Log

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**:



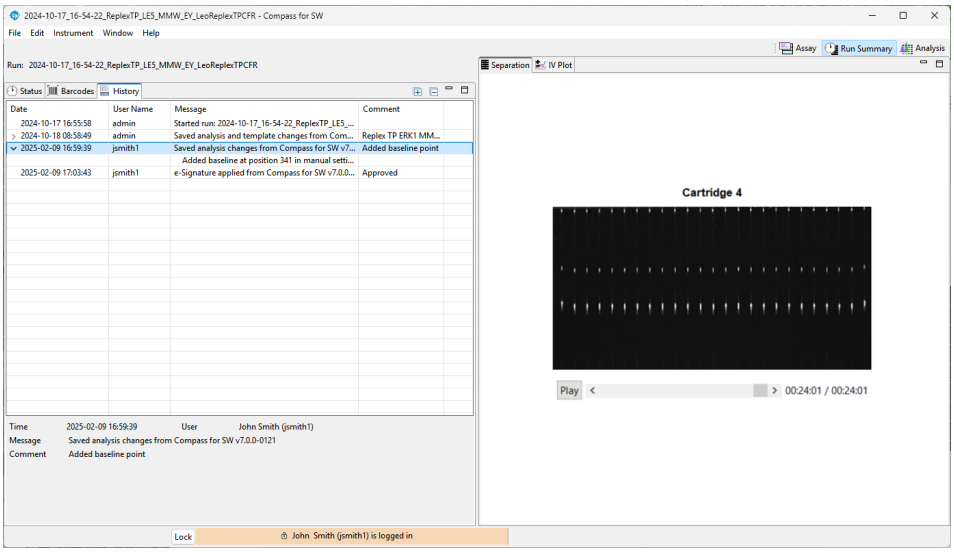


The Command Log lists all the commands sent to the instrument that were signed by the user who sent the command. If you want to copy the Command Log at any time, right click in the table and select **Copy**, then paste into another document.



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 126 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**, 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**, 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.

- **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 login.** Compass for Simple Western will only prompt for a login on launch when Access Control is enabled in Preferences. Enable Access Control by clicking **Edit**, then **Preferences** and **Access Control**. Select **Enable**, close Compass for Simple Western, then re-launch the software. You should now be prompted for a login.

Chapter 7:

Maintenance and Troubleshooting

Chapter Overview

- Software Updates
- Instrument Software (Embedded Updates)
- Maintenance
- Cleaning
- Basic Troubleshooting
- Leo Consumables

Software Updates

To check for software updates, first make sure the computer you're using has an active internet connection. Then go to Compass for Simple Western Software, select **Help** in the main menu and click **Check for Updates**. If you don't have internet access, contact ProteinSimple Technical Support for assistance on getting the latest update.

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 the system software will be displayed.

To check for embedded updates, first make sure the computer you're using has an active internet connection and is also connected to the Leo instrument. If it is not connected to Leo, go to Compass for Simple Western Software and connect to your instrument. Select **Instrument** in the main menu, then click **Update** and then click **Network**. If you don't have internet access, contact ProteinSimple Technical Support for assistance on getting the latest update.

Maintenance

Daily

Clean the Cartridge Holders and Trays: Dispose of the used capillary cartridge(s), Pre-filled Reagent Plate(s), and Sample Plate after each run.

Yearly

We recommend that the Leo Instrument has annual preventive maintenance performed by an authorized ProteinSimple representative. Please contact your Simple Western Sales Specialist or Technical Support to schedule a visit toll-free in the US and Canada at 1-888-607-9692. For customers in Europe, please contact Technical Support at +44 1235 529449.

As Needed

Clean the Door Liner: Clean the door liner if you notice contaminants in the fluorescent (standards) image or in your Fluorescence Immunoassay data. For optimal results, we recommend cleaning the door liner before performing a fluorescence assay. Contact ProteinSimple Technical Support to confirm contaminants if needed.

