Sally Sue and Peggy Sue User Guide

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Let's Get Started

Chapter Overview

- Welcome
- Simple Western Assays

Welcome

Congratulations on bringing Sally Sue™ or Peggy Sue™ 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 how Simple Western assays work as well as other useful operating and installation information.

To help you get the most from Sally Sue and Peggy Sue, we've added some attention phrases to guide you through the user guide:

NOTE	Points out useful information.
IMPORTANT	Indicates information necessary for proper operation of Sally Sue and Peggy Sue.
CAUTION	Cautions you about potentially hazardous situations that could result in injury to you or damage to Sally Sue and Peggy Sue.
!WARNING!	Warns you that serious physical injury can result if the listed precautions aren't followed.

Simple Western Assays

A Simple Western assay is an automated Western - no gels, no transfer devices, no blots, no film and no manual analysis.

Simple Westerns take place in a capillary. Sally Sue and Peggy Sue automate all steps of the process for you including sample loading, protein separation, immunoprobing or labeling, washing, detection and data analysis. Up to 12 samples can be analyzed simultaneously in a single cycle and up to 8 cycles (96 samples) can be processed in a single run.

Sally Sue performs Simple Western size-based assays (Immunoassay and Total Protein assay) and Peggy Sue can do both size-based and charge-based assays. All you have to do is pipette your samples, separation reagents and immunoreagents into the plate, load the capillaries and press start.

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 Sally Sue or Peggy Sue. Please have the space, electrical, vacuum and environmental requirements ready prior to scheduling your installation.

NOTE: Please wait for the ProteinSimple representative to unpack and install Sally Sue or Peggy Sue. Don't try to lift or unpack either of them on your own, you could cause injury to yourself or damage to the system.

Space Requirements

You'll need a lab bench or table that can support 200 lbs (91 kg) and that has enough space for both the instrument and computer. There should be sufficient clearance for both heat ventilation and to provide access if Sally Sue or Peggy Sue ever need service.

IMPORTANT

Sally Sue and Peggy Sue need a stable surface and must remain level to work properly. The lab bench or table can't shift or wobble under heavy weight. Also, anti-vibration tables can't be used as they can alter the instrument level when Sally Sue or Peggy Sue are running.

Dimension	Meters	Feet
Width	2.13	6.98
Depth	0.91	2.99
Height	1.00	3.28

Table 2-1: *Recommended space requirements for Sally Sue and Peggy Sue.*

Physical Specifications

Dimension	Meters	Feet
Peggy Sue		
Width	0.94	3.08
Width with doors open	1.27	4.17
Depth	0.61	2.00
Height	0.84	2.76
Auxiliary Module		
Width	0.33	1.08
Depth	0.30	1.00
Height	0.51	1.67
Tubing between Auxiliary Module and instrument	3.00	9.84
Computer Workstation		
Width	0.66	2.17
Depth	0.76	2.49
Height	0.41	1.35

For indoor use only. Use up to altitudes of 1524 meters (5000 feet).

Table 2-2: *Physical specifications.*

Electrical Requirements

Sally Sue and Peggy Sue need a dedicated, grounded circuit capable of delivering the appropriate current and voltage for your country. The power requirements for select countries are listed below:

Region	Volts (AC)	Frequency (Hz)	Amps
US and Canada	120	60	5.5
Europe	240	50	2.7
Japan	100	50/60	6.5

Table 2-3: *Power requirements.*

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

Environmental Requirements

Sally Sue and Peggy Sue like a consistent temperature in the lab. They work best when conditions stay within these ranges:

Requirement	Specification
Operating temperature range	18 - 24 °C (64 - 75 °F)
Operating humidity range	20-60% relative, non-condensing

Table 2-4: *Environmental requirements.*

Software and Computer Requirements

Sally Sue and Peggy Sue bring their own computer to the lab with Compass for Simple Western pre-installed. Compass software is used to run assays and analyze resulting data. Just in case you need it, a CD containing Compass for Simple Western also comes in the box. If you don't want to analyze your data at the instrument workstation in the lab, Compass software can also be installed on a separate workstation, such as your desktop computer. Your computer must meet the minimum requirements listed in the table below to run the software and process data.

Component	Minimum Recommended
Operating system	Windows 7
Processor	Core 2 Duo
Memory	2 GB
Free disk space	10 GB

Table 2-5: *Computer requirements.*

General Guidelines and Information

Intended Use

NOTE: Sally Sue and Peggy Sue are for research use only. Not for use in diagnostic procedures.

Lifting and Moving the System: Lift Sally Sue and Peggy Sue Correctly

IMPORTANT

Take all the standard precautions when lifting or moving Sally Sue or Peggy Sue. Since they each weigh 82 kg (180 lbs), two or more people are needed to lift them onto the lab bench.

Chapter 3:

Sally Sue and Peggy Sue

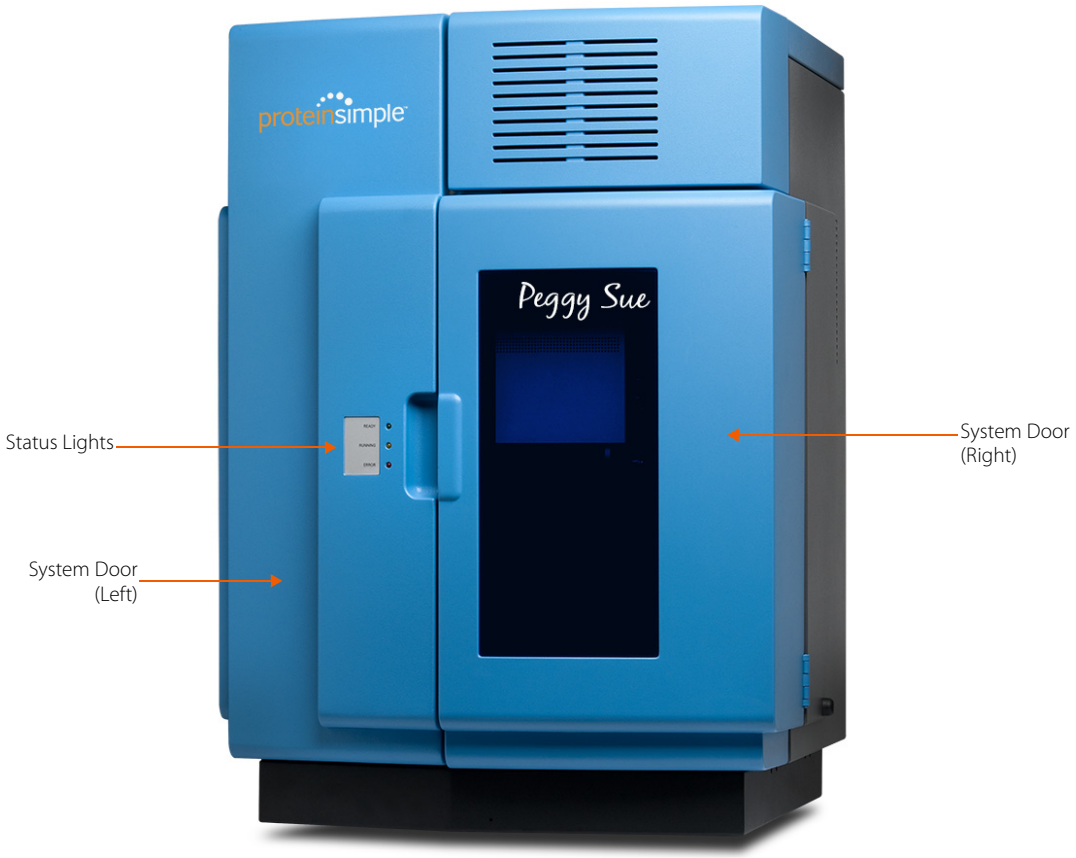
Chapter Overview

- Instrument Overview
- Auxiliary Module

Instrument Overview

Sally Sue's and Peggy Sue's individual hardware components are described in this section.

External Components



System Doors

Sally Sue's and Peggy Sue's doors give you access to the inside of the instrument to load samples, reagents and capillaries. To open the doors, make sure the green **System Status Ready Indicator** is on. Using the handle to the right of the **System Status Indicators**, pull the right door open first. Open the left door by pulling forward on the top and bottom latches. To close the doors, close the left door first and make sure both latches engage, then close the right door. The right door uses a magnet so it closes properly.

NOTE: Sally Sue's and Peggy Sue's doors must be closed before starting a run or cleaning protocol.

Door Interlock

A door interlock engages once you've started a run to prevent the doors from being opened during the run. This prevents you from being exposed to any dangerous interactions with the robot as well as protecting you from exposure to high voltage and UV light. The yellow indicator light will be on when the instrument is running and the door interlock is engaged.

When the system status is ready (green) and the doors are open, the robot's system tray, Z-stage motors and UV power supply are disabled.

!WARNING!

Don't override the door interlock. The interlock protects you from exposure to UV light and a potential pinch hazard that could be caused by moving system trays.

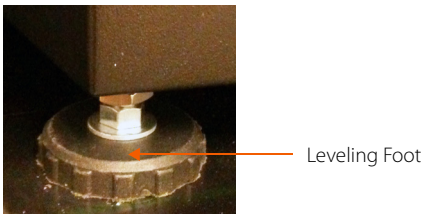
Status Lights

The LEDs on Sally Sue's and Peggy Sue's front panel tell you what their doing:

- **Ready (green)**: Sally Sue and Peggy Sue are powered on and ready for use.
- **Running (yellow)**: We're running an assay or a cleaning protocol.
- **Error (red)**: We've detected an error. To get more information, check the Status window of the Run Summary Screen in Compass for Simple Western.

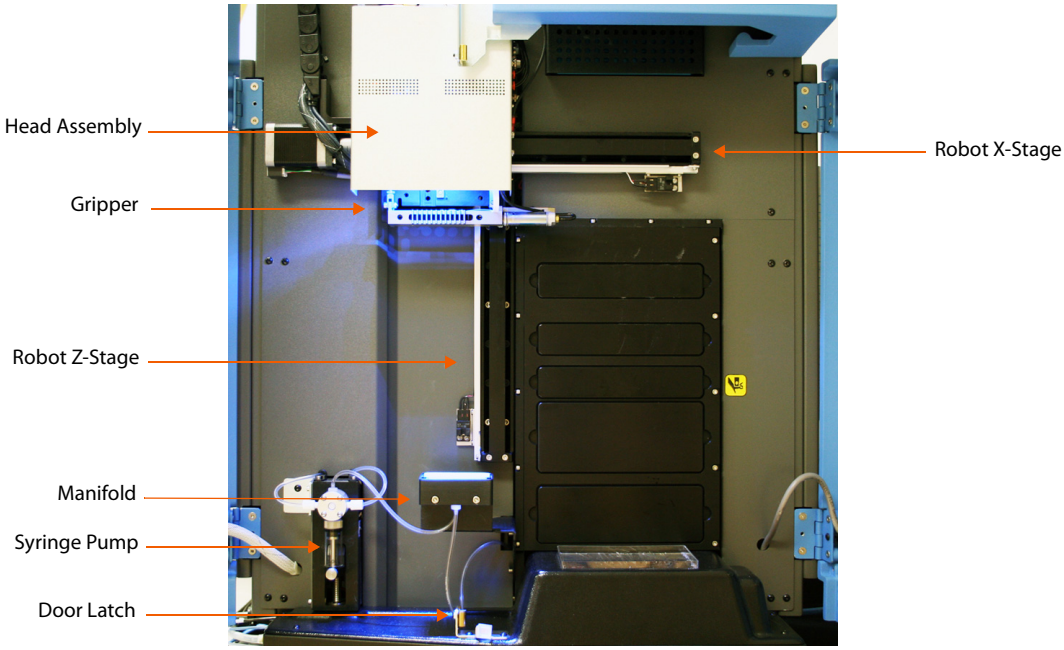
Leveling Feet

Four leveling feet are located at the base of the instrument. Sally Sue and Peggy Sue must be level to operate properly. When they are installed, the ProteinSimple representative will make adjustments to make sure Sally Sue and Peggy Sue are level, and their level will also be checked during preventive maintenance visits. The level status of Sally Sue and Peggy Sue are monitored internally and reported to you through Compass for Simple Western.



NOTE: If Sally Sue or Peggy Sue need to be moved from their original install location or require leveling, please see "Leveling the Instrument" on page 64 for leveling procedures or contact ProteinSimple Technical Support.

