USER GUIDE

Proteograph™ Product Suite

Proteograph™ Assay

FOR USE WITH
SP100 Automation Instrument
Proteograph™ Instrument Control Software
Proteograph™ Assay Kit
Proteograph™ PQR Labware Kit

seer™

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Revision history

DOCUMENT	DATE	DESCRIPTION OF CHANGE
CF-1016 F	October 2023	Updated instructions for the storage of controls. Miscellaneous minor corrections.
CF-1016 E	June 2023	Added new tips and important guidelines to ensure customer success. Improved readability by moving some content into two new chapters, SP100 Automation Instrument Overview (page 14) and Materials (page 25). Made miscellaneous minor corrections and clarifications.
CF-1016 D	August 2022	Updated instructions for the preparation of files needed for the Peptide Reconstitution method. Updated instructions for running the MPE Flush method. Made miscellaneous minor corrections and clarifications.
CF-1016 C	March 2022	Updated instructions for the storage and preparation of controls. Updated instructions for the Tip Counter stage of applicable methods. Made miscellaneous improvements to and clarifications in methods instructions. Expanded the index.
CF-1016 B	December 2021	General availability release Supports ICS version 2.1 and later
CF-1016 A	June 2021	Limited (early access) release

Contents

Chapter 1

Proteograph Overview

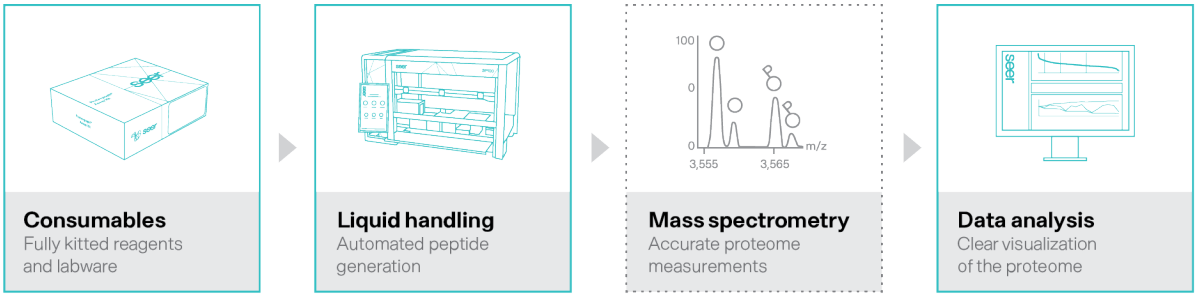
This chapter introduces you to the main components of the Seer Proteograph™ Product Suite.

Introduction

The Seer Proteograph™ Product Suite provides an integrated workflow for unbiased, deep, and large-scale access to the proteome. Combined with proprietary engineered nanoparticles, the Seer SP100 Automation Instrument prepares peptides for analysis on most mass spectrometry (MS) platforms configured for proteomics. The Proteograph™ Analysis Suite (PAS) easily integrates the analysis of raw MS data, quality control (QC) reporting, and data visualization to empower proteomics insights quickly and at scale.

Paired with MS, the Proteograph Product Suite simplifies proteome analysis, offering a rapid, integrated workflow to convert proteins into peptides for deep analysis of the proteome. The following figure shows the high-level steps in the workflow with the products used at each step.

Figure 1. Proteograph workflow



Highlights

The Proteograph Product Suite features the following products:

- **SP100 Automation Instrument**
  - Intuitive Proteograph Instrument Control Software (ICS) for experiment setup and instrument operation
  - Custom Proteograph Assay method that processes proteins in a typical 8-hour workday
  - Dedicated methods for instrument maintenance and troubleshooting
- **Proteograph Assay Kit**
  - Proprietary panel of five engineered nanoparticles for processing up to 16 samples in one 96-well plate
  - Buffers and reagents for protein lysis, digestion, and peptide purification
  - Quality controls to easily compare results across assays or troubleshooting for a specific assay
- **Proteograph Analysis Suite**
  - This product is documented separately in the *Proteograph Analysis Suite* Help system.

Proteograph Assay steps

The Proteograph Assay method runs on the SP100 Automation Instrument and outputs MS-ready peptides. The following table summarizes the method steps and durations.

Table 1. Summary of the Proteograph steps

DESCRIPTION	APPROXIMATE TIME	CUMULATIVE TIME (HOURS)*
Proteograph Assay method setup	30 minutes (all hands-on)	0.5
Sample dispensing and nanoparticle transfer	29 minutes	1.0
Protein corona formation	1 hour	2.0
Protein corona wash	36 minutes	2.6
Protein corona denaturation	40 minutes	3.3
Protein digestion	3 hours and 13 minutes	6.5
Peptide cleanup, wash, and elution	49 minutes	7.3
Deck cleanup	10 minutes (all hands-on)	7.5
Peptide quantification	30 minutes (including approximately 10 minutes hands-on)	8.0
Dry peptides	15 minutes (all hands-on) *THEN DRY OVERNIGHT*	8.2
STOPPING POINT		
Peptide reconstitution	25 minutes	8.7

*Rounded up to the nearest tenth of an hour.

SP100 Automation Instrument

The SP100 Automation Instrument automates the pipetting of liquids to prepare peptides for downstream analysis. A custom table holds the instrument and stores the monitored multi-flow positive pressure evaporative extraction ("MPE") power unit, chiller unit, and instrument computer.

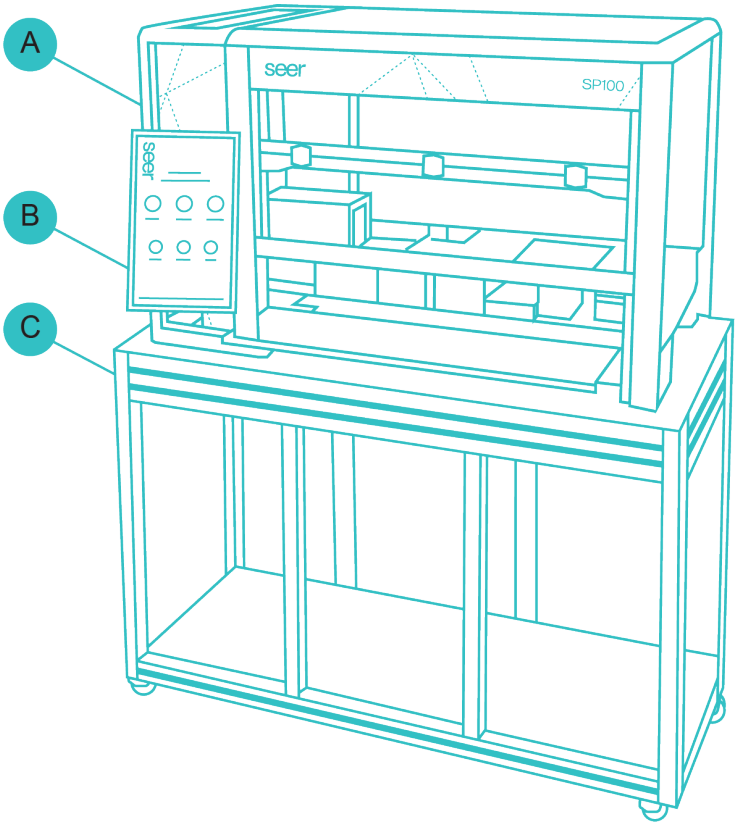
An adjustable arm affixed to the custom table presents the touchscreen monitor. The touchscreen is glove-compatible and displays the ICS user interface.

For a detailed overview of the SP100, including the instrument computer, work deck, and hardware components, see SP100 Automation Instrument Overview (page 14). Additional specifications are described in the *Site Preparation Guide* (CF-1017 or CF-1015 International).

TIP

Provide a staging area (e.g., a cart, a designated portion of a lab bench) near the instrument on which to set plasticware, reagents, and other materials.

Figure 2. SP100 installed on the custom table

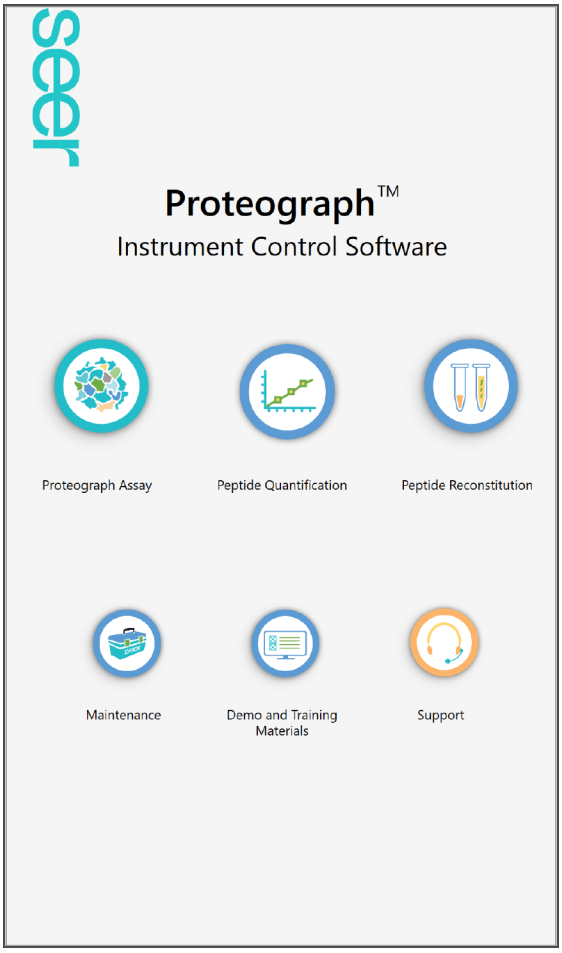


- **A** Instrument
- **B** Touchscreen monitor
- **C** Custom table

Proteograph Instrument Control Software

The instrument includes dedicated software, ICS, that controls instrument operations and provides an interface for running a method and maintaining the instrument.