1. Open the Leo Instrument door to reveal the door liner (glossy black finish area behind the door).

2. Spray 70% isopropyl alcohol onto a Polyester Cleanroom line-free wipe (P/N 104-0031) or equivalent.
3. Carefully wipe down the door liner with the moist wipe. Avoid touching the door liner with your hands.
4. Wait 30 seconds for the solvent to evaporate before using your Leo System.

NOTE: If your instrument is failing the Self Test due to background or filter checks, clean the door liner as instructed above. For further assistance, contact Technical Support at **support@bio-techne.com** (US/Canada) or **Instrument.Support.EMEA@bio-techne.com** (Europe).

Cleaning

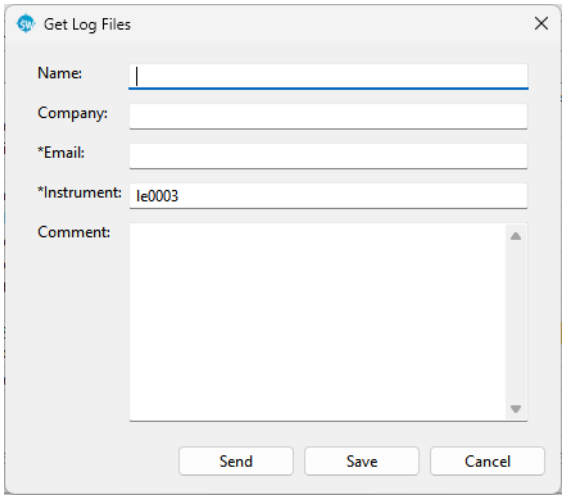
- **Touch pad** - Clean as needed with a soft cloth lightly moistened with dilute detergent.
- **Drip tray, Sample Plate tray, and Pre-Filled Reagent Plate tray** – Clean as needed with DI water.

Basic Troubleshooting

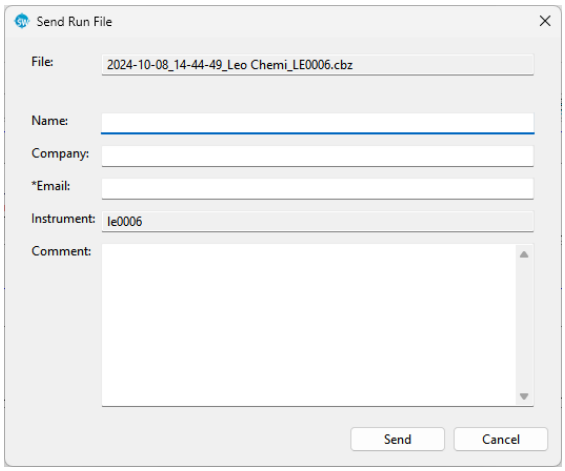
Problem	Solution
The Leo Instrument doesn't power on	• Make sure the power cord is still securely plugged into the outlet and on the back of the instrument.
The Leo System door won't open	• Use the manual release button under the Leo System base at the lower left side to open the door. The system status should resume to normal when the door is closed.
Red error light	• Click the **Reset** button in Compass for Simple Western Software. • Power the Leo System down and turn it back on. If the error persists, contact Technical Support at **support@bio-techne.com** (US/Canada) or **Instrument.Support.EMEA@bio-techne.com** (Europe).
Purple/magenta error light for more than 60 seconds	• The Leo Instrument can't initialize or the hard drive has failed. Power the Leo Instrument down and turn it back on. • If the error persists, contact Technical Support at **support@bio-techne.com** (US/Canada) or **Instrument.Support.EMEA@bio-techne.com** (Europe).
The Leo Instrument won't connect to the computer	• Confirm the static IP address for the ethernet port is set to 172.30.1.2. • Use a direct ethernet to ethernet connection. USB to ethernet adapters can be unreliable. • If the error persists, contact Technical Support at **support@bio-techne.com** (US/Canada) or **Instrument.Support.EMEA@bio-techne.com** (Europe).
I can't access my data	• By default, the first run file will save to C:\Users\Documents\Compass for SW\Runs. For subsequent runs, the software will use the last location saved. If the last location can't be accessed, the save path will default to C:\Users\Documents\Compass for SW\Runs. • If you don't see your file in the default folder, connect to Leo System's embedded drive. While connected to the instrument in Compass for Simple Western, select **Instrument** > **Runs...** **NOTE:** Run files are organized chronologically.