Internal Components

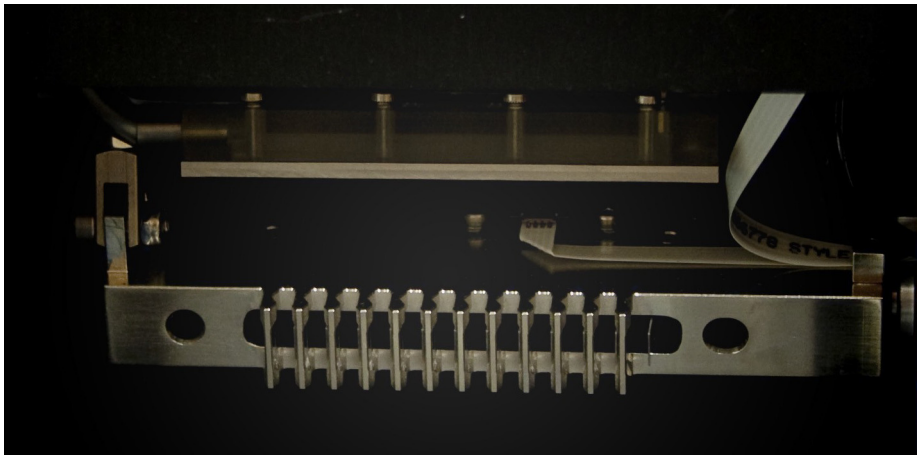


Head Assembly

Fluidic components in the head assembly handle all bulk reagent dispensing and sample tray reagent loading. Pneumatic components provide control for the gripper, which is used to pick up and transfer capillaries.

Gripper

The gripper sits at the base of the head assembly, and is used to transfer capillaries between system trays. The gripper picks up 12 capillaries at a time from the capillary box and moves them to and from each tray during each cycle in a run. The gripper rotates 90 degrees to allow vertical transfer and horizontal placement of capillaries.



X-Z Stage Robot

The X-Z stage robot moves the head assembly to and from the sample, resource, incubator, separation and capillary discard trays during the run. It also moves the pipettor that's used to transfer fluids and perform washes inside the instrument.

Syringe Pump

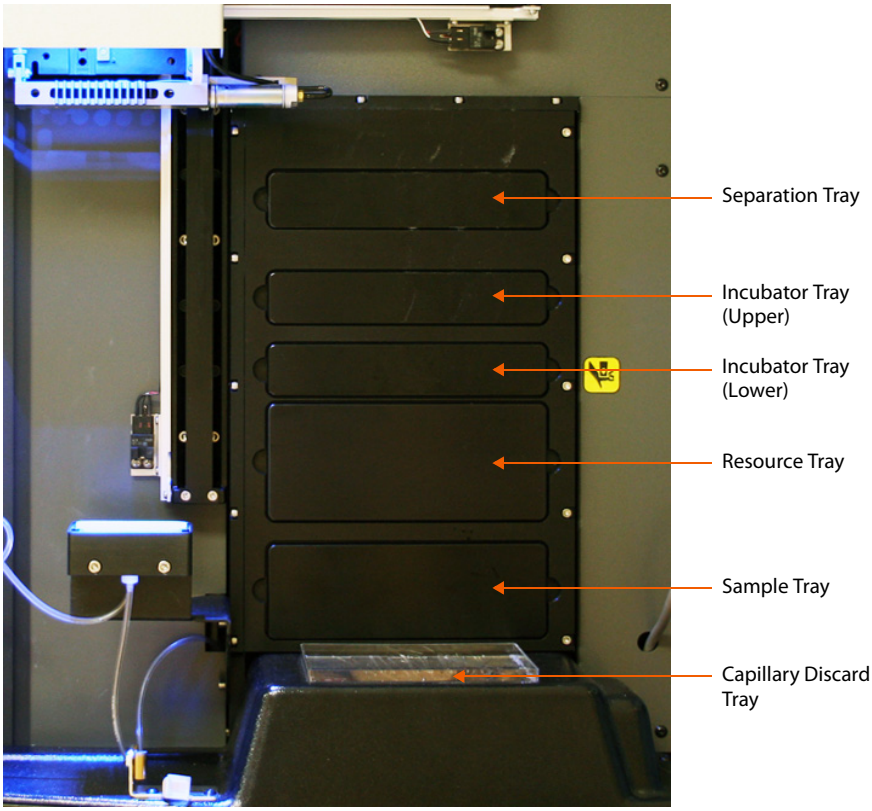
The syringe pump pipettes DI water, reagents in the resource tray cups, and also assists in washing the manifold head per the assay protocol.

Ionizer Fans

Anti-static ionizer fans are located inside Sally Sue's or Peggy Sue's right door. They're used to circulate ionized air inside the system. The uniform static charge they provide makes sure capillaries are always transferred properly between system trays during a run.

System Trays

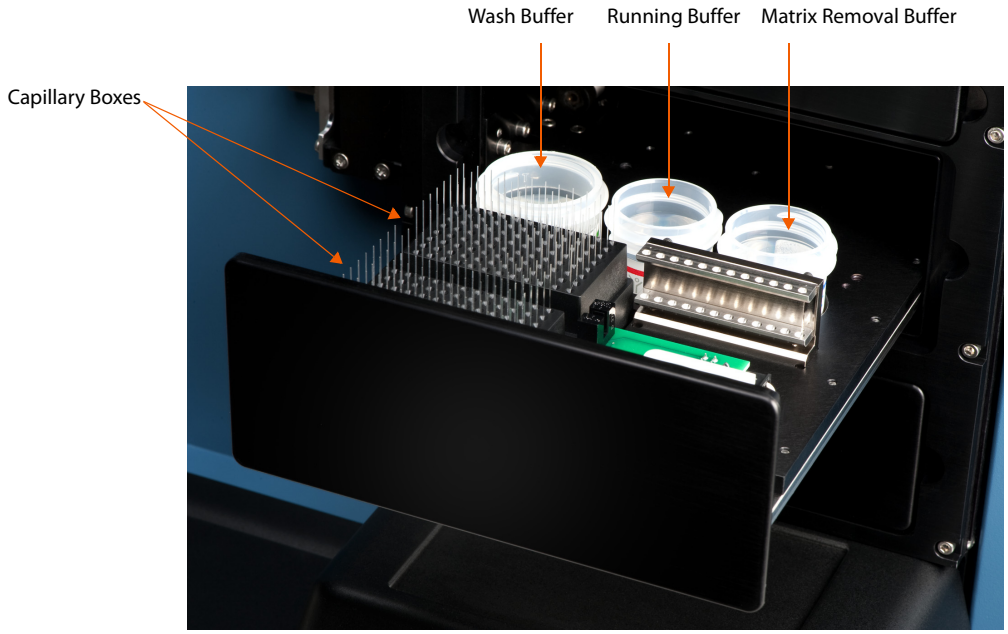
Sally Sue and Peggy Sue each have five trays that are used for capillary and reagent storage and to carry out different phases of the assay. You can open and close any tray by selecting **Open Trays** from the **Instrument** menu in Compass for Simple Western. For more details, please refer to the "Opening Trays" on page 53.



Resource Tray

Size-based Assays

For size assays, the resource tray holds Running Buffer, Wash Buffer, Matrix Removal Buffer, and up to two capillary boxes.



Insert reagents as shown: Wash Buffer (left), Running Buffer (middle), Matrix Removal Buffer (right). If you'll only be using one box of capillaries, put it in the primary box location, closest to the reagents.

NOTE: You can also use the labels on the resource tray as a guide to where to insert reagents and capillaries.

Charge-based Assays (Peggy Sue only)

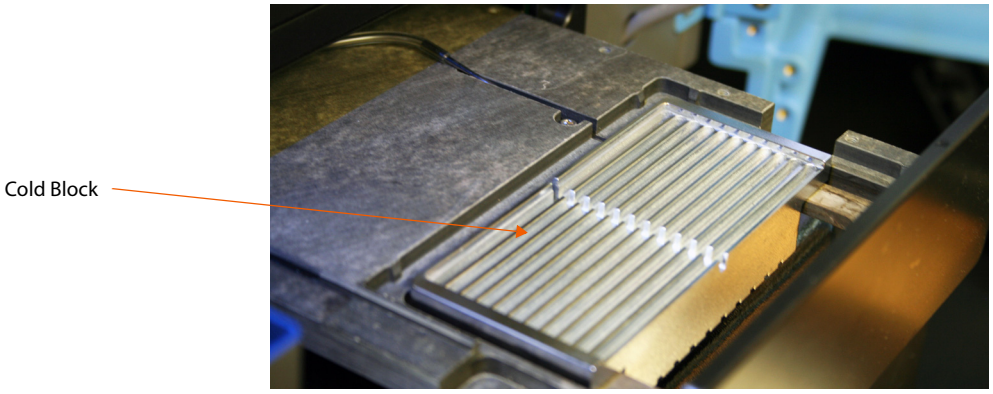
For charge assays, the resource tray holds Wash Buffer, Anolyte, Catholyte and up to two capillary boxes.

Insert reagents as shown: Wash Buffer (left), Anolyte (middle), Catholyte (right). If you'll only be using one box of capillaries, put it in the primary box location, closest to the reagents.

NOTE: You can also use the labels on the resource tray as a guide for where to insert reagents and capillaries.

Sample Tray

The sample tray holds the 384-well assay plate with your samples and reagents. It uses a cold block to keep a portion of the plate at 10 °C. The remaining portion of the plate stays at ambient instrument temperature.



All sample plates must have lids and should be inserted into the sample tray so the A1 well position is at the upper left corner of the cold block.

NOTES:
*Sally Sue and Peggy Sue require plate lids on sample plates. If a lid isn't detected, a message will display in the **Start Run Wizard**. Compass for Simple Western will then open the sample tray to allow you to put a lid on the plate.*

When you insert the sample plate, make sure it's firmly seated and level on the cold block. When plates aren't level they can interfere with the movement of the sample tray.

Incubation Trays

Sally Sue and Peggy Sue use two incubator trays to perform the incubation steps in an assay protocol. Each tray holds up to 48 capillaries.

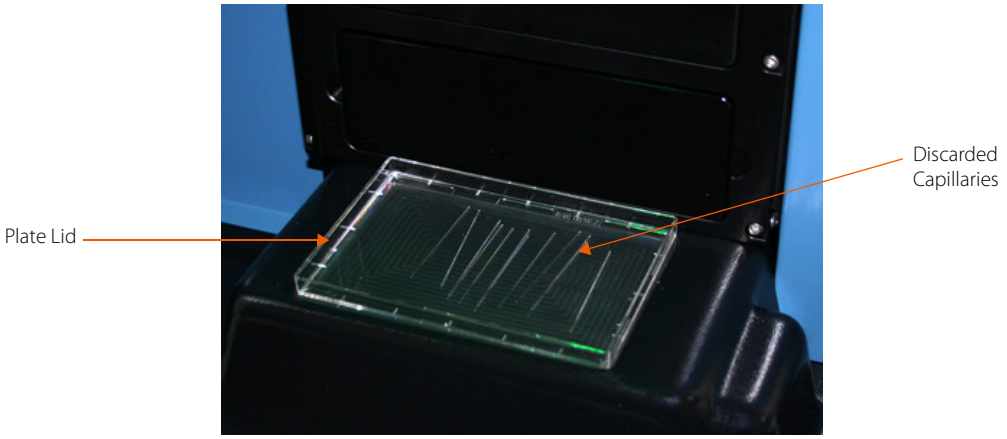
Separation Tray

The separation tray holds 12 capillaries and is used for separation and detection steps in the assay protocol. The separation block has two troughs which are automatically filled with buffer(s), and Sally Sue and Peggy Sue will also remove the buffer(s) and wash the troughs once the separation step is complete. Chemiluminescent and fluorescent CCD images are taken of the separation in the capillaries through the detection window.

- Size-based assays - both front and rear troughs are used for Running Buffer
- Charge-based assays - the front trough is used for Anolyte and the rear for Catholyte

Capillary Discard Tray

The capillary discard tray collects used capillaries as each cycle completes. We recommend placing an inverted plate lid in the tray area to catch capillaries, and emptying the lid on a daily basis.