Figure 3. Proteograph ICS main menu



Methods

ICS includes the following methods to prepare peptides for MS, maintain the instrument, train on the instrument, and troubleshoot problems.

ICON	METHOD	PURPOSE
	Proteograph Assay	Peptide preparation
	Peptide Quantification	Peptide preparation
	Peptide Reconstitution	Peptide preparation
	Daily Maintenance	Instrument maintenance
	Weekly Maintenance	Instrument maintenance
	MPE Flush	Instrument maintenance
	Water Run	Training and troubleshooting

Required Seer kits

Use of the SP100 requires the following Seer kits:

- **Proteograph Assay Kit** – Includes all consumables for assay runs on the SP100.
- **Proteograph PQR Labware Kit** – Includes consumables for peptide quantification and reconstitution runs on the SP100.

For detailed information about the contents and storage requirements of these kits, as well as additional required third-party equipment and materials, see Materials (page 25).

Chapter 2

SP100 Automation Instrument Overview

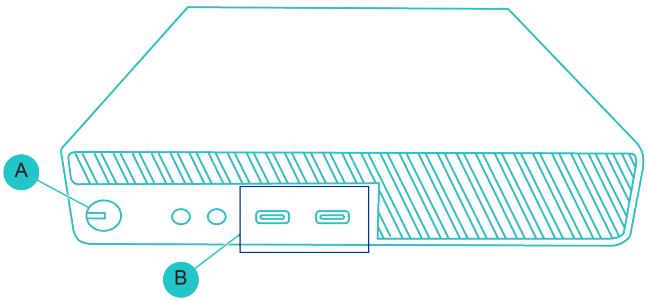
This chapter provides a detailed overview of the SP100 Automation Instrument, including the instrument computer, the work deck and autoload tray, and other hardware components.

Instrument computer

The front panel of the instrument computer includes a power switch and USB ports for transferring data to a portable device.

NOTE
The touchscreen monitor also has USB ports that are more readily accessible than those on the instrument computer.

Figure 4. Instrument computer with power switch and USB ports



A Power switch

B USB ports

Software packages

The following software packages are preinstalled on the instrument computer and appear on its desktop.

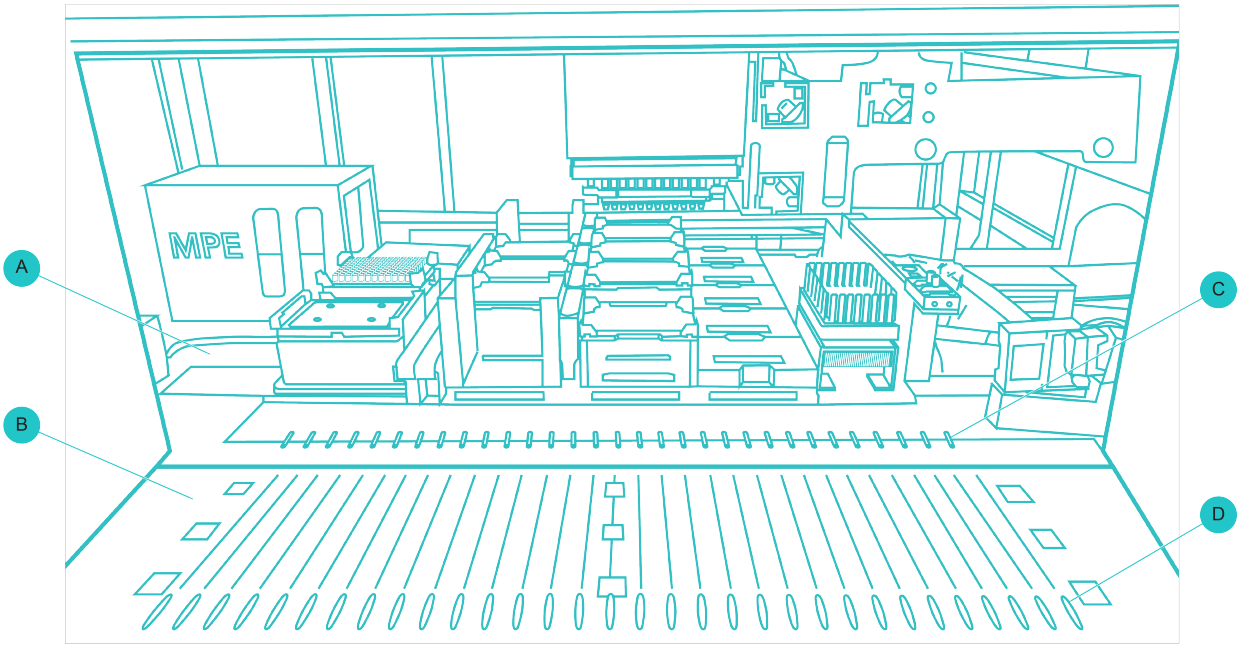
- For end-user use:
  - Proteograph Instrument Control Software (ICS)
  - Seer Support Package Creator (generates files used for troubleshooting)
  - Seer Trace Collector (generated files used for troubleshooting)
- For Seer support personnel use:
  - Microlab Star Maintenance & Verification
  - Microlab STAR Verification 2
  - Hamilton Method Editor
  - Hamilton Run Control
  - Hamilton CO-RE Liquid Editor
  - DataMan Setup Tool

Work deck and autoload tray

The SP100 has two work surfaces, the work deck and the autoload tray. Enclosed by a front protective cover, the work deck is the area inside the instrument that holds the carriers. The autoload tray is the surface in front of the instrument. It includes 30 tracks, labeled in increments of five, that guide carriers as they move in and out of the instrument.

The side blocks on the front of the autoload tray guide the carriers as they move along tracks between the work deck and the autoload tray. Stop hooks attached to the back of the autoload tray secure carriers and provide a stop point so that carriers are completely inserted.

Figure 5. Work surfaces



A Work deck **B** Autoload tray **C** Stop hooks **D** Side blocks

Work deck layout

The following figure shows how the carriers and built-in components are arranged on the work deck. Table 2 indicates the placement of labware for the Proteograph Assay method.