For Leo Instrument and Simple Western assay troubleshooting information, please contact ProteinSimple Technical Support toll-free in the US and Canada at (888) 607-9692, **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 **https://www.bio-techne.com/resources/literature** or the **Bio-Techne Academy**, or contact your local Field Application Scientist for help.

If the computer connected to your Leo Instrument is connected to the internet, send log files directly to Technical Support through the **Help** menu using the **Export Logs** feature.



You can also send a run file that is open in Compass for SW Software directly to Technical Support through the **Help** menu using the **Send Run File** feature.



Leo Consumables

Click here for a full list of **Leo consumables**.

Chapter 8:

Safety and Regulatory Compliance

Chapter Overview

- Regulatory Compliance
- Safety Guidelines
- Safety Warning Labels
- Safe Use Specifications
- Chemical Hazards
- Biohazard Waste
- Safety Data Sheets
- Surface Decontamination
- Transport
- Customer Service and Technical Support
- Legal Notices

Regulatory Compliance

Leo Systems comply with:

- **UL 61010-1:2001:** Safety requirements for electrical equipment for measurement, control and laboratory use - Part 1: General requirements (US)
- **EN 61010-1:2001:** Safety requirements for electrical equipment for measurement, control and laboratory use - Part 1: General requirements (EU)
- **CAN/CSA 22.2 No. 61010-1-04:** Safety requirements for electrical equipment for measurement, control and laboratory use - Part 1: General requirements (CA)
- **EN 61326-1:2013:** Electrical equipment for measurement, control and laboratory use. EMC Requirements. General requirements (EU)

Safety Guidelines

!WARNING! If your Leo System is not used as specified by ProteinSimple, overall safety may be impaired.

!WARNING! If your Leo Instrument is damaged and doesn't function properly, stop the instrument safely and contact ProteinSimple Technical Support right away toll-free in the US and Canada at 1-888-607-9692 for customers in the USA or +44 1235 529449 for customers in Europe.

CAUTION Avoid using your Leo System in ways not specified by ProteinSimple to avoid personal injuries or damage to the instrument. Although the instrument has been designed to protect you, this protection may not be effective if the instrument isn't used properly. Only appropriated trained users should operate the system. Your Leo Instrument should only be serviced by a Bio-Techne Field Service Engineer.

Safety Warning Labels

The following safety labels are located on Leo Instruments. Each label will display a safety alert symbol indicating a potential safety hazard.

Symbol	Description
	Risk of Electric Shock
	Refer to the Leo Instrument User Guide before proceeding.
	Danger of hazardous waste. Use caution in these areas. This warning only applies if using hazardous material. Leo System reagents are not considered hazardous waste. If you are using hazardous materials, please contact your field service representative to place labels in the appropriate locations.

Safe Use Specifications

Personal Protective Equipment

When operating your Leo System, always use the following personal protective equipment:

- Lab coat
- General purpose safety goggles
- Latex or nitrile gloves

NOTE: Additional PPE may be required depending on any biohazards associated with the sample.

Chemical Hazards

!WARNING! CHEMICAL HAZARD
Some chemicals used can be potentially hazardous and can cause injury or illness.

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, clothing). For additional safety guidelines, consult the SDS.
- Do not leave chemical containers open.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended by the SDS.
- Comply with all local, state/provincial and national laws and regulations related to chemical storage, handling and disposal.

Biohazard Waste

!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 environ-mental 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.