!WARNING! SHARPS HAZARD

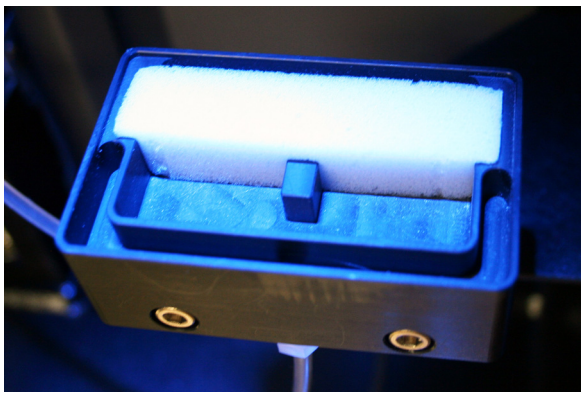
Capillaries may present a potential sharps hazard. Dispose of used capillaries according to your institution's sharps disposal policy.

NOTE: An overfilled capillary discard tray can cause the sample tray to jam. If this happens, call ProteinSimple Technical Support at (888) 607-9692 or (408) 510-5500, option 3.

Manifold Wash Station

The manifold wash station washes and cleans the instrument manifold after each separation, and prevents build-up of viscous materials in the head.

NOTE: Replace the manifold sponge with a new one after each run and whenever you start an experiment on a new assay plate.



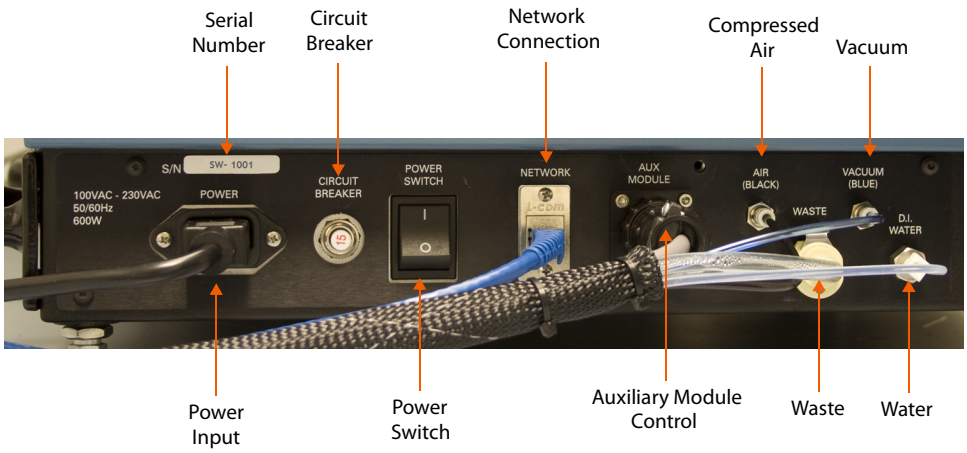
User Vacuum

There's a user vacuum line on the inside left panel of the instrument that can be used for instrument cleaning procedures or for cleaning liquid spills. The vacuum turns on whenever Sally Sue's or Peggy Sue's doors and system tray are open at the same time.

To use the vacuum, turn the valve to the **on** position. Turn the valve back to the **off** position when you aren't using it.

Instrument - Left Panel

Located on the lower left side of Sally Sue and Peggy Sue are their serial number, power entry, power switch and connector panel.



- **System Serial Number**: This label contains the serial number for the system.
- **System Power** - The main system power components include the power switch and power input.

!WARNING!
Only use the power supply cord provided with Sally Sue or Peggy Sue. If the cord is damaged, please contact ProteinSimple Technical Support.

!WARNING!
No user replaceable parts.

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

- **Network Connection** - A 10/100/BASE-T Ethernet (RJ-45 connector) is used to connect Sally Sue and Peggy Sue to a computer or local network.

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

Auxiliary Module Connections

- **Module Control**: Allows communication between Sally Sue or Peggy Sue and the auxiliary module.
- **Compressed Air**: Compressed air from the auxiliary module is used to power pneumatic actuations inside the instrument. The compressed air line is black.
- **Waste**: Waste produced during a run is emptied into the waste reservoir in the auxiliary module. This line is under vacuum when waste aspirations are performed.
- **Vacuum**: Vacuum is provided to the instrument by the auxiliary module and is used in pick and place operations inside the instrument.
- **Water**: Provides water to the instrument from the auxiliary module.

Instrument - Rear Panel

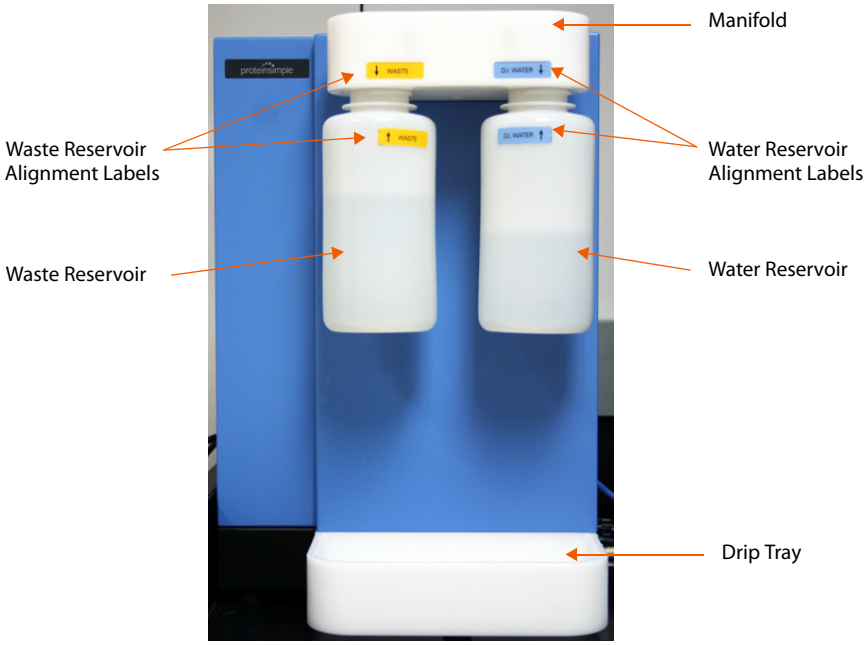
System Label

A system label can be found on Sally Sue's and Peggy Sue's rear panel. It includes the ProteinSimple location, system model, power requirements, serial number and certification markings. The serial number of the instrument can also be found on the lower left side panel above the power entry.

Auxiliary Module

The auxiliary module holds the water and waste bottles used by Sally Sue and Peggy Sue. The waste bottle has a yellow label, and the water bottle has a blue label. The volume in each reservoir is 1.0 L.

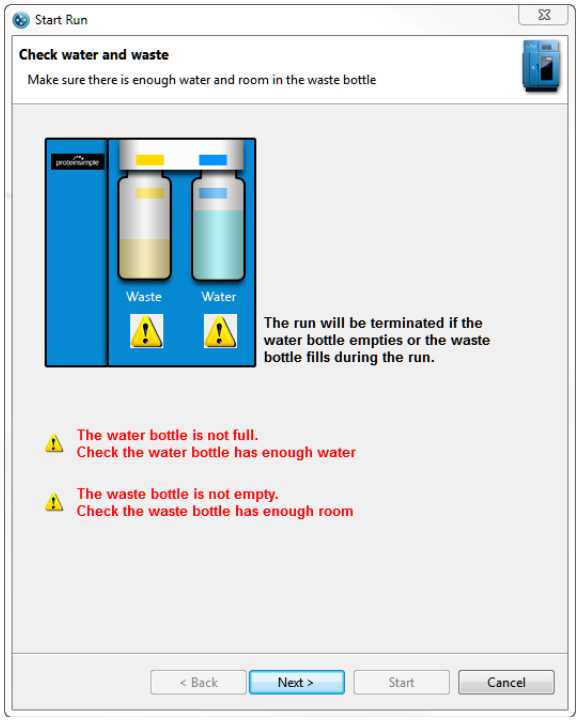
Deionized water is used by the instrument to clean the troughs in the separation tray and the fluidics in the head assembly. The waste bottle collects waste generated during the run. For more information on waste production, please refer to the "Waste Production and Disposal" on page 78.



There are two liquid level sensors in each bottle. The sensor at the bottom of the bottle detects when the bottle is empty, and the sensor at the top of the bottle determines if sufficient volume is available to complete an eight-cycle assay with the instrument.

NOTE: We recommend you check fluid levels in the bottles before starting a run. Empty the waste bottle if needed, and fill the water bottle up to the base of its neck. If the waste bottle fills up or the water bottle empties during a run, the run will be aborted.

The **Start Run Wizard** in Compass for Simple Western will also check the fluid levels in each bottle. When a full or empty status is detected, Compass will display an error in the **Start Run Wizard**:



If this happens, fill or empty each bottle using the procedures in the next section.

Removing and Installing Bottles

Bottles may be removed from the auxiliary module whenever Sally Sue or Peggy Sue are idle. To remove or install the waste or water bottle, use the following procedure:

IMPORTANT

Don't remove either bottle if Sally Sue or Peggy Sue are running, doing this may abort the run in progress.

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1. Remove the bottle by unscrewing it clockwise until it disengages from the manifold.
2. Pull the bottle down slowly until the sensor at the bottom of the bottle is clear.
3. Fill the water bottle to the base of its neck and empty the waste bottle as needed.

4. To reinstall the bottle, lift it up to the manifold making sure to enclose the sensor that sits at the bottom inside the bottle. Screw the bottle counter-clockwise until the arrow on the bottle's alignment label lines up with the arrow on the manifold's alignment label.

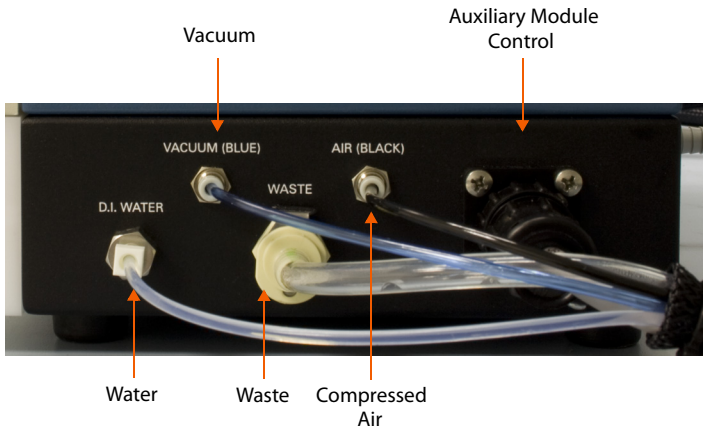
!WARNING! BIOHAZARD

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

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

Connection Panel

The auxiliary module's connector panel is on its lower left panel.



- **Compressed Air**: Compressed air is provided to the instrument to power pneumatic actuations. The compressed air line is black.
- **Waste:** Waste produced is emptied into the waste reservoir. This line is under vacuum when waste aspirations are performed.

- **Vacuum**: Vacuum is provided to the instrument and is used in pick and place operations inside the instrument.
- **Water**: This connection provides water to the instrument.

Chapter 4:

Operating Sally Sue and Peggy Sue

Chapter Overview

- Before You Throw the Switch
- Power Up
- Starting a Run: Size-based Assays (Sally Sue and Peggy Sue)
- Starting a Run: Charge-based Assays (Peggy Sue Only)
- Stopping a Run
- Waste and Water Bottles
- Controlling Sally Sue and Peggy Sue
- Instrument Status Modes
- Shutdown

Before You Throw the Switch

Ensure that everyone using Sally Sue or Peggy Sue has:

- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for Sally Sue or Peggy Sue.
- Received instruction on handling of biohazards (if biohazardous materials are to be used on the system).
- Read and understood all related SDSs.

Power Up

1. Turn on the computer connected directly to Sally Sue or Peggy Sue.
2. Turn on the instrument's main power switch.
3. Wait for the instrument to initialize.
4. Double-click the Compass for Simple Western icon to open the application.

Starting a Run: Size-based Assays (Sally Sue and Peggy Sue)

Step 1 - Get Ready

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

IMPORTANT

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

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4. While the plate is spinning, add Wash, Upper and Lower Running Buffers to resource tray cups and insert a capillary box in the resource tray.

IMPORTANT

Capillaries are light sensitive. Keep the cover on the box until you're ready to insert the capillary box into the resource tray.