Figure 6. Empty work deck

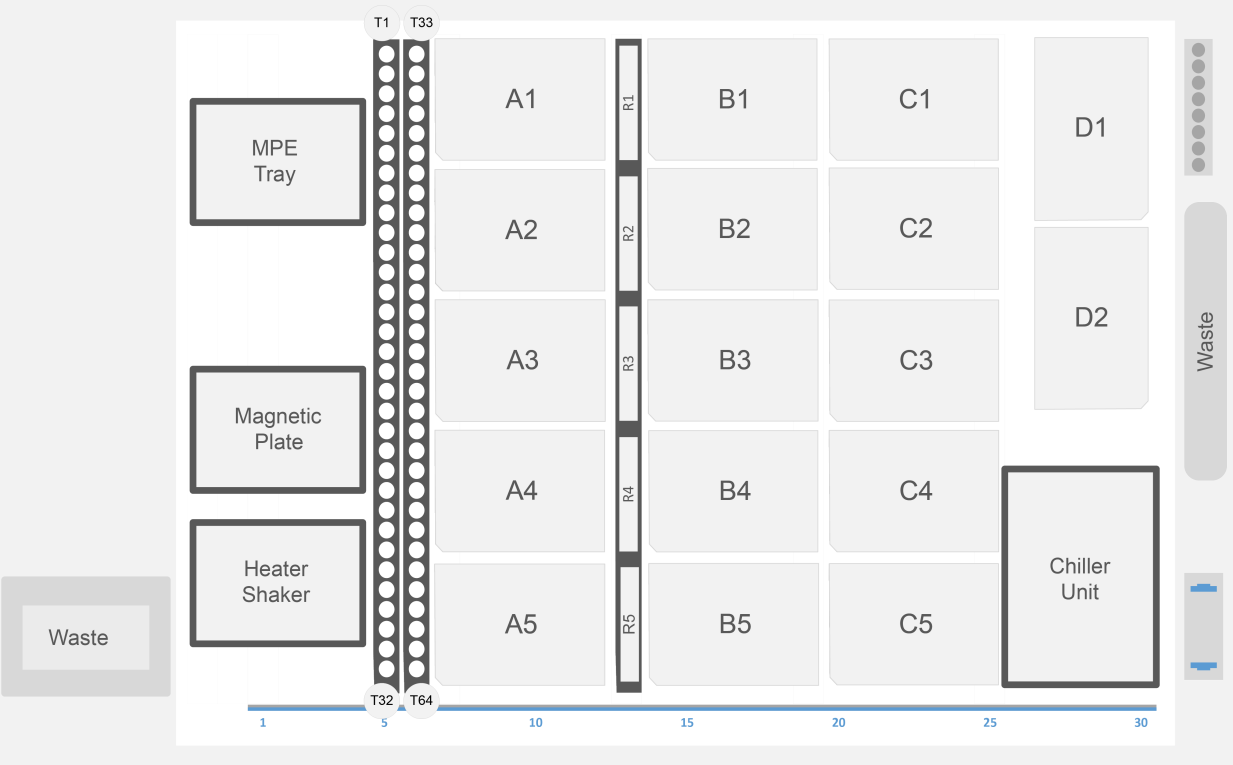


Table 2. Labware positions for the Proteograph Assay method

CARRIER	POSITION	ITEM	NOTES
Tube Carrier 1	T1–T16	Sample Tubes 01–16	
	T17	Plasma Control	
	T18	MPE Control	
	T19–T32	Empty	
Tube Carrier 2	T33–T37	Nanoparticle Tubes (NP #1–5)	
	T38–T64	Empty	
Plate Carrier A	A1	Peptide Cleanup Plate with holding microplate underneath	a
	A2	Wash C Solution Single Reservoir with black microplate lid	
	A3	Cleanup Reagents 4-Well Reservoir with black microplate lid	
	A4	Sample Prep Plate with clear microplate lid	a
	A5	Intermediate Plate	a, b

CARRIER	POSITION	ITEM	NOTES
Reservoir Carrier R	R1	Control Dilution Solution Reservoir	
	R2	Denaturing Solution Reservoir	
	R3	Reduction Solution Reservoir	
	R4	Alkylation Solution Reservoir	
	R5	Deionized Water Reservoir	
Plate Carrier B	B1	Empty	
	B2	Intermediate Plate	a
	B3	Intermediate Plate	a
	B4	Empty	
	B5	Peptide Collection Plate	a
Tip Carrier C	C1	Empty	
	C2	1 Stack of 300 µL Nested Conductive Tips (NCTs)	c
	C3	1 Stack of 300 µL NCTs	c
	C4	1 Stack of 300 µL NCTs	c
	C5	1 Stack of 300 µL NCTs	c
Stationary in Work Deck	D1	1 Stack of 300 µL NCTs	d
	D2	1 Stack of 300 µL NCTs	d

[a]This labware is loaded empty.
[b]One stack of four plates.
[c]One stack of four NCT racks.
[d]The stack must contain from a minimum of two full racks, up to a maximum of four racks.

Serial number

A nameplate is mounted on the inside of the instrument, on the left side of the work deck behind the protective front cover. The nameplate includes the instrument serial number, model, electrical information, and certification information.

Instrument hardware

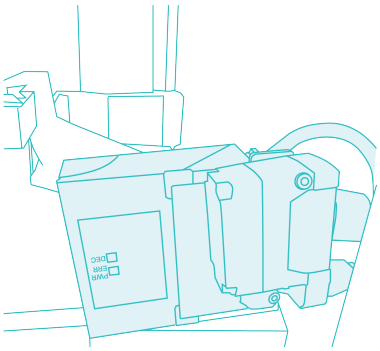
The SP100 is equipped with the following hardware, which pipettes liquids in labware and transports plates. All hardware components reside inside the instrument, except for the external waste containers and carriers, which move in and out of the instrument.

Labels affixed to the instrument hardware identify safety considerations and hazards. For a list of labels with descriptions, see Safety hazards (page 89).

Barcode reader

Before starting a method, the barcode reader scans all barcode-labeled plates, reservoirs, and tubes to ensure proper setup.

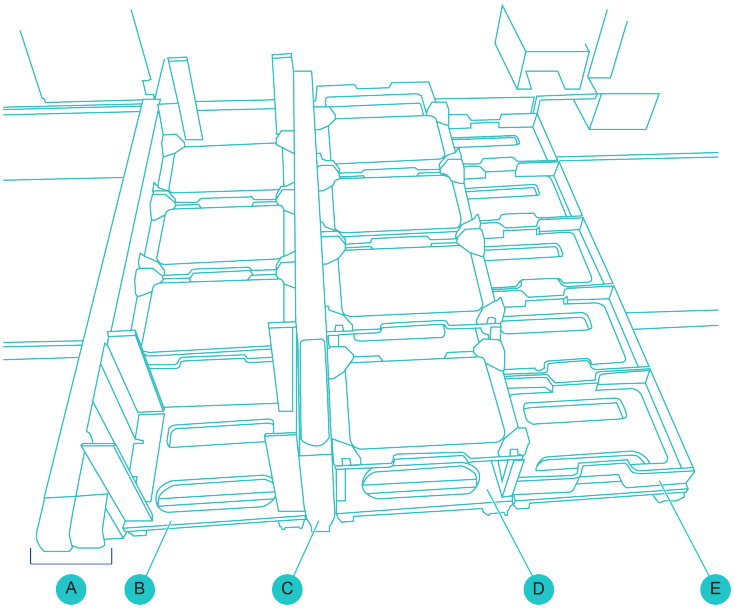
Figure 7. *Barcode reader*



Carriers

Guided to predetermined positions, the carriers are movable units that hold labware and move between the autoload tray and the work deck. The instrument uses different types of carriers for different types of labware (tubes, plates, reservoirs, and tips). For a schematic of the carriers, see Work deck layout (page 17).

Figure 8. *Tube, plate, reservoir, and tip carriers*



- **A** Tube Carrier 1 and Tube Carrier 2
- **B** Plate Carrier A
- **C** Reservoir Carrier R
- **D** Plate Carrier B
- **E** Tip Carrier C

Chiller unit and chiller power unit

During the method, the chiller unit maintains the Trypsin/LysC 8-Well reservoir at the appropriate temperature. The chiller power unit (an instrument peripheral, located beneath the instrument on the lower shelf of the custom table) controls power to the chiller unit.

Figure 9. Chiller unit

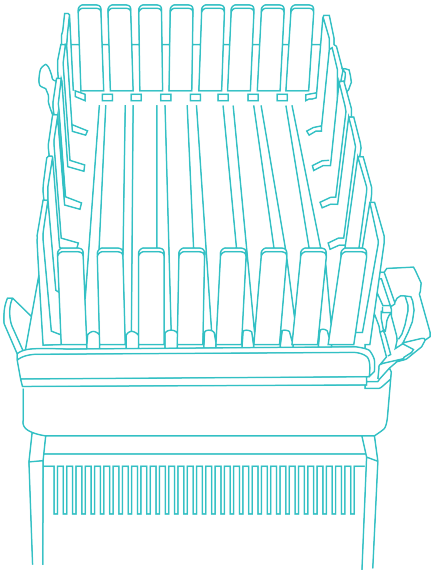
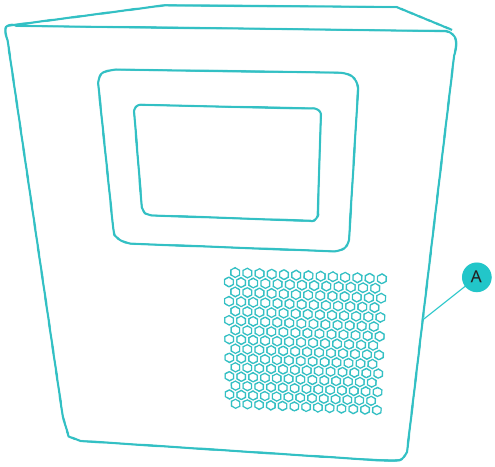


Figure 10. Chiller power unit



A Power switch (not shown; located in the upper-left corner of the back panel)

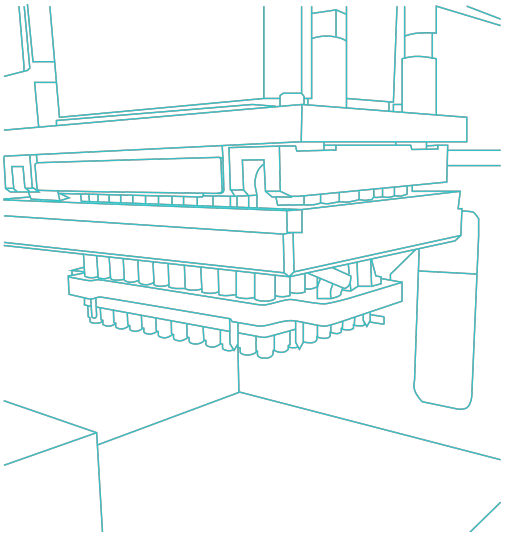
Compressed O-ring expansion head

Attached to the moving gantry, the compressed O-ring expansion (CO-RE) head enables simultaneous 96-well liquid handling throughout the method.