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in a waste container before you store, handle or dispose of chemical waste.
- Minimize contact with chemical waste. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, clothing).

Safety Data Sheets

Some chemicals used with the Leo Instrument may be listed as hazardous. Warnings are displayed on the labels of all chemicals when hazards exist.

Safety Data Sheets (SDSs) provide users with safety information needed to store, handle, transport and dispose of the chemicals safely. We recommend updating laboratory SDS records periodically.

SDSs for ProteinSimple reagents are available online on the reagent specific webpage, or by calling toll-free in the US and Canada 1-888-607-9692. Otherwise, call the chemical manufacturer directly or visit their web site.

Surface Decontamination

All assay steps are performed and contained within the capillary cartridge. Unless there is a spill, no surface decontamination is required.

Always consult the SDS for specific information on the chemical before attempting cleanup.

NOTES:
Do not use bleach to clean the instrument.

Contact your Field Service Engineer if you need to decontaminate the cartridge shield or cartridge holder.

Transport

Do not attempt to move the Leo Instrument yourself. Contact your Field Service Engineer for support.

Customer Service and Technical Support

Telephone

(408) 510-5500

(888) 607-9692 (toll-free in the US and Canada)

+44 1235 529449 (Europe)

Fax

(408) 510-5599

E-mail

US - **support@bio-techne.com**

Europe - **Instrument.Support.EMEA@bio-techne.com**

Web

https://www.bio-techne.com/instruments/simple-western

Address

ProteinSimple

3001 Orchard Parkway

San Jose, CA 95134

USA

Legal Notices

NOTE: Read the Legal Notices carefully before using your Leo System.

Leo System Disclaimer of Warranty

EXCEPT AS EXPRESSLY PROVIDED IN ANY ProteinSimple SOFTWARE LICENSE AGREEMENT OR QUOTATION, THE PRODUCTS SOLD AND SERVICES PROVIDED BY ProteinSimple ARE PROVIDED ON AN "AS IS" AND "AS AVAILABLE" BASIS WITHOUT WARRANTY OF ANY KIND. ProteinSimple AND ITS SUPPLIERS DO NOT WARRANT THE SECURITY, PRIVACY OR ACCURACY OF ANY DATA PROVIDED VIA THE PRODUCTS OR SERVICES, AND YOU AGREE THAT THE USE OF ANY SUCH DATA BY YOU IS AT YOUR SOLE RISK. TO THE MAXIMUM EXTENT ALLOWED UNDER APPLICABLE LAW, ProteinSimple AND ITS SUPPLIERS DISCLAIM ANY AND ALL WARRANTIES, WHETHER EXPRESS, IMPLIED OR STATUTORY, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY OF MERCHANTABILITY, TITLE, NON-INFRINGEMENT OR FITNESS FOR A PARTICULAR PURPOSE.

Compass for Simple Western Software and Authorization Server License

IMPORTANT - PLEASE READ CAREFULLY THE TERMS OF THIS COMPASS SOFTWARE AND AUTHORIZATION SERVER LICENSE AGREEMENT ("AGREEMENT"). BY CLICKING ON THE "I AGREE" BUTTON, (1) YOU ACKNOWLEDGE THAT YOU HAVE READ, UNDERSTAND AND AGREE TO BE BOUND BY THIS AGREEMENT AND (2) YOU REPRESENT THAT YOU HAVE THE AUTHORITY TO ENTER INTO THIS AGREEMENT, PERSONALLY OR IF YOU HAVE NAMED A COMPANY AS CUSTOMER, ON BEHALF OF THAT COMPANY (YOU OR ANY SUCH COMPANY, THE "CUSTOMER"), AND TO BIND THE CUSTOMER TO THE TERMS OF THIS AGREEMENT. IF YOU DO NOT AGREE TO ALL TERMS AND CONDITIONS OF THIS AGREEMENT, OR IF YOU DO NOT HAVE SUCH AUTHORITY, YOU SHOULD CLICK ON THE "CANCEL" BUTTON TO DISCONTINUE THE DOWNLOAD OF THE LICENSED SOFTWARE.