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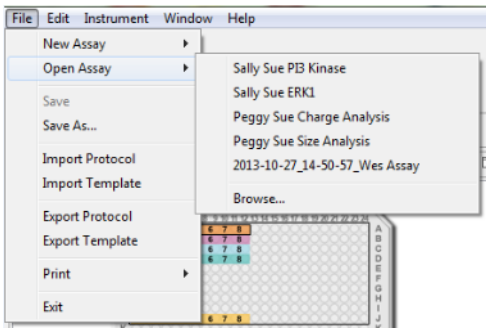
5. Place the assay plate into the sample tray.
6. Click **Start**.

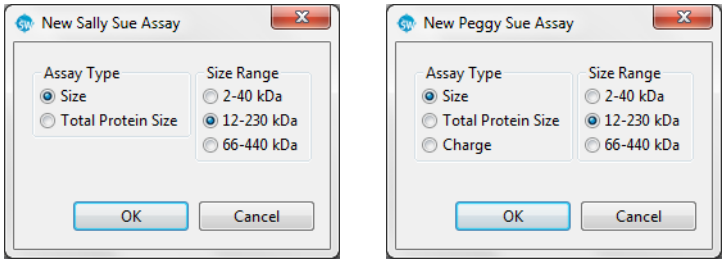
Step 2 - Start the Run

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

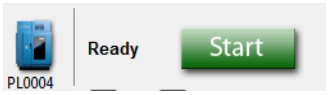
1. To start a new run with an existing assay:
   a. Select **File** in the main menu and click **Open Assay**.



   b. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different one.
2. To start a run with a new assay:
   a. Select **File** in the main menu, click **Open Assay** and select **Sally Sue Size** or **Peggy Sue Size**. From the pop up window, select the **Assay Type**: Size, Total Protein Size or Charge (Peggy Sue only) and the **Size Range** (Size or Total Protein Size assay types only): 2-40 kDa, 12-230 kDa or 66-440 kDa.



   b. The **Start** button will display which means an assay is already loaded.



c. Go to the Assay screen and make sure it's the one you want to use. If not, select **File** in the main menu, click **Open Assay**, and open the correct one.

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

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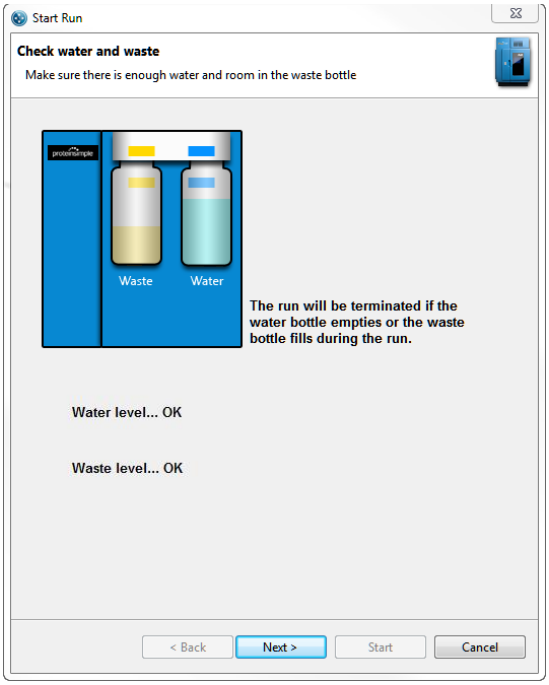
*NOTE: If you didn't clean the manifold in Sally Sue or Peggy Sue prior to starting the run, a message will appear. If you see this message, click **Yes** to cancel the run and perform the manifold cleaning.*

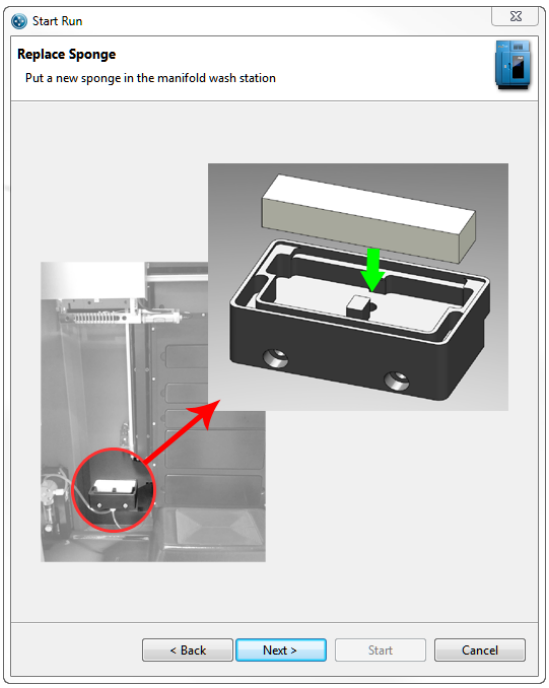
---

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



*NOTE: If the waste level is too high or the water level is too low to complete the run, a message will display in this screen. If this happens, fill or empty each bottle using the procedures in "Removing and Installing Bottles" on page 29. Once this is done, error status will automatically update and let the **Start Run Wizard** proceed.*

5. **Replace Sponge.** Each time you start a new run, you should use a new sponge. Remove and discard the old sponge and insert a new one in the manifold wash station.



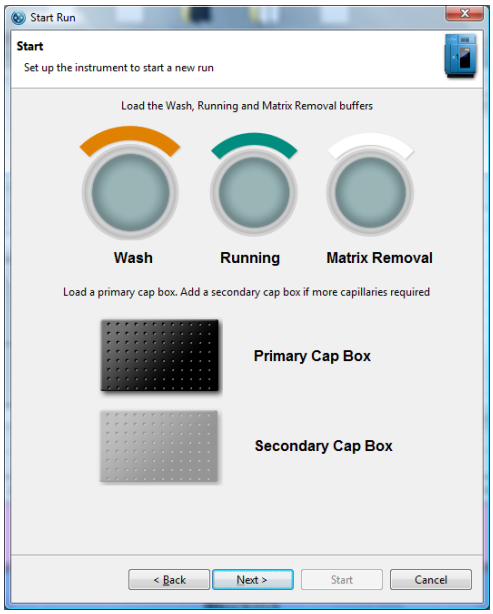
6. **Start.** The resource tray will open.
   a. **If you're running an assay with Running Buffer Rev B**: fill the cups with Wash Buffer, Running Buffer and Matrix Removal Buffer.

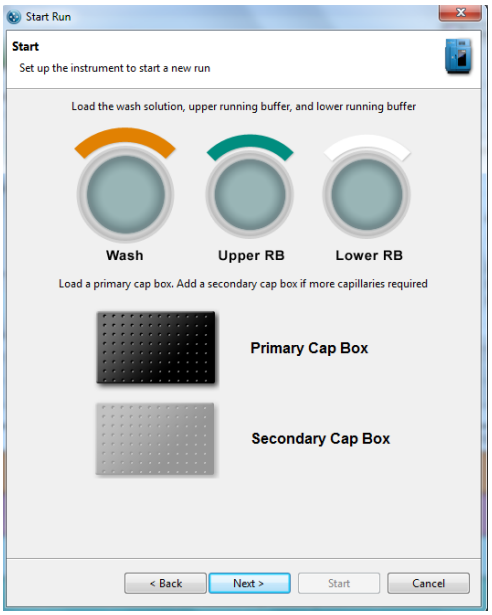
      If you're running a split Running Buffer assay: fill the cups with Wash Buffer, Upper Running and Lower Running Buffer.

   b. Insert a capillary box in the primary position.
   c. Add a second capillary box if more capillaries are required for your run.
   d. Click **Next** when you're done. The resource tray will close.

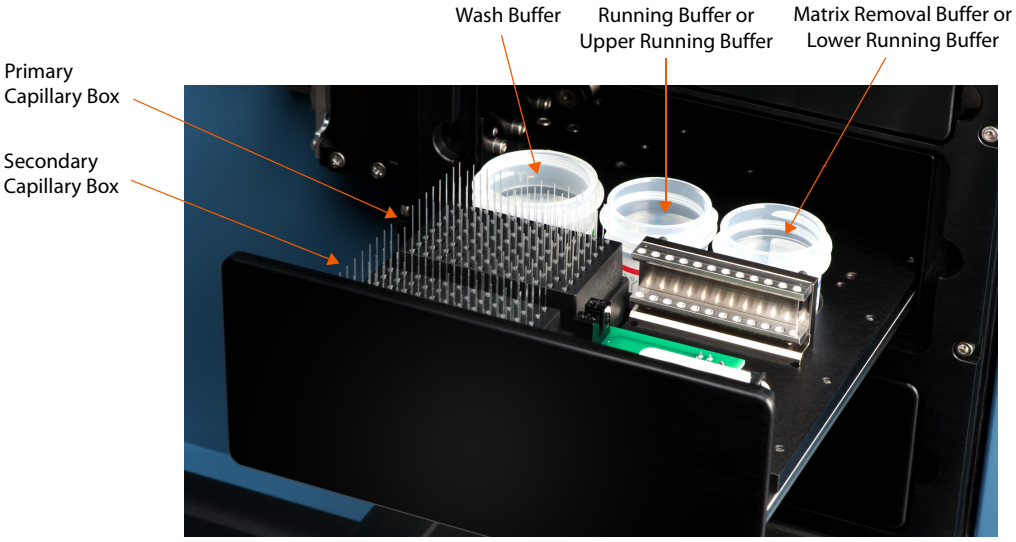
NOTES:
Make sure enough capillaries are loaded to complete the run. Insert a capillary box in the primary position first and if additional capillaries are needed, use the secondary position. When the primary box is empty, Sally Sue or Peggy Sue will automatically move to the secondary box.

Discard any leftover Running Buffer (Rev B, Upper or Lower), Wash Buffer and Matrix Removal Buffer in the resource tray cups before refilling them for new run.

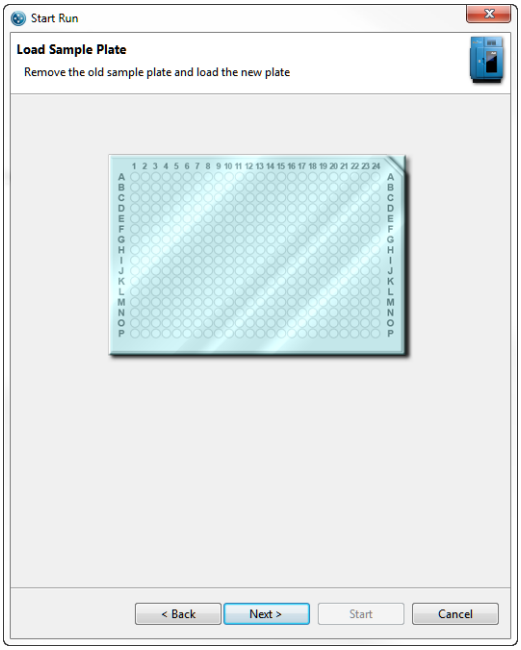


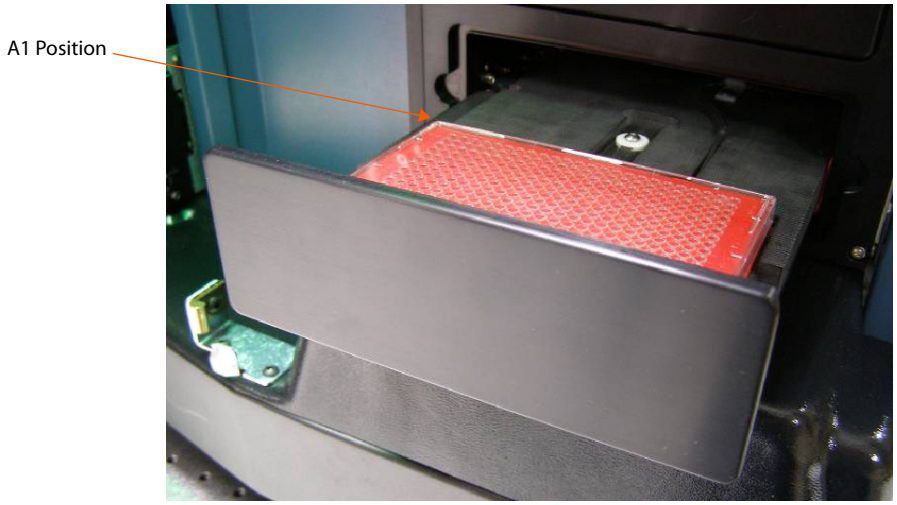


NOTE: You can also use the labels on the resource tray as a guide for where to insert reagents and capillaries.