WARNING

Do not touch the CO-RE head during operation.

Figure 11. Compressed O-ring expansion head

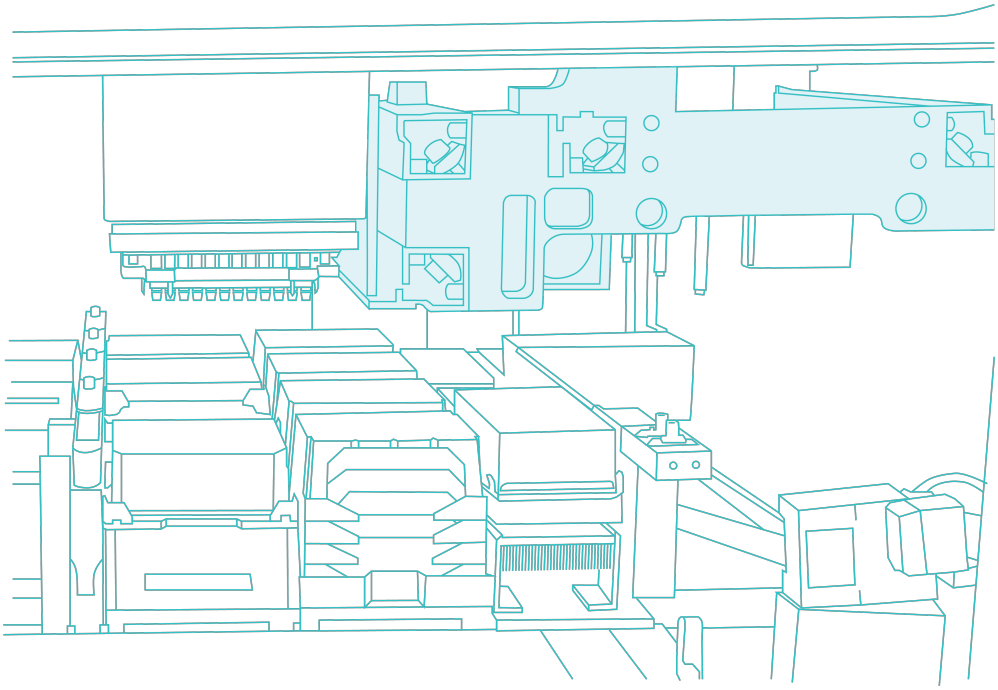


Gantry

During the method, the gantry moves the CO-RE head and independent 8-channel pipette heads.

WARNING
Do not touch the gantry during operation.

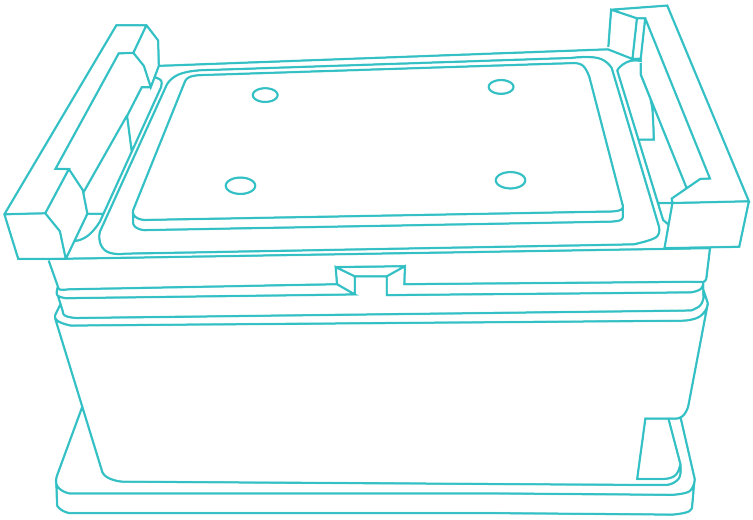
Figure 12. Gantry



Heater shaker

The heater shaker incubates and continuously mixes the Sample Prep Plate. During the Proteograph Assay method, the heater automatically heats to the appropriate temperature and reaches 95 °C for sample denaturing.

Figure 13. Heater shaker



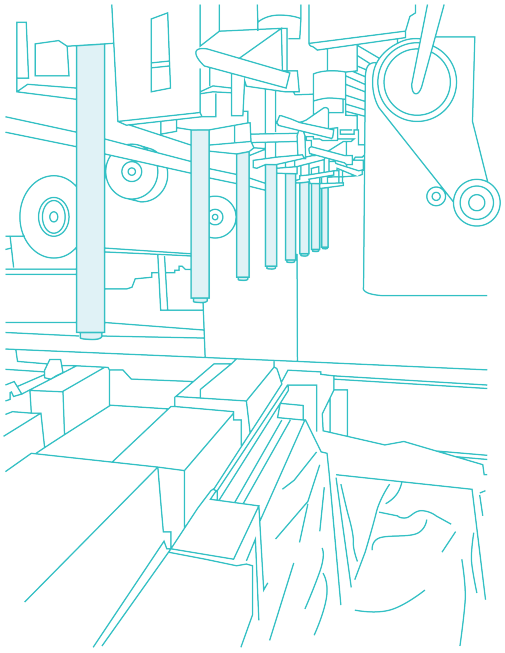
Independent 8-channel CO-RE pipette heads

Attached to the gantry, the independent 8-channel CO-RE pipette heads provide eight separate channels for liquid handling.

WARNING

Do not touch the heads during instrument operation.

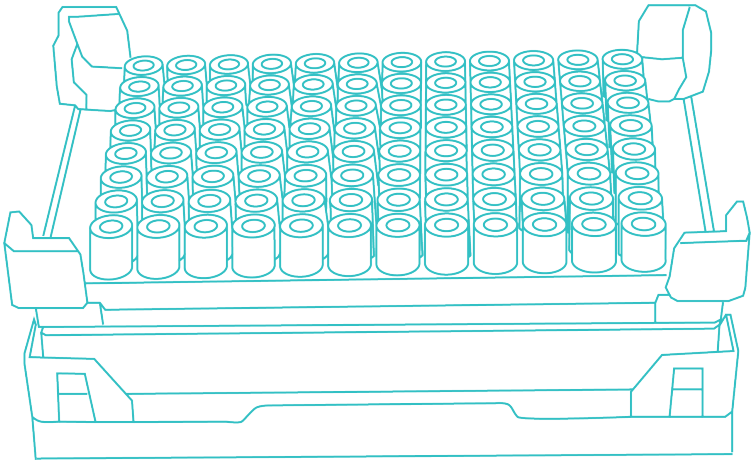
Figure 14. Independent 8-channel CO-RE pipette heads



Magnetic plate

The magnetic plate is a solid-core ring magnet used to pull down nanoparticles during corona washing and peptide transfer. Integrated spring-cushion technology optimizes performance. The magnetic plate always remains inside the instrument.

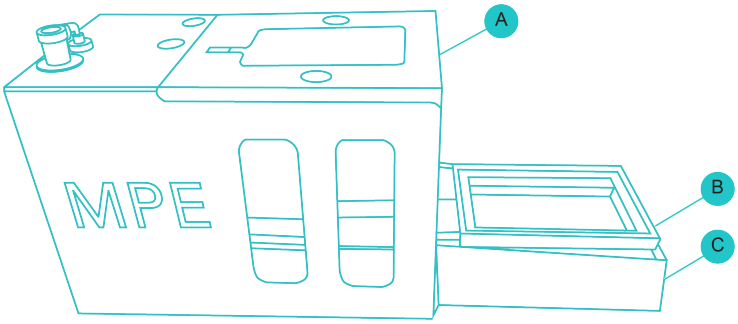
Figure 15. Magnetic plate



MPE module and MPE power unit

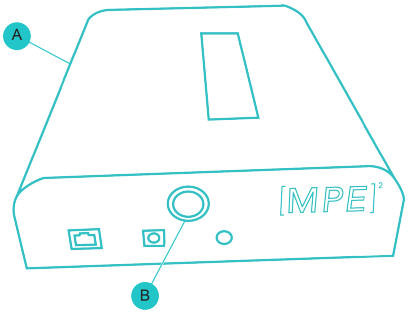
The MPE module uses positive pressure to collect and purify peptides from the Peptide Cleanup Plate. During wash steps, the MPE module sends liquid waste to an external container. The MPE power unit (an instrument peripheral) controls power to the MPE module.