1. **Definitions**

   1.1. **"Authorized Use Parameters"** means the following usage restrictions, which restrict the operation of the Licensed Software to a particular set of conditions: Customer shall (a) limit simultaneous use of the Licensed Software to a maximum of ten (10) Authorized Users; and (b) use the Licensed Software only in connection with the accompanying System purchased by Customer pursuant to the System Quotation and located at the Site.

   1.2. **"Authorized User"** means one (1) User who initiates the execution of the Licensed Software and/or interacts with or directs the Licensed Software in the performance of its functions. Multiple Authorized Users may work simultaneously with one installation of the Licensed Software, as on a server, or they may each have their own installation on single-user machines, or a mix of these, provided that in all cases the total number of simultaneous Users does not exceed the applicable Authorized Use Parameters.

   1.3. **"Company"** means ProteinSimple.

   1.4. **"Documentation"** means Company's then-current manuals, guides and online help pages, if any, applicable to the Licensed Software and made generally available by Company to its customers.

   1.5. **"Enterprise"** means those organizations that have Internet addresses located at top level and second-level domain names set forth in the System Quotation.

   1.6. **"Error"** means a reproducible error in the Licensed Software that prevents such Licensed Software from operating substantially in accordance with its Documentation.

1.7. **“Executable Code”** means the fully compiled binary version of Licensed Software that can be executed by a computer and used by an end user without further compilation.

1.8. **“Intellectual Property Rights”** means all copyrights, trade secrets, patents, patent applications, moral rights, contract rights and other proprietary rights, but specifically excluding any trademarks or service marks.

1.9. **“Licensed Software”** means the Compass for Simple Western software program in Executable Code form, and any Updates that Company makes available to Customer in accordance with this Agreement.

1.10. **“Site”** means the facility or campus set forth in the System Quotation.

1.11. “**System”** means the proprietary NP1000, NP100, Simon, Sally, Peggy, Wes, Jess, Abby, Leo, Sally Sue and Peggy Sue protein analysis system or any future model or successor thereto that is provided to Customer by Company pursuant to a separate agreement between the parties (the “System Quotation”).

1.12. **“Update”** means those releases of the Licensed Software that Company provides to customers to correct Errors, fix bugs, or create minor improvements, incremental features or enhancements of existing features which Company designates by a change in the number to the right of the first or second decimal point. Updates do not include those releases of the Licensed Software that provide substantial new features or additional functionality which Company designates by a change in the number to the left of the first decimal point.

1.13. **“User”** means any individual that has an e-mail address within the Enterprise.

2. **License and Restrictions**

2.1. **License Grant.** Subject to the terms and conditions of this Agreement and the payment of the required fees set forth in the System Quotation, Company grants to Customer a nontransferable, nonexclusive, royalty-free, revocable, worldwide license (without the right to sublicense) to (a) install the Licensed Software on any computer located at any Site; (b) use, execute and display the Licensed Software, in Executable Code form only; and (c) copy the Licensed Software and Documentation, solely as necessary to support Authorized Users; in each of the foregoing, solely in accordance with the Documentation and the Authorized Use Parameters. Customer agrees that it will comply with the Authorized Use Parameters.

2.2. **License Restrictions.** Customer acknowledges that the Licensed Software and its structure and organization constitute valuable trade secrets of Company. Accordingly, the license granted in this Agreement is subject to the following restrictions: Customer and its Authorized Users (a) may not reverse engineer, disassemble, decompile or otherwise attempt to derive the source code of Licensed Software; (b) may not modify, adapt, alter, translate or create derivative works from the Licensed Software; (c) may not merge the Licensed Software with other software; (d) may not use the Licensed Software in any service bureau or time-sharing arrangement, license, sell, rent, lease, transfer, assign, distribute, host, outsource, disclose or otherwise commercially exploit or make the Licensed Software or Documentation available to any third party; (e) shall only make that number of exact copies of the Licensed Software and Documentation as delivered by Company that are necessary to support Customer's use of the Licensed Software in accordance with this Agreement; (f) shall include any titles, trademarks and copyright and restricted rights notices that are included on or in the Licensed Software as delivered by Company on and in any copies of the Licensed Software that it makes; and (g) shall ensure that Customer's use of the Licensed Software does not exceed the scope of the license that Customer has purchased pursuant to this Agreement.