Wash Buffer
Running Buffer or
Upper Running Buffer
Matrix Removal Buffer or
Lower Running Buffer
Primary
Capillary Box
Secondary
Capillary Box

7. **Load Sample Plate.** The sample tray will open. Place the lidded 384-well plate on the cooling block in the tray, making sure the A1 well position is in the upper left corner of the cold block. Once this is done, click **Next**. The sample tray will close.



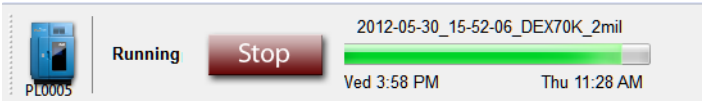


NOTES:
*Sally Sue and Peggy Sue require plate lids on sample plates. If a lid isn't detected, a message will display in the **Start Run Wizard**. Compass for Simple Western will then open the sample tray to allow you to put a lid on the plate.*

When you insert the sample plate, make sure it's firmly seated and level on the cold block. When plates aren't level they can interfere with the movement of the sample tray.

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

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



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

Step 3 - Post-Run Procedures

When the run is done, you'll need to:

1. Empty the capillary discard tray.
2. Remove the assay plate.

Capillary and sample plate disposal depends on the samples that were assayed. If you aren't sure what your sample origins are, we recommend used capillaries and plates be disposed of in biohazard waste.

!WARNING! SHARPS HAZARD

Capillaries may present a potential sharps hazard. Dispose of used capillaries according to your institution's sharps disposal policy.

!WARNING! BIOHAZARD

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

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

Starting a Run: Charge-based Assays (Peggy Sue Only)

Step 1 - Get Ready

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

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

---

4. While the plate is spinning, add Wash Buffer, Anolyte and Catholyte to resource tray cups and insert a capillary box in the resource tray.

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

---

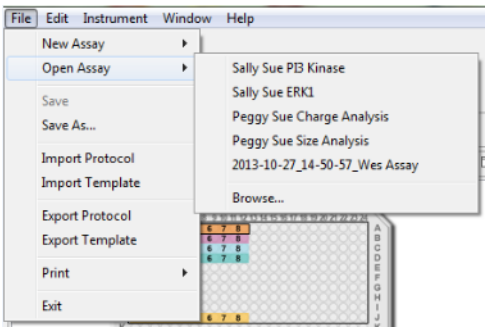
5. Place the assay plate into the sample tray.
6. Click **Start**.

Step 2 - Start the Run

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

1. To start a new run with an existing assay:

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

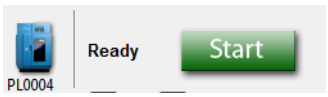


b. A list of the last five assays opened will display. Select one of these assays or click **Browse** to open the Assay folder and select a different one

2. To start a run with a new assay:

a. Choose **New Assay** and select **Peggy Sue Charge** to get the default Peggy Sue assay conditions.

b. The **Start** button will display which means an assay is already loaded.



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

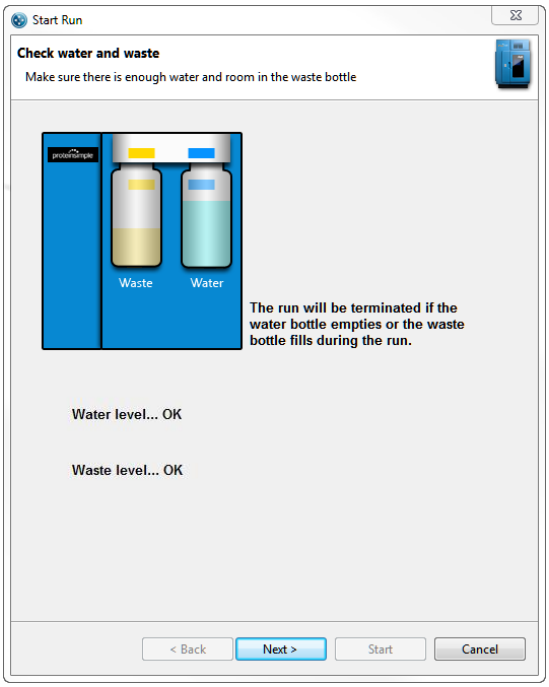
3. Click **Start**. This will launch the **Start Run Wizard**.

---

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

---

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

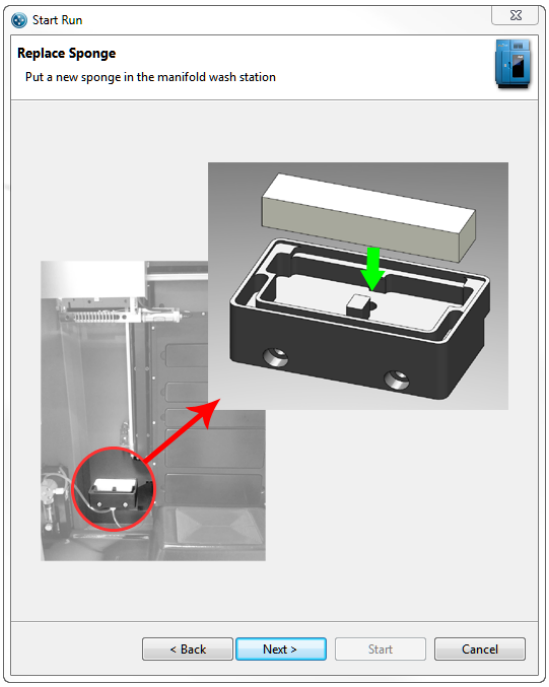


---

*NOTE: If the waste level is too high or the water level is too low to complete the run, a message will display in this screen. If this happens, fill or empty each bottle using the procedures in "Removing and Installing Bottles" on page 29. Once this is done, error status will automatically update and let the **Start Run Wizard** proceed.*

---

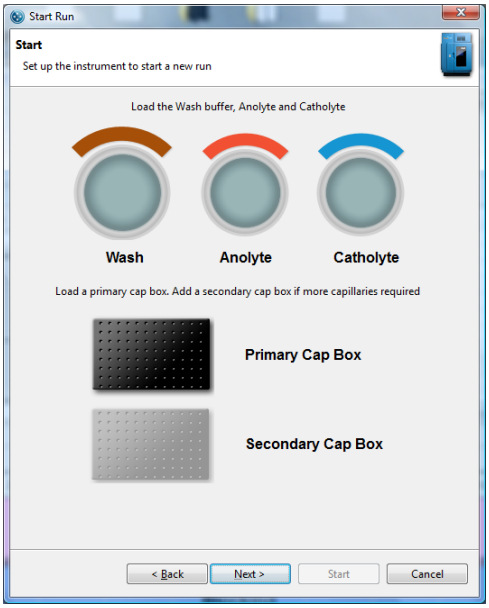
5. **Replace Sponge.** Each time you start a new run, you should use a new sponge. Remove and discard the old sponge and put a new one in the manifold wash station.



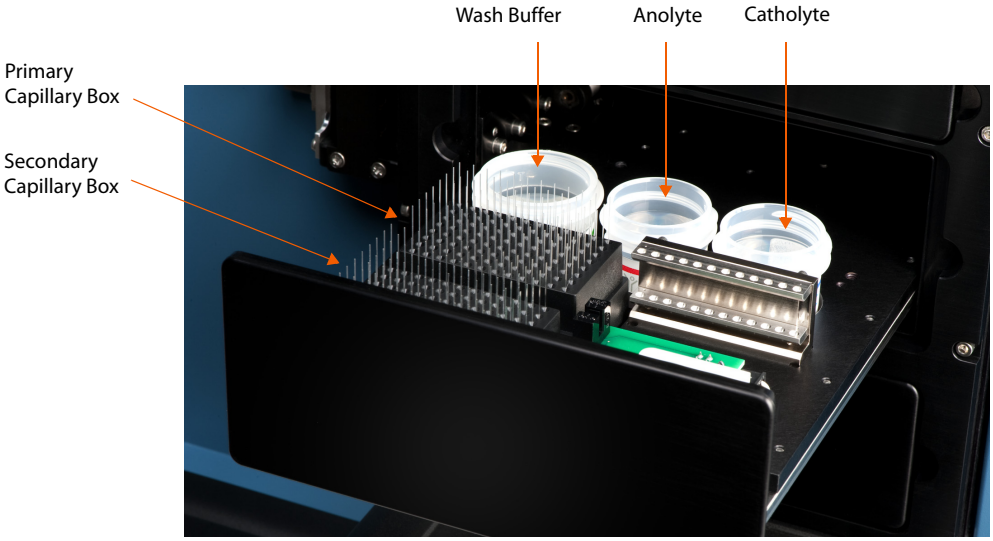
6. **Start.** The resource tray will open. Fill the Wash Buffer, Anolyte and Catholyte cups and insert a capillary box in the primary position. Add a secondary capillary box if more capillaries are required for you run. Click **Next** when you're done. The resource tray will close.

NOTES:
Make sure enough capillaries are loaded to complete the run. Insert a capillary box in the primary position first and if additional capillaries are needed, use the secondary position. When the primary box is empty, Peggy Sue will automatically move to the secondary box.

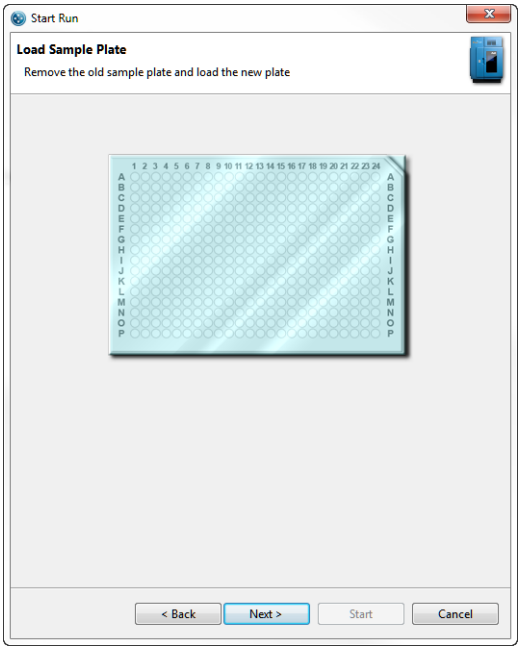
Discard any leftover Running Buffer, Wash Buffer and Matrix Removal Buffer in the resource tray cups before refilling them for new run.

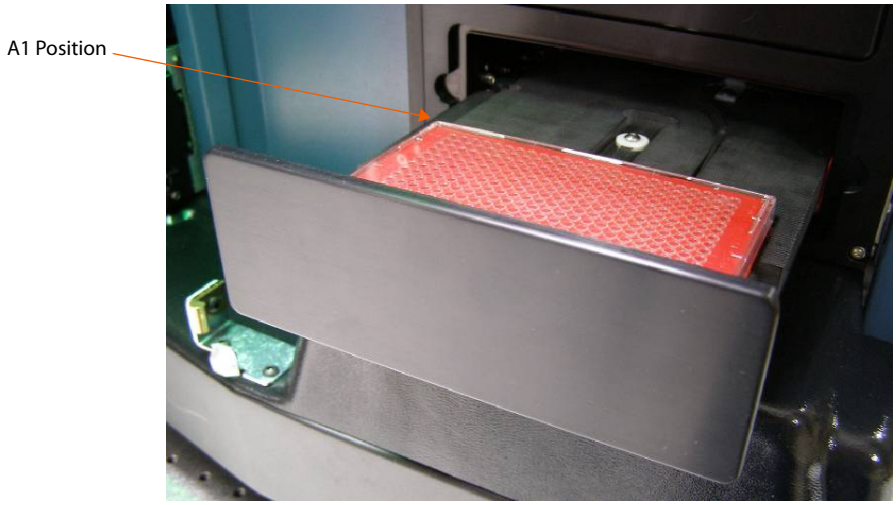


NOTE: You can also use the labels on the resource tray as a guide for where to insert reagents and capillaries.



7. **Load Sample Plate.** The sample tray will open. Place the lidded 384-well plate on the cooling block in the tray, making sure the A1 well position is in the upper left corner of the cold block. Once this is done, click **Next**. The sample tray will close.