Figure 16. MPE module



A MPE module
B Filter tray
C Waste tray

Figure 17. MPE power unit



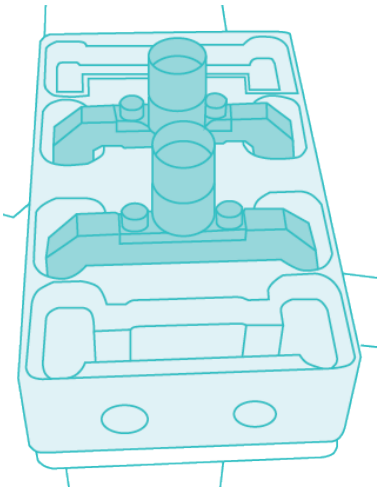
A MPE power unit
B MPE power button (which glows green when on)

Plate grippers

Channels 7 and 8 of the independent 8-channel pipette heads use the plate grippers.

CAUTION
Do not move the plate grippers from positions 2 and 3 of the plate gripper tray.

Figure 18. Plate grippers

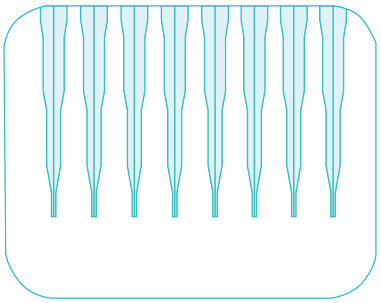


Teaching needles

A set of eight teaching needles are used to check pressure tightness of the pipetting channels during maintenance.

CAUTION
Avoid removing the teaching needles from the work deck. Without the teaching needles, the Daily Maintenance and Weekly Maintenance methods fail.

Figure 19. Teaching needles



Waste containers

The following containers collect waste from the SP100. Clear bags in the pipette and core waste bins direct waste to a separate biohazard container. Each biohazard container is lined with a red, labeled biohazard bag.

- **Independent 8-channel pipette head waste** — Waste bin for the 8-channel conductive 300 µL tips, lids, and some empty NCT racks.
- **CO-RE head waste** — Waste bin for 300 µL NCTs from the CO-RE head.
- **MPE waste** — Liquid waste container for all liquid waste, the majority being the MPE waste from wash steps (wash buffers and plasma).

Chapter 3

Materials

This chapter describes the contents and storage requirements of the Seer kits needed for peptide preparation on the SP100 Automation Instrument, as well as additional required third-party equipment and materials.

Kit contents and storage requirements

The Proteograph Assay method requires a Seer Proteograph Assay Kit, while the Peptide Quantification and Peptide Reconstitution methods require a Seer Proteograph PQR Labware Kit. The following table summarizes the attributes of these kits.

For information about additional third-party equipment and materials needed for peptide preparation on the SP100, see Required equipment (page 29) and Additional required materials (page 30).

Table 3. *Seer Proteograph kits*

KIT	PART NUMBER	NUMBER OF BOXES	CONTAINS	NOTES
Proteograph Assay Kit – 16 Samples	S55R1100	1	All consumables for one automated assay run (16 samples) on the SP100.	
Proteograph Assay Kit – 64 Samples	S55R1117	4	All consumables for four automated assay runs (64 samples) on the SP100.	
Proteograph PQR Labware Kit	S55R1108	1	Consumables for four quantification runs and four reconstitution runs on the SP100.	a

[a]In addition to the consumables included in the Proteograph PQR Labware Kit, peptide reconstitution runs require a user-prepared Peptide Reconstitution Buffer. See Peptide Reconstitution Buffer preparation (page 61).

Proteograph Assay Kit

The Proteograph Assay method requires a Seer Proteograph Assay Kit. Each box of 16 kit samples in the Proteograph Assay Kit contains two components: a refrigerated box of reagents, and a room-temperature box of labware.

Refrigerated box (reagents)

The refrigerated box includes two separately packaged pouches: Proteograph XT Control Panel and the Proteograph Nanoparticle Panel.

When you receive a Proteograph Assay Kit, ***promptly store*** the contents at 4 °C.

Table 4. *Contents of the Seer Proteograph Assay Kit refrigerated box*

DESCRIPTION	QUANTITY	CAP COLOR / LABEL	VOLUME	NOTES
Alkylation Solution	1	Green	7 mL	
Control Dilution Solution	1	White	10 mL	
Denaturing Solution	1	Denature	25 mL	
Digestion Stop Solution	1	Red	7 mL	
Elution Solution	1	Elution	20 mL	
Enzyme Reconstitution Solution	1	White	7 mL	
MPE Control	1	Clear with black ring	n/a	a
MS Control	1	Clear with black ring	n/a	a

DESCRIPTION	QUANTITY	CAP COLOR / LABEL	VOLUME	NOTES
Nanoparticle #1 (NP #1)	1	Clear with black ring	n/a	b
Nanoparticle #2 (NP #2)	1	Clear with black ring	n/a	b
Nanoparticle #3 (NP #3)	1	Clear with black ring	n/a	b
Nanoparticle #4 (NP #4)	1	Clear with black ring	n/a	b
Nanoparticle #5 (NP #5)	1	Clear with black ring	n/a	b
Peptide Wash Solution A	1	Wash A	40 mL	
Peptide Wash Solution B	2	Wash B	27 mL	
Plasma Control	1	Clear with black ring	n/a	a
Reduction Solution	1	Blue	7 mL	
Trypsin/LysC Protease MS Grade	6	Gray	n/a	
Wash Solution C	2	White	100 mL	

[a]Packaged in the Proteograph XT Control Panel pouch.
[b]Packaged in the Proteograph Nanoparticle Panel pouch.

Room-temperature box (labware)

Table 5. Contents of the Seer Proteograph Assay Kit room-temperature box

DESCRIPTION	QUANTITY
Alkylation Solution Reservoir	1
Black Plate Lid	3
Cleanup Reagents 4-Well Reservoir	1
Clear Plate Lid	1
Control Dilution Solution Reservoir	1
Deionized Water Reservoir	1
Denaturing Solution Reservoir	1
Intermediate Plate	6
Peptide Cleanup Plate (with plate holder)	1
Peptide Collection Plate	1
Reduction Solution Reservoir	1
Sample Prep Plate	1

DESCRIPTION	QUANTITY
Sample Tubes 01–16	16
Trypsin/LysC 8-Well Reservoir	1
Wash C Solution Single Reservoir	1

Proteograph PQR Labware Kit

The Seer Proteograph PQR Labware Kit (PN S55R1108) provides labware and reagents needed for four Peptide Quantification runs and four Peptide Reconstitution runs on the SP100.

NOTE

When you receive this kit, immediately store the Peptide Elution Solution at 4 °C. All other contents should be stored at room temperature.

Table 6. Contents of the Seer Proteograph PQR Labware Kit

DESCRIPTION	QUANTITY	NOTES
Black Plate Lid	4	a
Cleanup Reagents 4-well Reservoir	4	a
Empty 2 mL Tube	32	a
Intermediate Plate	8	a
Microplate, 96 well, Black	4	a
Peptide Assay Buffer Reservoir	4	a
Peptide Assay Reagent Tube	32	a
Peptide Digest Assay Standard Tube	4	a
Peptide Elution Solution (20 mL)	2	a, b
Reconstitution Buffer Reservoir	4	c

[a]For the Peptide Quantification method.
[b]Must be stored at 4 °C.
[c]For the Peptide Reconstitution method.

Required equipment

Peptide preparation, quantification, and reconstitution on the SP100 require the following third-party equipment. For additional information about these equipment, refer to the *Site Preparation Guide* (CF-1017 or CF-1015 International).

Table 7. Required equipment

DESCRIPTION	SUPPLIER	NOTES
Acid-Resistant CentriVap Centrifugal Vacuum Concentrator	Labconco, catalog # 7810016	a
Fluorescence intensity microplate reader	Molecular Devices, SpectraMax, M2[e]	a
Refrigerated Microcentrifuge	Thermo Fisher Scientific, catalog # 75002441	a

[a]Or equivalent.

Additional required materials

Peptide preparation, quantification, and reconstitution on the SP100, as well as instrument maintenance, require the third-party consumables and other materials listed in the following table.

NOTE

The Pierce Quantitative Fluorometric Peptide Assay, required for peptide quantification, should be stored at 4 °C.

NOTE

Suggested suppliers for these materials are listed below. Where indicated, you may use alternate suppliers whose materials meet the stated requirements. Confer with your Seer field service representative about any substitutions.