2.3. **Open Source Software.** Certain items of independent, third-party code may be included in the Licensed Software that are subject to open source licenses (“Open Source Software”). Such Open Source Software is licensed under the terms of the license that accompanies such Open Source Software. Nothing in this Agreement limits Customer's rights under, or grants Customer rights that supersede, the terms and conditions of any applicable end user license for such Open Source Software. In particular, nothing in this Agreement restricts Customer's right to copy, modify and distribute such Open Source Software that is subject to the terms of such open source licenses.

2.4. **Ownership.** Company reserves all rights not expressly granted to Customer in this Agreement. Without limiting the generality of the foregoing, Customer acknowledges and agrees that, except as expressly set forth in this Agreement, Company and its suppliers retain all Intellectual Property Rights, title and interest in and to the Licensed Software and Documentation.

3. **Support and Maintenance Services**

   3.1. **Services.** Subject to Customer's payment of the Services fees, as set forth in the System Quotation, and to the terms and conditions herein, Company will use commercially reasonable efforts to provide to Customer the following support and maintenance services (the "Services") for the Licensed Software: (a) Company will answer technical questions concerning functions and features of the Licensed Software; (b) Company will provide Error verification, analysis and corrective efforts for the Licensed Software; and (c) Company will provide, without charge, Updates of the software released during the term of this Agreement. Customer will be responsible for providing, in a manner consistent with good industry practice, all Services to Users. Customer acknowledges that Company may not be able to correct all reported Errors. Any Update of the Licensed Software will be deemed part of the Licensed Software and Customer will use such Updates in accordance with the requirements and obligations in this Agreement.

   3.2. **Service Conditions.** Company's obligation to provide the Services is conditioned on Customer: (a) notifying Company of any Error within a reasonable period of time; (b) providing Company all information relating to the Error; (c) providing access to the Licensed Software and Customer's facility where the Licensed Software is located and informing Company of any potential hazards which may be encountered while servicing the Licensed Software. Customer may contact Company via telephone toll-free in the US and Canada 1-888-607-9692 or e-mail at **support@bio-techne.com** during the hours of 8:00 AM (Pacific Time) and 5:00 PM (Pacific Time) Monday through Friday, excluding holidays, to report any Error. A list of standard holidays will be provided to Customer upon request. Company shall have the right to determine in its sole discretion what corrective action Company will perform to support the Licensed Software. Company may subcontract the Services to a third party contractor provided that Company will be responsible for the third party contractor's compliance with this Agreement.

   3.3. **Service Exclusions.** Company will not be obligated to provide the Services if (a) Company determines that an Error is caused by malfunction of any hardware (other than malfunction of the System) or third party software used with the Licensed Software; or (b) Customer has failed to incorporate the latest Update previously released to Customer.

4. **Warranty**

   4.1. **Licensed Software Warranty.** Company warrants that the Licensed Software, as properly installed, and under normal use, will perform substantially in accordance with its Documentation during the Warranty Period. The "Warranty Period" for the Licensed Software begins on date Customer downloads the Licensed Software and ends twelve (12) months thereafter.