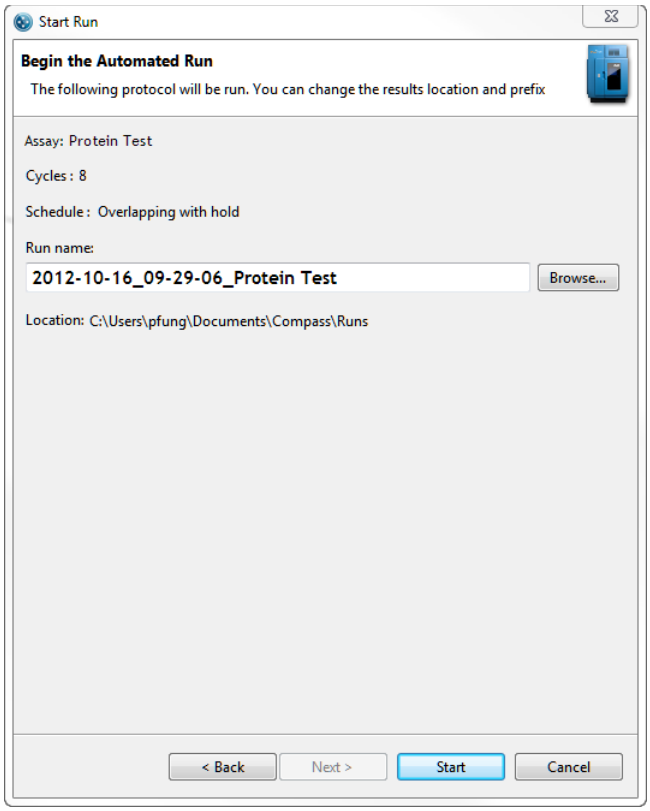




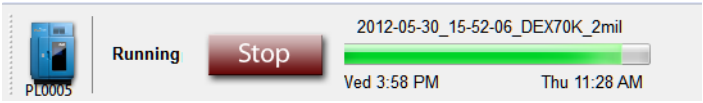
NOTES:
Peggy Sue requires plate lids on sample plates. If a lid isn't detected, a message will display in the ***Start Run Wizard****. Compass for Simple Western will then open the sample tray to allow you to put a lid on the plate.*

When you insert the sample plate, make sure it's firmly seated and level on the cold block. When plates aren't level they can interfere with the movement of the sample tray.

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



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



The run will continue until complete (~11 hours depending on the assay).

Step 3 - Post-Run Procedures

When the run is done, you'll need to:

1. Empty the capillary discard tray.
2. Remove the assay plate.

Capillary and sample plate disposal depends on the samples that were assayed. If you aren't sure what your sample origins are, we recommend used capillaries and plates be disposed of in biohazard waste.

!WARNING! SHARPS HAZARD

Capillaries may present a potential sharps hazard. Dispose of used capillaries according to your institution's sharps disposal policy.

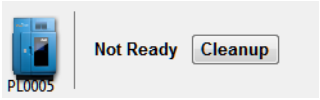
!WARNING! BIOHAZARD

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

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

Stopping a Run

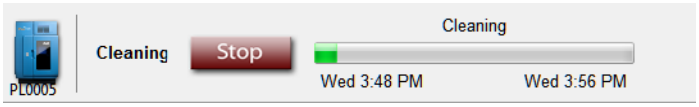
1. To stop a run, click **Stop**. When the run stops, instrument status will change to not ready and a cleanup button displays:



NOTE: If a run is stopped before it completes, Sally Sue and Peggy Sue must perform a cleaning protocol to remove used capillaries from system trays. This prepares them for the next run.

---

2. Click **Cleanup**.



Let Sally Sue or Peggy Sue finish the cleaning protocol, which takes about ten minutes. Once cleaning is done, instrument status will change to ready and you can start a new run.

Waste and Water Bottles

You should empty the waste bottle and refill the water bottle in the Auxiliary Module before starting a new run. To remove or install bottles:

IMPORTANT

Do not remove either bottle if Sally Sue or Peggy Sue are running, doing this may abort the run in progress.

---

1. Remove the bottle by unscrewing it clockwise until it disengages from the manifold.
2. Pull the bottle down slowly until the sensor at the bottom of the bottle is clear.
3. Fill the water bottle to the base of its neck and empty the waste bottle as needed.
4. To reinstall the bottle, lift it up to the manifold making sure to enclose the sensor that sits at the bottom inside the bottle. Screw the bottle counter-clockwise until the arrow on the bottle's alignment label lines up with the arrow on the manifold's alignment label.

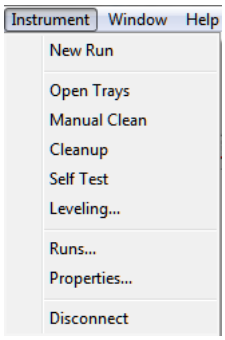
!WARNING! BIOHAZARD

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

Depending on the samples used, waste bottle contents may constitute a biohazard. Use precaution when emptying the waste bottle. Dispose of waste bottle contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Controlling Sally Sue and Peggy Sue

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



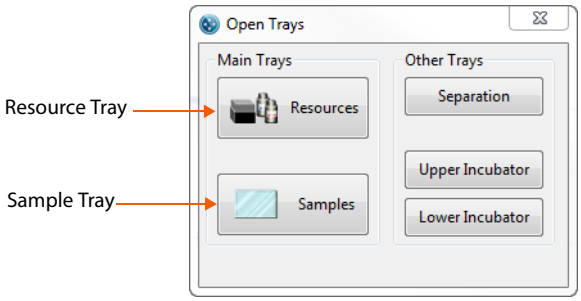
NOTE: Instrument menu options are only active when a computer with Compass for Simple Western is connected directly to Sally Sue and Peggy Sue.

Starting a New Run

To start a new run, select **Instrument** in the main menu and click **New Run**. Then follow the steps in "Step 2 - Start the Run" on page 35 for size assays or "Step 2 - Start the Run" on page 43 for charge assays.

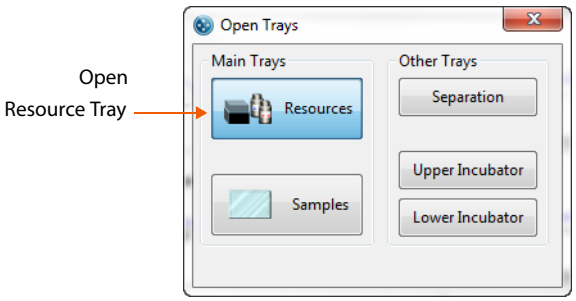
Opening Trays

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



Open a tray by clicking its button. The button will change color when a tray is open.

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

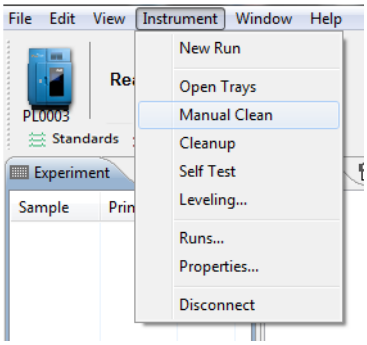


To close a tray, click its tray button again.

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

Cleaning

Instrument cleaning can be performed either manually or automatically in Compass for Simple Western.



Manual Clean

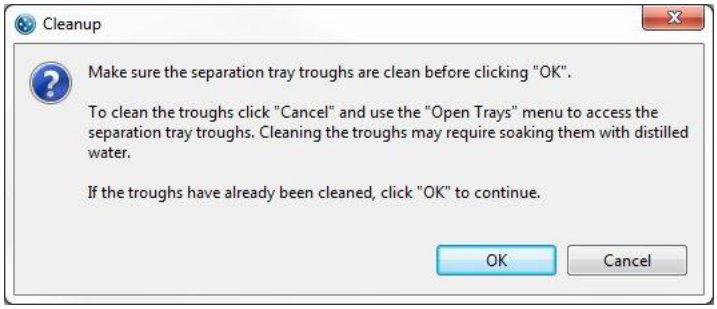
Use this option for general manual cleaning and to clean the manifold head. Select **Instrument** and click **Manual Clean**. The manifold head will move to a safe position for easy access and the vacuum will turn on. See "NOTES: You can follow the recipe below to make your own wash solution, or order the Peggy Sue/Sally Sue Pipet Wash Solution Kit, P/N 043-120. It includes Pipet Wash Solution (180 mL) and three empty reservoir cups. To run the pipet wash, you'll need Compass for Simple Western v2.6.7 and up." on page 65 for the manual manifold cleaning procedure.

NOTE: Please contact Protein Simple Technical Support if you have any questions on the manifold cleaning procedure.

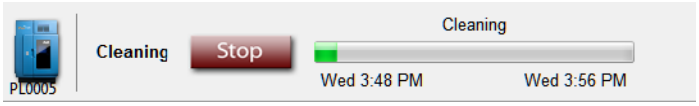
Cleanup

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

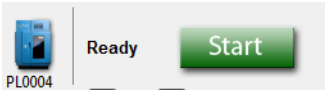
Select **Instrument** and click **Cleanup**. These instructions will display:



Instrument status will change to cleaning, and the stop button and cleaning progress bar display. The Assay screen provides cleaning status details:



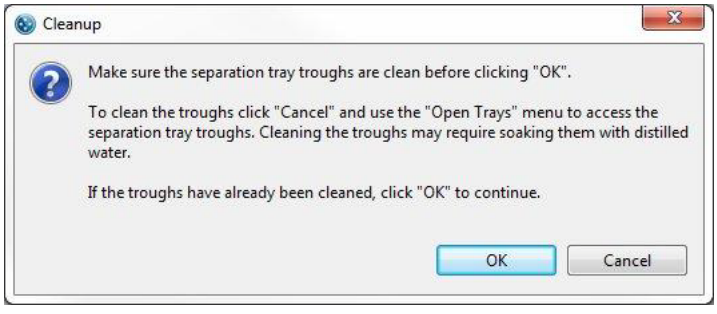
When cleaning is done, instrument status will change to ready.



Cleaning After a Run Error

Additional cleaning steps are needed if the run is stopped because of an error. If this happens, the red Error status light on Sally Sue's or Peggy Sue's front panel will come on (see "Status Lights" on page 12).

Click **Reset**. These instructions will display:

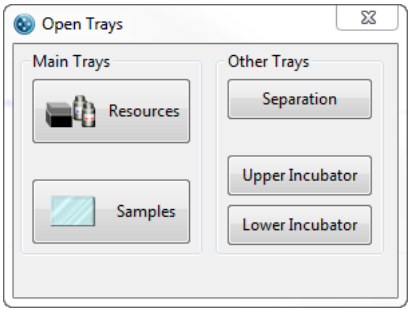


If the troughs in the separation tray are empty, click **OK** and proceed with "Cleanup" on page 54.

If Running Buffer is present in the separation tray, click **Cancel** and remove the buffer manually. When Running Buffer evaporates, it leaves a highly viscous residue that the automatic cleaning feature can't remove.

To remove Running Buffer:

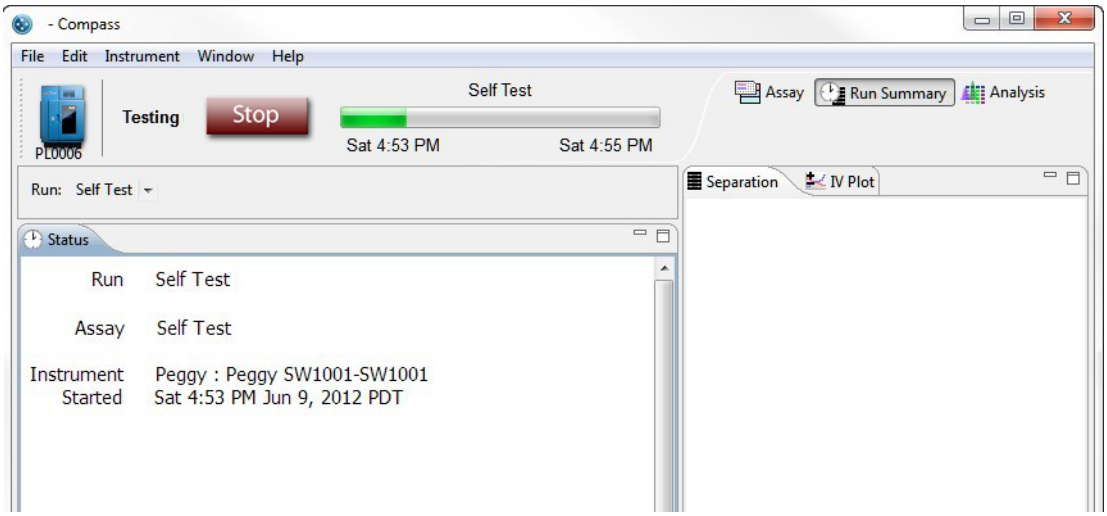
1. Select **Instrument** and click **Open Trays**.