Table 8. Additional required materials

DESCRIPTION	SUPPLIER	REQUIREMENTS	NOTES
1–10 mL pipette with tips	Rainin, material # 17011783		a
20–200 µL multichannel pipette with tips	Rainin, material # 17013810		a
20–200 µL pipette with tips	Rainin, material # 17014391		a
70% isopropyl alcohol or 70% ethanol	General lab supplier		
100–1000 µL pipette with tips	Rainin, material # 17014382		a
300 µL Nested Conductive Tips (NCTs)	Hamilton, part # 235950		
Aluminum Sealing Foil 5 × 3 Inch	VWR, catalog # 60941-126	Must be able to withstand freezing at -80 °C.	a
Axygen AxyMats 96 Round Well Sealing Mat for PCR Microplates	VWR, product # AM-96-PCR-RD		
Deionized water	General lab supplier; see Deionized water quality requirements (page 32)		
Disposable latex gloves	General lab supplier		
Eppendorf twin.tec PCR Plates 96 LoBind, semi-skirted, 250 µL, PCR clean, colorless	Thermo Fisher Scientific,0030129504		b
Kimwipes or similar lint-free tissues	General lab supplier		
Lab coats	General lab supplier		
Peptide Reconstitution Buffer	Laboratory prepared		c

DESCRIPTION	SUPPLIER	REQUIREMENTS	NOTES
Pierce Quantitative Fluorometric Peptide Assay	Thermo Fisher Scientific, catalog # 23290		d
Protective goggles	General lab supplier		
Waste Bags with Biohazard Labeling	Hamilton, part # 199203	Dimensions suitable for placement inside the Waste Container Biohazard Box.	a
Waste Container Biohazard Box	General lab supplier	Required dimensions: • W: 24" (61 cm) • L: 24" (61 cm) • H: Maximum 20" (51 cm)	a

[a]Or equivalent.
[b]Or equivalent proteomics-compatible 96-well plate.
[c]Or equivalent laboratory-prepared reconstitution buffer. For information about preparing the reconstitution buffer, see Peptide Reconstitution Buffer preparation (page 61).
[d]Must be stored at 4 °C.

Deionized water quality requirements

In its own testing laboratory, Seer uses a PURELAB Chorus 1 Complete water purification system to produce deionized water for the assay. Seer recommends that the water purification system you use to produce deionized water meets or exceeds the following specifications.

PRODUCT SPECIFICATIONS	PURELAB CHORUS 1 COMPLETE 10L/HR	NOTES
Dispense Flowrate	>1.5 L/min	
Inorganics (resistivity at 25 °C)	18.2 MΩ.cm	
Organics (TOC)	<5 ppb	
Bacteria	<0.001 CFU/mL	a
Bacterial Endotoxin	<0.001 EU/mL	a
pH	Effectively neutral	
Particles	0.2 µm	a
DNase	< 5 pg/mL	
RNase	< 1 pg/mL	
Daily Usage (max)	100 L/day	
Daily Usage (min)	1 L/day	
Delivery Flow Rate	10 L/hr	

[a]With point-of-use filter fitted.

Trap column recommendation

Seer recommends the use of a trap column coupled to the LC-MS system used for analysis. A trap column removes contaminants and unwanted analytes that could interfere with the analysis of analytes of interest. For assistance with selecting a suitable trap column for your organization's use, contact the manufacturer of your liquid chromatography (LC) system.

Chapter 4

Proteograph Assay

This chapter provides instructions on how to prepare and load the Proteograph Assay labware and reagents on the Seer SP100 Automation Instrument.

Proteograph Assay method

The Proteograph Assay method on the SP100 Automation Instrument prepares peptides for analysis. To run the method, prepare the instrument and materials and follow the ICS prompts to set up the work deck. After the method starts, the instrument automatically performs the steps to convert proteins into peptides. Subsequent quantification and reconstitution ensure the appropriate volume and concentration for downstream LC-MS.

Before proceeding, complete the following prerequisites:

- Review safety and regulatory information to ensure safe and correct instrument operation. See Safety and Compliance (page 85).
- Confirm you have all Proteograph Assay Kit items and other materials. See Additional required materials (page 30).
- Review best practices to help load the work deck correctly and efficiently. See the information in Best practices (page 37).

Required materials

The Proteograph Assay method is designed to work with the Seer Proteograph Assay Kit (page 26).

Additional required equipment and materials are listed below. (For supplier information, see Required equipment (page 29) and Additional required materials (page 30).)

Required equipment

- Refrigerated Microcentrifuge

Additional required materials

- 300 µL Nested Conductive Tips (NCTs)
- Deionized water
- Personal protective equipment:
  - Disposable latex gloves
  - Lab coats
  - Protective goggles
- Pipettes with tips:
  - 1–10 mL pipette with tips
  - 20–200 µL pipette with tips
  - 100–1000 µL pipette with tips

Number of samples and controls

The Proteograph Assay method requires 16 plasma samples. Each sample incubates separately with each of the five nanoparticles, resulting in 80 wells of peptides in a 96-well plate.

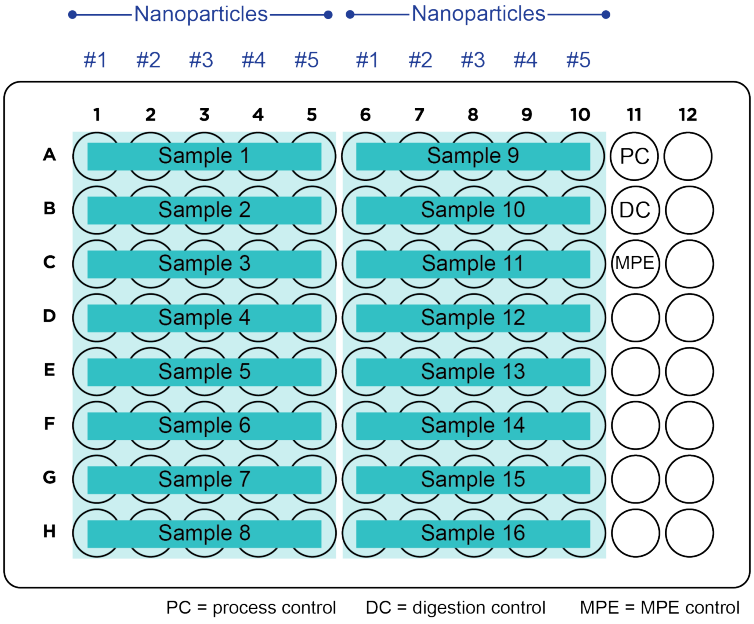
To ensure consistent quality and help with troubleshooting, three additional wells are dedicated to the following controls:

- **Process Control** — A mixture of pooled plasma samples that is subsequently mixed with Nanoparticle #1. It is exposed to every step, including corona formation, trypsin digestion, and peptide cleanup.
- **Digestion Control** — A mixture of the plasma control and the control dilution solution. It is added to the plate after corona formation and exposed to the trypsin digestion and peptide cleanup steps.
- **MPE Control** — A purified mixture of commercially available pre-digested peptides. It is added to the plate after tryspin digestion and exposed to the peptide cleanup step.

Peptide Collection Plate

The output of the Proteograph Assay method is the Peptide Collection Plate. Samples and nanoparticles occupy all wells in columns 1–10 and controls occupy wells A11, B11, and C11. For example, Sample 1 occupies well A1 with NP #1, well A2 with NP #2, and so on.

Figure 20. Layout of the final Peptide Collection Plate



Best practices

When setting up the work deck, apply the following best practices to ensure the proper techniques for pipetting liquids, placing labware and reagents, and placing lids. Improper work deck setup can cause errors. (For information on resolving setup errors, see Troubleshooting (page 79).)

Pipetting

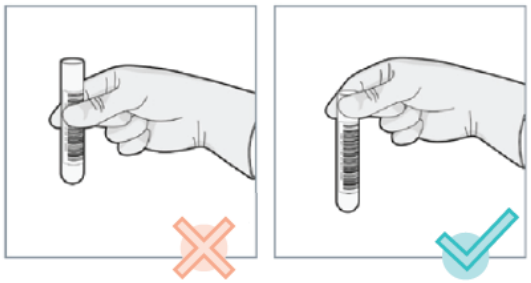
When pipetting samples to Proteograph sample tubes, observe the following best practices to avoid the formation of bubbles.

- After spinning down the tube, carefully aspirate 250 µL from it, avoiding lipid layers and residuals at the bottom of the tube.
- Slowly dispense plasma at the bottom of the sample tube with the tip touching the side of the tube.
- While dispensing, avoid using the second stop of the pipette so as not to introduce bubbles.
- If a bubble is present after dispensing, use a dry pipette tip to pop the bubble. For small bubbles, use a small pipette tip, e.g., a p200 or p20 tip.
- If a bubble will not burst, carefully aspirate just on top of the bubble to remove it.

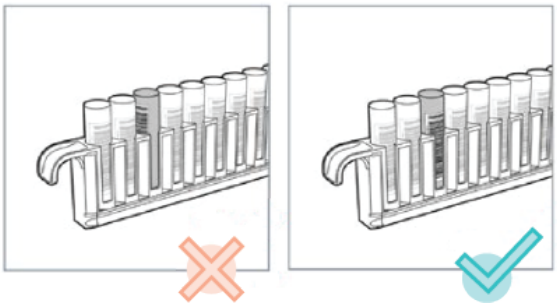
Loading labware

When loading labware onto the work deck, observe the following best practices for holding tubes, placing labware, loading carriers, barcode orientation, tube placement, and labware orientation.