   4.2. Remedy. If Customer notifies Company in writing during the Warranty Period of an Error, Company will, at its expense and as its sole obligation for any breach of the foregoing warranty, use commercially reasonable efforts to correct the Error or replace the Licensed Software. Any Error correction or replacement of the Licensed Software will not extend the original Warranty Period. The warranty and the remedies provided above will not apply to the Licensed Software if (a) Company determines that an Error is caused by accident, abuse, misuse, negligence, fire, earthquake, flood, other force majeure event, failure of electrical power, the use of unauthorized products or unauthorized repairs or modifications; (b) Company determines that an Error is caused during or as a result of delivery; (c) a problem arises from or is based on Company's compliance with Customer's specifications; or (d) Company determines that an Error is caused by malfunction of any hardware (other than malfunction of the System) or third party software used with the Licensed Software.

   4.3. **Disclaimer.** THE WARRANTIES ABOVE ARE EXCLUSIVE AND IN LIEU OF ALL OTHER WARRANTIES, WHETHER EXPRESS, IMPLIED OR STATUTORY, INCLUDING WITHOUT LIMITATION THE IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, TITLE AND NONINFRINGEMENT.

5. **Limitation of Liability.** NEITHER COMPANY NOR ITS SUPPLIERS SHALL BE RESPONSIBLE OR LIABLE WITH RESPECT TO ANY SUBJECT MATTER OF THIS AGREEMENT OR TERMS OR CONDITIONS RELATED THERETO UNDER ANY CONTRACT, NEGLIGENCE, STRICT LIABILITY OR OTHER THEORY (A) FOR LOSS OR INACCURACY OF DATA, LOSS OF PROFITS OR COST OF PROCUREMENT OF SUBSTITUTE GOODS, SERVICES OR TECHNOLOGY, OR (B) FOR ANY INDIRECT, INCIDENTAL OR CONSEQUENTIAL DAMAGES INCLUDING, BUT NOT LIMITED TO LOSS OF REVENUES AND LOSS OF PROFITS. COMPANY'S AGGREGATE CUMULATIVE LIABILITY HEREUNDER SHALL NOT EXCEED THE GREATER OF FIVE HUNDRED DOLLARS ($500.00).

6. **Term and Termination**

   6.1. **Term of Agreement.** The Agreement is effective on the date Customer downloads the Licensed Software and shall remain in effect until terminated by either party as provided in this section.

6.2. **Termination For Material Breach.** Either party may terminate this Agreement upon written notice if the other party materially breaches this Agreement and fails to cure such breach within thirty (30) calendar days following receipt of written notice from the other party specifying the breach in detail. Notwithstanding the foregoing, Company may immediately terminate this Agreement and all licenses granted hereunder if Customer breaches Section 2 (License and Restrictions) hereof or upon termination of the System Quotation. The foregoing rights of termination are in addition to any other rights and remedies provided in this Agreement or by law.

6.3. **Effect of Termination.** Upon termination of this Agreement (or termination or expiration of any license granted hereunder), all rights of Customer to use the Licensed Software and Documentation will cease and (a) all license rights granted under this Agreement will immediately terminate and Customer shall promptly stop all use of the Licensed Software and Documentation; (b) all Services will terminate immediately; (c) Customer shall promptly erase all copies of the Licensed Software from Customer's computers, and destroy all copies of the Licensed Software and Documentation on tangible media in Customer's possession or control or return such copies to Company; and (d) upon request by Company, Customer shall certify in writing to Company that it has returned or destroyed such Licensed Software and Documentation. The parties' rights and obligations under Sections 1 (Definitions), 2.4 (Ownership), 4.3 (Disclaimer), 5 (Limitation of Liability), 6 (Term and Termination), and 7 (General) shall survive termination of this Agreement.

7. **General**

7.1. **Assignment.** This Agreement and Customer's rights hereunder may not be assigned to any third party by Customer except with the prior written approval of Company. Any attempted assignment of this Agreement or any rights or obligations hereunder will be null and void.