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

Self Test

Sally Sue and Peggy Sue can perform a set of self tests to make sure they're operating properly. To start the test, select **Instrument** and click **Self Test**. The test takes about two minutes.



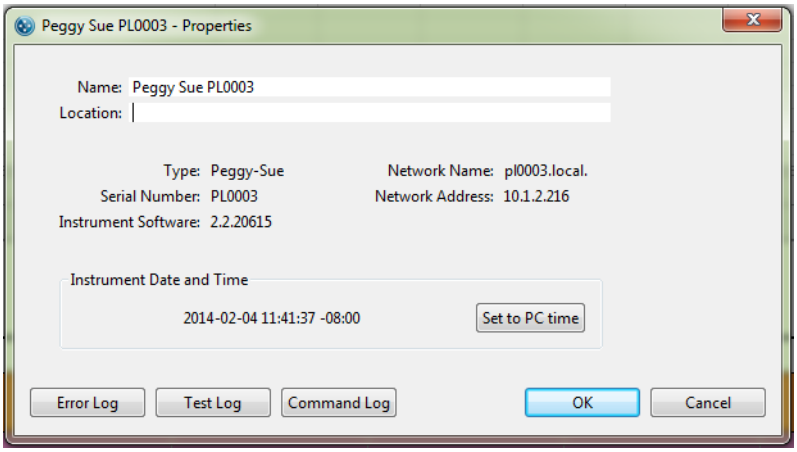
NOTE: We recommend performing the self test before you start a run.

To view the log when the test is done, select **Instrument**, click **Properties** and **View Test Log**.

Viewing and Changing System Properties

Select **Instrument** and click **Properties** to see system properties:

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



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

Viewing Error and Test Logs

Select **Instrument** and click **Properties**. To view a log, you can either click **View Error Log** or **View Test Log**.

Instrument Status Modes

The status bar shows status, buttons and progress bars depending on what Sally Sue or Peggy Sue are doing.

- **Ready/Start button** - We're ready and an assay is loaded. Click **Start** to begin a run.
- **Not Ready/Clean button** - We're not ready and need to do a system cleaning. Click **Clean** to start the cleaning protocol.
- **Not Ready/Reset button** - We're not ready and need to reinitialize. Click **Reset** to start the initialization protocol.
- **Running/Stop button** - We're running an assay. The run name, time the run started and when it will done show in the progress bar. Click **Stop** to stop the run.
- **Cleaning/button not active** - We're running a cleaning protocol. The time the cleaning protocol started and when it will be done show in the progress bar.
- **Error/Reset button** - We've detected an error. You can go to the Status window in the Run Summary screen to get more details. Once you've been able to correct the error, click **Reset**.

Shutdown

1. Close Compass for Simple Western and shut down the system computer.
2. Sally Sue and Peggy Sue can remain on unless you don't think they'll be used for more than a week. In that case, you can just turn the power off.

Chapter 5:

Maintenance and Troubleshooting

Chapter Overview

- Software Updates
- Maintenance
- Preparation for Storage or Shipment
- Spare Parts
- Troubleshooting

Software Updates

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

Maintenance

Daily

Empty the Capillary Discard Tray

Empty the capillary discard tray on a daily basis after each run. The discard tray is located under the instrument's sample tray.

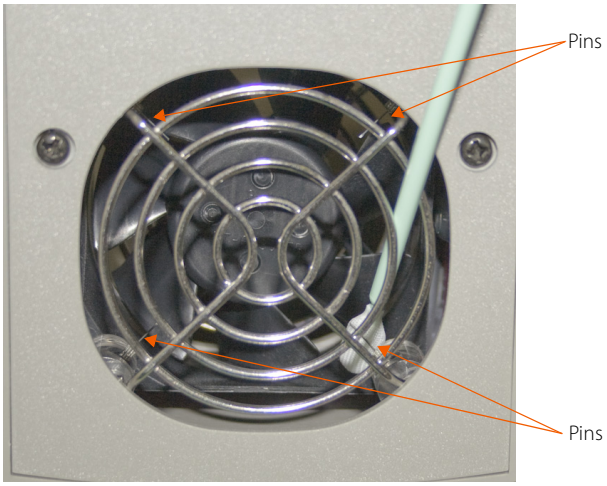
Monthly

Ionizer Cleaning

Items needed: Isopropyl alcohol, cleaning swab (lint and fiber-free, VWR P/N 89133-806) and the screwdriver (P/N 040-994) provided in the Sally Sue or Peggy Sue accessory kit.

1. Open the instrument's right door.
2. Remove the thumbscrew holding the protective cover over the fans.

3. Using the cleaning swab, rub isopropyl alcohol over each of the 4 ionizing pins on the fan to remove any build-up.



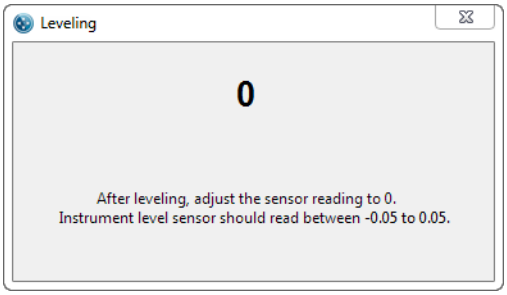
4. Replace the panel and thumbscrew.

Yearly

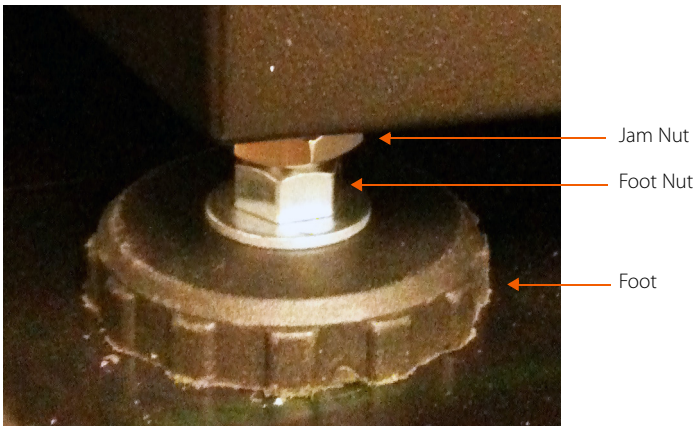
We recommend Sally Sue and Peggy Sue have annual preventive maintenance performed by an authorized ProteinSimple representative. Please contact Customer Support to schedule a visit.

Leveling the Instrument

This option allows you to see if the instrument is properly leveled. In the Compass for Simple Western main menu, select **Instrument** and click **Leveling**. A screen will display showing the level status:



NOTE: If the reading is outside the -0.05 to 0.05 range, you'll need to level the instrument. Follow the instructions below or contact ProteinSimple Technical Support or your Customer Service Engineer if you need help.



1. Loosen the jam nut (11/16") on both front feet.

2. Adjust the foot nut (1/2") on both front feet with a wrench until the level indicates 0 (+/- 0.05).

NOTE: Turning the wrench clockwise will raise the front and make the sensor read more negative.

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3. Once level, lock the position by holding the foot nut in place with the wrench and tightening the jam nut by hand.

Instrument Cleaning

You can run instrument cleaning automatically. See "Cleaning" on page 53.

Pipet Wash

This procedure washes the pipet, liquid paths and troughs in the instrument. Perform the wash as soon as you see precipitate in any of the reservoir tray cups or in the separation tray trough, but wait until after a run is completed. Whenever you see precipitate in a cup, you'll need to run the pipet wash before you use the system again.

NOTES:
You can follow the recipe below to make your own wash solution, or order the Peggy Sue/Sally Sue Pipet Wash Solution Kit, P/N 043-120. It includes Pipet Wash Solution (180 mL) and three empty reservoir cups.

To run the pipet wash, you'll need Compass for Simple Western v2.6.7 and up.

Items needed: DTPA (Sigma P/N 32319), 50% NaOH, 10% (v/v) Triton X-100, Milli-Q water, 0.2 µm filter, 1L vessel and three dry, clean resource tray cups.

Pipet Wash Solution

The wash solution is 0.1 M DTPA and 0.1% Triton X-100. Here's the recipe to make 1000 mL:

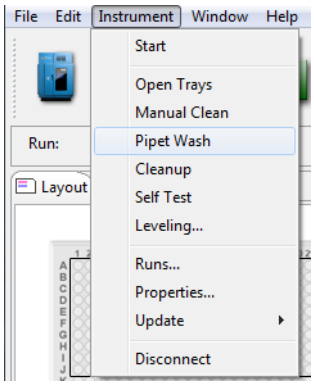
1. Weigh 39.3 g of DTPA in a 1 L vessel.
2. Add 800 mL of Milli-Q water. At this point the DTPA will not dissolve.
3. Adjust the pH to 7-8 with 50% NaOH to bring the DTPA into solution.
4. Add more Milli-Q water to a volume of 1 L. Stir to fully dissolve.
5. Filter the solution with a 0.2 µm filter.

6. Add 10 mL of 10% (v/v) Triton X-100 stock.
7. Gently stir to mix completely.

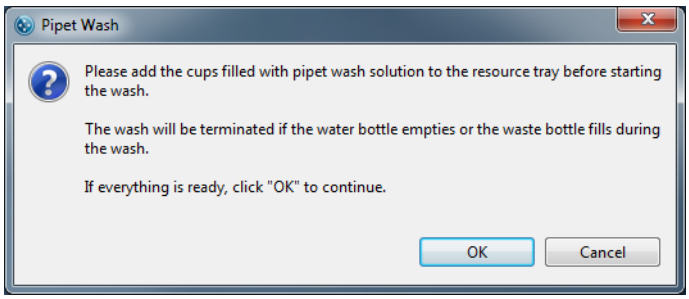
Wash Procedure

1. Check the Auxiliary Module and make sure the water bottle is full and the waste bottle is empty.
2. Place a new sponge in the manifold wash station.
3. Open all system trays and remove anything found (capillaries, capillary boxes, cups, etc.).
4. Take three empty, clean and dry cups and fill each with 20 mL of the pipet wash solution. Place the filled cups in the resource tray.
5. Launch Compass for Simple Western. Select **Instrument** in the main menu and click **Pipet Wash**.

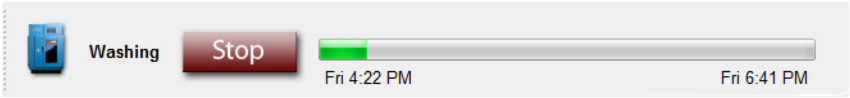
NOTE: To run the pipet wash, you'll need Compass for Simple Western v2.6.7 and up.



The following window will display:



6. If everything is ready, press **OK** to start the wash protocol. The estimated wash time displays at the top of the screen:



7. After the wash is complete, remove all three cups, rinse them with distilled water and air dry.

Cleaning the Manifold

Items needed: 5% Contrad 1000 solution (Decon Labs), bleach (diluted 1:10 in DI water), DI water, 60 mL disposable syringe (BD, PN 309653), 45 degree bent, stainless steel dispensing tip, (Nordsen EFD PN 7018123, 18 gauge) and paper towels.

1. In Compass for Simple Western, select **Instrument** > **Manual Clean**.
2. Fill the 60 mL disposable syringe with 5% Contrad solution and place the tip on the syringe.
3. Insert the syringe tip into the first hole on the bottom of the manifold and slowly inject ~15 mL of the 5% Contrad solution. Repeat this flush for each hole in the manifold.