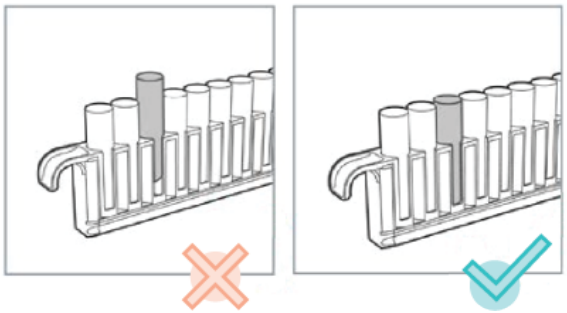
- **Tubes** — Hold tubes at the top to avoid warming samples in your hand. Avoid placing your fingers over barcodes.



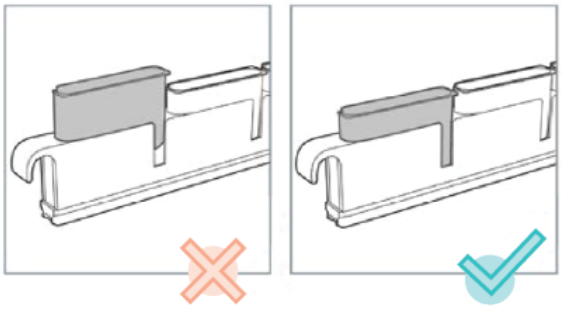
Face all tube barcodes to the right so the barcode reader can scan them. Incorrect orientation makes the barcodes unreadable.



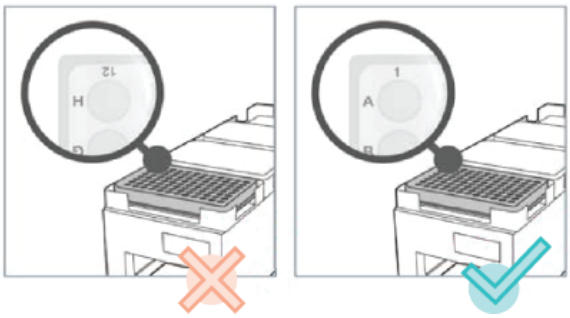
Fully insert tubes into the tube carrier. Improper seating can cause malfunction.



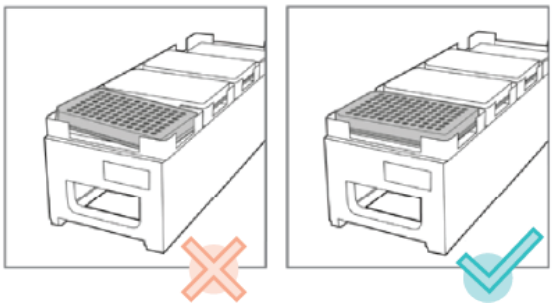
- **Reservoirs** — Fully insert reservoirs into the Reservoir Carrier R.



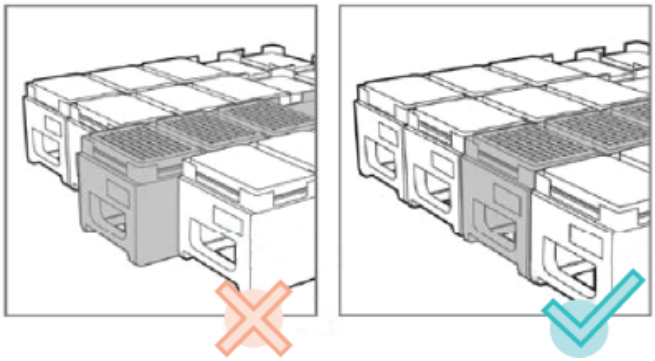
- **Wells** — Position wells on plate carriers so that well A1 is in the upper-left corner.



- **Plates** — Fully seat plates on the plate carriers. Misaligned plates cause collision or malfunction.



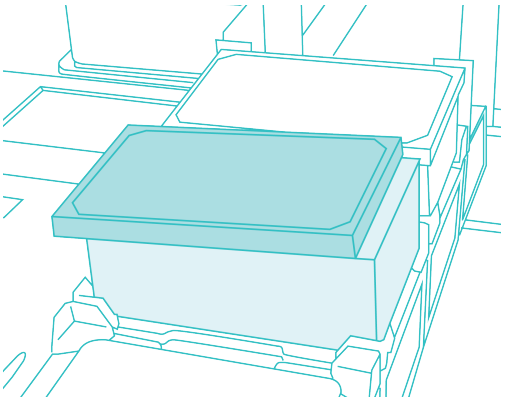
- **Carriers** — Push all carriers to the stop hooks at the back of the autoload tray. Incorrect positioning causes collision or malfunction. Do not pull carriers out during method runs.



Placing lids

Properly place lids on top of plates. The plate grippers cannot pick up a plate with a misaligned lid.

Figure 21. Misaligned lid



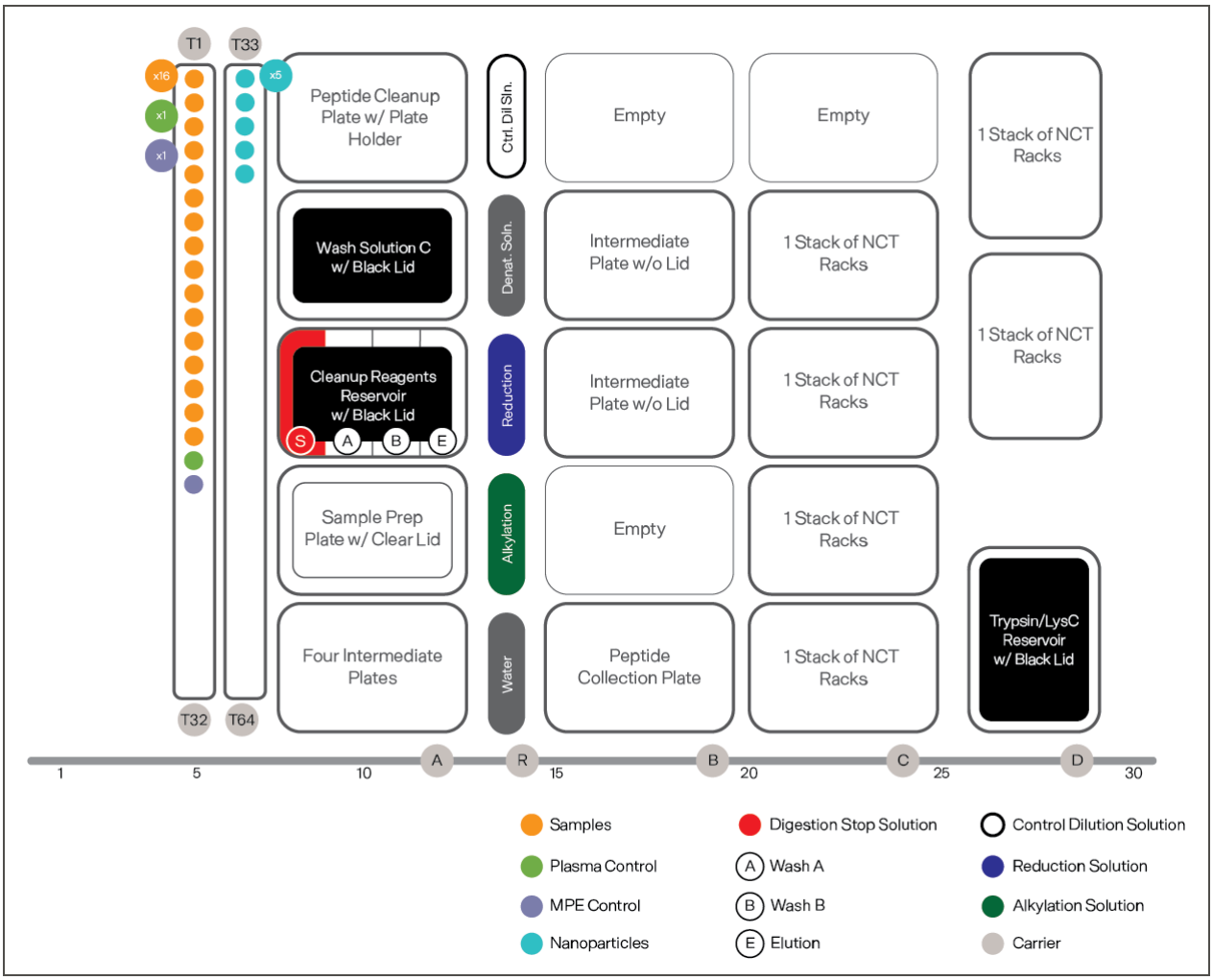
Work deck quick reference

The following figure shows the layout of the work deck after it is loaded with labware and reagents for the Proteograph Assay method.

If you are:

- A new user, refer to the figure to confirm proper work deck layout before starting the method.
- An experienced user, use the figure as a quick reference when loading the work deck.