7.2. **Governing Law.** This Agreement is made in, governed by, and shall be construed in accordance with the laws of the State of California, without regard to any conflicts of law principles that would result in application of laws of any other jurisdiction. The United Nations Convention on Contracts for the International Sale of Goods does not apply to this contract. Any legal action or other legal proceeding relating to this contract or the enforcement of any provision of this contract must be brought in any state or federal court located in Santa Clara County, California. Customer and Company expressly and irrevocably consents and submits to the jurisdiction of such courts.

7.3. **Injunctive Relief.** Customer acknowledges that the Licensed Software contains valuable trade secrets and proprietary information of Company, that any actual or threatened breach of this Agreement will cause harm to Company for which monetary damages would be an inadequate remedy, and that injunctive relief is an appropriate remedy for such breach.

7.4. **Modifications.** Company reserves the right to change the terms and conditions of this Agreement or its policies relating to the Licensed Software at any time. Company will notify Customer of any material changes to this Agreement by sending Customer an e-mail to the last e-mail address Customer provided to Company or by prominently posting notice of the changes on Company's website. Any material changes to this Agreement will be effective upon the earlier of thirty (30) calendar days following Company's dispatch of an e-mail notice to Customer or thirty (30) calendar days following Company's posting of notice of the changes on Company's website. These changes will be effective immediately for new users of our Licensed Software. Please note that at all times Customer is responsible for providing Company with its most current e-mail address. In the event that the last e-mail address that Customer has provided Company is not valid, or for any reason Company is not capable of delivering to Customer the notice described above, Company's dispatch of the e-mail containing such notice will nonetheless constitute effective notice of the changes described in the notice. If Customer does not agree with the changes to this Agreement, Customer must notify Company prior to the effective date of the changes that Customer wishes to terminate its license to the Licensed Software. Continued use of the Licensed Software, following notice of such changes, shall indicate Customer's acknowledgment of such changes and agreement to be bound by the terms and conditions of such changes.

7.5. **Severability.** In the event any provision of this Agreement is held to be invalid or unenforceable, the remaining provisions of this Agreement will remain in full force.

7.6. **Waiver.** The waiver by either party of any default or breach of this Agreement shall not constitute a waiver of any other or subsequent default or breach.

7.7. **Export.** Customer agrees not to export, reexport or transfer, directly or indirectly, any U.S. technical data acquired from Company, or any products utilizing such data, in violation of the United States export laws or regulations.

7.8. **Force Majeure.** Company shall not be liable, directly or indirectly, for any delay or failure in performance of any obligation under this Agreement, including any delivery obligation, where such delay or failure arises or results from a cause beyond Company's reasonable control, or beyond the reasonable control of Company's suppliers or contractors, including, but not limited to strike, boycott or other labor disputes, embargo, governmental regulation, inability or delay in obtaining materials, acts of God, war, earthquake, fire or flood. In the event of such force majeure, the time for delivery or other performance will be extended for a period equal to the duration of the delay caused thereby, provided that Company notifies Customer of the nature and duration of such force majeure event.

7.9. **Entire Agreement; Notice.** This Agreement constitutes the complete agreement between the parties and supersedes all prior or contemporaneous agreements or representations, written or oral, concerning the subject matter of this Agreement. Except as otherwise expressly provided in this Agreement, any modifications of this Agreement must be in writing and agreed to by both parties. Company may provide any notice to Customer by e-mail. Customer may provide notice to Company by sending an e-mail to **info@biotechne.com** or a letter by United States mail to ProteinSimple, 3001 Orchard Parkway, San Jose, CA 95134, or to such other address as Company may specify in writing by posting the new address on the Company website.

7.10. **Relationship of the Parties.** The parties are acting hereunder as independent contractors and not as partners, agents, fiduciaries or joint venturers. Neither party has the power or authority represent, act for, bind or otherwise create or assume any obligation on behalf of the other party.

Contact Us

Global info@bio-techne.com, bio-techne.com/find-us/distributors
North America TEL 800 343 7475
Europe // **Middle East** // **Africa** TEL +44 (0)1235 529449
China info.cn@bio-techne.com, TEL 400.821.3475

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