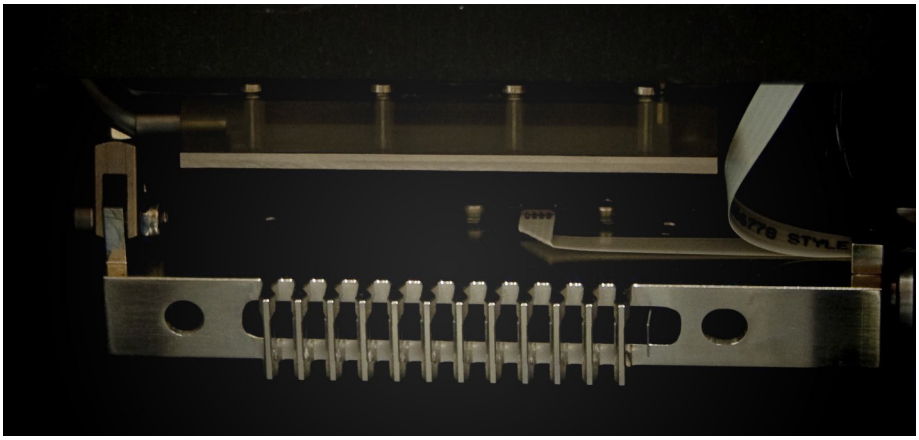
4. Repeat step 3 with DI water.
5. Fill the 60 mL syringe with 10% bleach solution and place the tip on the syringe.
6. Insert the syringe tip into the first hole on the bottom of the manifold and slowly inject ~15 mL of the 10% bleach solution. Repeat this flush for each hole in the manifold.
7. Repeat step 6 with DI water.

8. When all flush steps are complete, click **OK** in Compass.
9. In Compass for Simple Western, select **Instrument** > **Cleanup** to run the automated cleanup procedure two times.

Gripper Cleaning

Items needed: 0.22-micron filtered deionized water (molecular biology grade or better), cleaning swab (lint and fiber-free, VWR P/N 89133-806)

1. Select **Instrument** in the Compass for Simple Western main menu and click **Cleanup**. Let the cleaning protocol complete, this will take about one minute.
2. Power down the instrument.
3. Open the right and left system doors on Sally Sue or Peggy Sue.
4. Wet a cleaning swab with deionized water and clean in between the 12 vertical slots in the gripper.



5. Close both doors.
6. Turn the instrument power back on.

System Decontamination

You should perform the decontamination procedure after analysis of hazardous samples on Sally Sue or Peggy Sue.

Prior to Hazardous Sample Analysis

Perform the following procedure to prepare Sally Sue or Peggy Sue for hazardous sample analysis:

Items needed: 10% bleach solution

1. Remove and empty the waste bottle.
2. Add 10% bleach solution to the bottle until the liquid level is 1 cm from the bottom of the bottle.
3. Reinstall the waste bottle.

Post-Run Decontamination

When running hazardous samples, perform the following procedures monthly at minimum, or as often as needed based on the type of samples being run. You should also perform this procedure before system shipment.

Items needed: 10% bleach solution, 5% Contrad solution, 0.22-micron filtered deionized water (molecular biology grade or better), 50-mL disposable syringe, 1-mm syringe tip, rubber gloves and paper towels

1. Perform the **Wipe Down** procedure below.
2. Perform the **Internal Instrument Cleaning** procedure on page 69.
3. Perform the **Waste Stream Cleaning** procedure on page 70.

Wipe Down Procedure

Items needed: 10% bleach solution and towels

NOTE: You'll need to wear rubber gloves for the wipe down procedure.

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1. Completely saturate a towel with 10% bleach solution.
2. Wipe down the exterior of the instrument.
3. Select **Instrument** in the Compass for Simple Western main menu and click **Open Trays**. Click the tray buttons one at a time to open each for cleaning. Wipe down all accessible areas and pay particular attention to:
   a. The upper and lower levels of the capillary box racks in the resource tray.
   b. The underside of the wash station in the resource tray.
4. Close all trays.

Internal Instrument Cleaning Procedure

1. Empty and rinse the Running, Wash and Matrix Removal Buffer cups.
2. Fill each cup with 10% bleach solution and insert into the resource tray.

Waste Stream Cleaning Procedure

1. Select **Instrument** from the Compass for Simple Western main menu and click **Manual Clean**. This will position the manifold for easy access and turn on the vacuum.
2. Fill the 50-mL disposable syringe with 10% bleach solution. Place the 1-mm tip on the syringe.
3. Flush each hole on the bottom of the manifold. Refer to the picture in "NOTES: You can follow the recipe below to make your own wash solution, or order the Peggy Sue/Sally Sue Pipet Wash Solution Kit, P/N 043-120. It includes Pipet Wash Solution (180 mL) and three empty reservoir cups. To run the pipet wash, you'll need Compass for Simple Western v2.6.7 and up." on page 65 for proper insertion of the syringe, then position the syringe tip into the first hole and inject 15 mL of the 10% bleach solution. Repeat for each manifold hole.
4. Repeat step 3 with 5% Contrad solution.
5. Repeat step 3 with water.
6. Click **OK**.

NOTE: Contact Protein Simple Technical Support if you have any questions on the manifold cleaning procedure.

Preparation for Storage or Shipment

Storage

We recommend storing Sally Sue and Peggy Sue if they will not be used for two weeks or longer. To prepare them for storage:

1. Open the resource tray and remove any remaining capillaries along with the Wash, Running and Matrix Removal Buffer cups.
2. Discard the unused buffers.
3. Rinse the cups with 0.22-micron filtered deionized water (molecular biology grade or better) and let them air dry. When dry, cap and store at room temperature.

NOTE: If you don't remove and cap the reagent cups, this can lead to evaporation and unnecessary concentration of reagents which will affect assay performance the next time you run the system.

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4. Open the sample tray and remove the sample plate.

5. If hazardous samples have been analyzed on Sally Sue or Peggy Sue, perform the "Post-Run Decontamination" on page 69.
6. Empty the waste bottle and reinstall.

Shipment Preparation

To prepare Sally Sue or Peggy Sue for shipment:

1. Perform "Instrument Cleaning" on page 65.
2. Perform "Post-Run Decontamination" on page 69.
3. Empty the waste bottle and reinstall.

Spare Parts

If you need help with replacement parts, contact ProteinSimple Technical Support at (888) 607-9692.

Description	Part Number
Auxiliary Module Waste Bottle	002-611
Wrench, Combination, 1/2" - 5/8", Open End	040-992
Wrench, Driver, Allen M2.5	040-993
Sally Sue and Peggy Sue Instrument Cup Set (3)	041-595
Waste/Water Bottle Replacement Kit	040-710

Troubleshooting

For Sally Sue, Peggy Sue or Simple Western assay troubleshooting information, please contact ProteinSimple Technical Support at (888) 607-9692 (option 3), support@proteinsimple.com or visit http://www.proteinsimple.com/technical_support.html. You can also contact your local Field Application Specialist for help.

Chapter 6:

General Information

Chapter Overview

- Compliance
- Safety Guidelines
- Customer Service and Technical Support
- Legal Notices

Compliance

Sally Sue and Peggy Sue 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 61326-1:2006**: Electrical equipment for measurement, control and laboratory use. EMC Requirements. General requirements (EU)

Safety Guidelines

!WARNING!

If Sally Sue and Peggy Sue are not used as specified by ProteinSimple, overall safety will be impaired.

!WARNING!

If Sally Sue or Peggy Sue are damaged and don't function properly, stop them safely and contact ProteinSimple Technical Support immediately.

!WARNING!

You cannot replace or service any parts except for the ionizer fan and separation block.

CAUTION

Avoid using Sally Sue or Peggy Sue in ways not specified by ProteinSimple. Although the instruments have been designed to protect you, this protection may not be effective if they aren't used properly.

Door Interlock

A door interlock engages once you've started a run to prevent the doors from being opened during the run. This prevents you from being exposed to any dangerous interactions with the robot as well as protecting you from exposure to high voltage and UV light. The yellow indicator light will be on when the instrument is running and the door interlock is engaged.

When the system status is ready (green) and the doors are open, the robot's system tray, Z-stage motors and UV power supply are disabled.

!WARNING!

Do not override the door interlock. The interlock protects you from exposure to UV light and a potential pinch hazard that could be caused by moving system trays.

System Trays

CAUTION

Don't open or close any of the system trays manually. Instead, use the **Open Trays** command in the **Instrument** menu in Compass for Simple Western.

Auxiliary Module Reservoirs

IMPORTANT

Don't remove either bottle if Sally Sue or Peggy Sue are running, doing this may abort the run in progress.

Waste Disposal Hazard

!WARNING! BIOHAZARD

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

Depending on the samples used, waste bottle contents may constitute a biohazard. Use precaution when emptying the waste bottle. Dispose of waste bottle contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

!WARNING! SHARPS HAZARD

Capillaries may present a potential sharps hazard. Dispose of used capillaries according to your institution's sharps disposal policy.

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, or 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 on the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazards

!WARNING! BIOHAZARD

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

Depending on the samples used, waste bottle contents may constitute a biohazard. Use precaution when emptying the waste bottle. Dispose of waste bottle contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the 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, or clothing).
- Use precaution when emptying the waste bottle.
- Dispose of waste bottle contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Production and Disposal

Please make sure you dispose of waste properly. The aqueous bulk waste stream from is approximately pH 8. For reference, ProteinSimple disposes of waste under the non-RCRA Hazardous Waste Liquid code 141 (buffer containing sodium chloride surfactants). A typical waste profile for one cycle (12 capillaries) is:

NOTE: Be sure to adjust waste volumes up when running more than one cycle.

Waste Origin: Size-based Assays	Volume
Trough Reagents for 1 Cycle	
Matrix Removal Buffer	2.0 mL
Wash Buffer	2.0 mL
Running Buffer	1.6 mL
Water (for trough and manifold cleaning)	80.0 mL
Total waste per cycle	**85.6 mL**
Assay Plate Reagents*	
Separation Matrix	0.240 mL
Stacking Matrix	0.240 mL
Biotinylated Ladder	0.005 mL
Antibody Diluent	0.200 mL
Primary Antibodies in Antibody Diluent	0.180 mL
Streptavidin-HRP	0.010 mL
Secondary HRP Conjugate	0.180 mL
Luminol/Peroxide Mix	0.180 mL
Prepared Sample – contains Sample Buffer, DTT, fluorescent Standards and water	0.055 mL

**Assumes 1 row of sample and 1 row of antibody.*

Waste Origin: Charge-based Assays	Volume
Trough Reagents for 1 Cycle	
Anolyte (10 mM)	1.0 mL
Catholyte (100 mM)	1.0 mL
Wash Buffer	3.0 mL
Water (for trough and manifold cleaning)	80.0 mL
Total waste per cycle	**85.0 mL**
Assay Plate Reagents*	
Luminol/Peroxide Solution	0.025 mL
Antibodies in Antibody Diluent	0.075 mL
Example Cell Lysate Sample Cell lysate Sample Diluent Premix Protease inhibitors Phosphatase inhibitors Labeled peptides	 0.036 mL

**Assumes 1 row of sample and 1 row of antibody.*

Safety Data Sheets

Some chemicals used with Sally Sue and Peggy Sue may be listed as hazardous. Warnings are displayed on the labels of all chemicals when hazards exist.

SDSs provide you with safety information needed to store, handle, transport and dispose of the chemicals safely. We recommend updating laboratory SDS records periodically.

Safety Data Sheets for ProteinSimple reagents are available online at www.proteinsimple.com/literature or by calling (888) 607-9692. Otherwise, call the chemical manufacturer directly or visit their web site.

Instrument Safety Labels

The following safety labels are located on Sally Sue and Peggy Sue. Each label displays a safety alert symbol to let you know there's a potential safety hazard.

Symbol	Description
	Risk of electric shock.
	Refer to user guide before proceeding.
	Danger of hazardous waste. Use caution in these areas. This warning only applies if using hazardous material. Sally Sue and Peggy Sue 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.

Customer Service and Technical Support

Telephone
(408) 510-5500
(888) 607-9692 (toll free)

Fax
(408) 510-5599

E-mail
support@proteinsimple.com

Web
proteinsimple.com

Address
ProteinSimple
3001 Orchard Parkway
San Jose, CA 95134
USA

Legal Notices

NOTE: Read the Legal Notices carefully before using Sally Sue or Peggy Sue.

Sally Sue and Peggy Sue Disclaimer of Warranty

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

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

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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.

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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.

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be encountered while servicing the Licensed Software. Customer may contact Company via telephone at 1-888-607-9692 or e-mail at support@proteinsimple.com during the hours of 8 a.m. (Pacific Time) and 5 p.m. (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.

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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.

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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 acknowledgement 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@proteinsimple.com or a letter by United States mail to ProteinSimple, 3040 Oakmead Village Drive, Santa Clara, CA 95051, 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.