Figure 22. Work deck loaded for the Proteograph Assay method



Turn on the instrument

Turning on the SP100 ensures that the instrument and peripherals are communicating and ready to start the method. When not in use, the instrument and peripherals should be turned off.

1. Turn on the following hardware:
   - **SP100** — Press the green switch on the front of the instrument.
   - **MPE power unit** — Press the power button on the front of the MPE power unit.
   - **Chiller power unit** — Press the power switch on the back panel of the chiller power unit.
   - **Instrument computer** — Press the power button on the front of the instrument computer.
2. Check the gas supply for appropriate pressure (≥110 psi).
3. Confirm that the MPE regulator (located near the MPE waste container) is set between 105–110 psi.
4. After the computer has initialized, select the **Proteograph ICS** desktop shortcut to launch ICS.

5. Open the front protective cover and pull the carriers onto the autoload tray.
6. Push the carriers to the stop hooks at the back of the autoload tray.

CAUTION
Failure to push the carriers to the stop hooks completely can result in an autoload failure.
7. Close the front protective cover.
8. Run the Daily Maintenance method. For instructions, see Run the Daily Maintenance method (page 72).

   When the Daily Maintenance method is complete, ICS returns to the main menu.

Prepare the assay materials

1. Set the centrifuge to 4 °C.
2. Verify that the chiller unit is turned on and is set to 4–5 °C.
3. If plasma samples are frozen, remove them from the freezer and thaw in an ice-water bath.
4. Remove labware from room-temperature storage and arrange as follows.
   - Place the Black Plate Lid and Trypsin/LysC 8-Well Reservoir on ice.
   - Check all labware for appropriate barcode labels.

NOTE
Intermediate Plates and the Peptide Cleanup Plate do not have a barcode label.

5. Remove the refrigerated box from 4 °C storage and arrange contents as follows.
   - Place the Enzyme Reconstitution Solution on ice.
   - Place the six tubes of Trypsin/LysC Protease MS Grade on ice.
   - Set aside the remaining contents at room temperature.

Set up the Proteograph Assay method

1. On the ICS main menu, select **Proteograph Assay**.

2. At the **Sign In** stage:
    a. Enter your username (up to 20 alphanumeric characters, including commas, dashes, periods, spaces, and underscores).
    b. Select **Continue**.
3. At the **Experiment Registration** stage:
    a. In the **Experiment Name** field, enter a name for your experiment.
    b. In the **Plate Name** field, enter a unique name for the run.
    c. Select **Continue**.

    Each name can contain up to 20 alphanumeric characters and underscores.

    TIP

    If an experiment requires multiple plates, use the same experiment name for each plate to group the plates for analysis.

4. At the **Device Initialization** stage:
    a. Open the front protective cover.
    b. Pull the carriers onto the autoload tray.
    c. Push the carriers to the stop hooks at the back of the autoload tray.

    CAUTION

    Failure to push the carriers to the stop hooks completely can result in an autoload failure.

    d. Close the front protective cover.
    e. Confirm that the pressure regulator reads between 105–110 psi.
    f. Select **Continue**.
5. At the **MPE Initialization** stage:
    a. Verify there is no plate in the MPE module's filter tray.
    b. Select **Initialize**.
6. Open the front protective cover.

7. At the **Tip Counter** stage:
    a. Confirm that each of positions D1 and D2 has at least two full racks of 300 µL Nested Conductive Tips (NCTs) (four full racks, minimally).
        - Under **NTR Details**, confirm that the **Stack Height** shown matches the number of racks in each stack.
        - Under **Top Stack Details**, confirm that the arrangement of 300 µL NCTs in the top rack of each stack is correct.
        - Under **NTR Details**, confirm that the **Total Tips Remaining** shown matches the total number of 300 µL NCTs.

        NOTE

        If ICS shows an incorrect arrangement or insufficient number of 300 µL NCTs needed for the method, perform a refill operation before continuing. See Refill the 300 µL NCTs (below).

    b. Having confirmed the 300 µL NCTs (and refilled, if necessary), select **Continue**. Continue to Set up the work deck (next page).

Refill the 300 µL NCTs

During the method's **Tip Counter** stage, ICS may show an incorrect arrangement or insufficient number of 300 µL Nested Conductive Tips (NCTs). When this happens, you will need to perform a refill operation.

NOTE

At any time, you can discontinue the refill operation by selecting **Reset** at the top of the **Tip Counter** screen. That will return the **NTR Details** and **Top Stack Details** to their original configuration.

1. Replace each of the stacks in positions D1 and D2 with a stack of four full racks of 300 µL NCTs (eight racks in all).
2. Select the **Refill All Eight (8) Nested Tip Racks (NTR)** checkbox.
3. Select **Refill** at the top of the **Tip Counter** screen.

    When the refill operation is finished, ICS updates the **NTR Details**.
4. Return to the **Tip Counter** stage step in Set up the Proteograph Assay method (previous page).

Set up the work deck

A series of Deck Setup stages guide you through setting up the work deck.

CAUTION

For all pipetting steps, make sure all liquid is in the bottom of the tube. Do not leave droplets on the sides of the tube or in caps.

Load reagents and plates

At the **Deck Setup 1 of 3** stage:

1. Place the Peptide Cleanup Plate (including the microplate holder underneath it) into position A1.
2. Place the Wash C Solution Single Reservoir into position A2.
3. Add two bottles of Wash Solution C to the Wash C Solution Single Reservoir. Cover with a Black Plate Lid.
4. Load the Cleanup Reagents 4-Well Reservoir.

   CAUTION

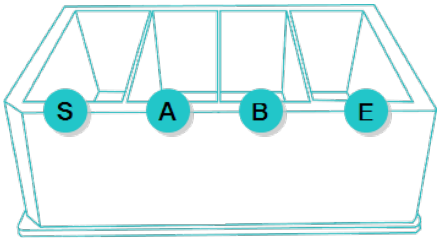
   Ensure correct orientation of the reservoir ***before adding reagents*** to the reservoir. The barcode should be facing to the right (toward the barcode reader).

   a. Place the reservoir into position A3.

   b. Pouring reagents from the ***front*** of the reservoir, add reagents to each well as follows.

   TIP

   Pouring reagents from the front of the reservoir prevents cross-contamination between wells.

   - **Well 1** — Digestion Stop Solution (1 bottle)
   - **Well 2** — Peptide Wash Solution A (1 bottle)
   - **Well 3** — Peptide Wash Solution B (2 bottles)
   - **Well 4** — Elution Solution (1 bottle)

   c. Cover the reservoir with a Black Plate Lid.

5. Place the Sample Prep Plate into position A4. Cover with a Clear Plate Lid.

6. Load the Intermediate Plates:
    a. Stack four plates in position A5.
    b. Place one plate into position B2.
    c. Place one plate into position B3.
7. Place one Peptide Collection Plate into position B5.
8. Place a stack of 300 µL NCTs into each of positions C2–C5.
9. Perform the following steps on ice:
    a. Add 500 µL Enzyme Reconstitution Solution to one tube of Trypsin/LysC Protease MS Grade for a final concentration of 0.2 ug/µL.
    b. Pipette 2–3 times to mix.
    c. Repeat steps a and b for the remaining five Trypsin/LysC tubes.
    d. Transfer 500 µL from each Trypsin/LysC tube into Well A of the Trypsin/LysC 8-Well Reservoir.

    CAUTION

    Ensure correct orientation of the reservoir before transferring solution into the reservoir. The barcode should be facing to the right (toward the barcode reader). Well A is the leftmost well, furthest from the barcode.

    e. Cover the reservoir with a Black Plate Lid.
10. Verify that the chiller unit is cold. If it is not, verify that it is turned on and is set to 4–5 °C. Wait until that temperature is achieved.

    TIP

    If the chiller unit is set to but is not achieving the set temperature, contact your field service representative or Seer support. See Technical Support (page 100) for contact information.
11. Place the Trypsin/LysC 8-Well Reservoir (with black microplate lid) into the 5 °C chiller unit. Face the barcode to the right.
12. Select the checkbox for each item.
13. Select **Continue**.

Load solutions and reservoirs

At the **Deck Setup 2 of 3** stage:

1. Place each of the following reservoirs into its labeled position, fill it with its corresponding reagent, and cover it with a black CO-RE lid.

   NOTE

   For each of these reservoirs, you may pour the reagent into either half of the reservoir, as the two halves are connected at the base.

RESERVOIR	POSITION	REAGENT
Control Dilution Solution Reservoir	R1	Control Dilution Solution
Denaturing Solution Reservoir	R2	Denaturing Solution
Reduction Solution Reservoir	R3	Reduction Solution
Alkylation Solution Reservoir	R4	Alkylation Solution

2. Place an empty Deionized Water Reservoir into position R5 and fill with 15 mL of deionized water. (For guidance on water quality, see Deionized water quality requirements (page 32).) There is no black CO-RE lid for R5.

   The Deionized Water Reservoir holds the black CO-RE lids during instrument operation.

3. Select the checkbox for each item.
4. Select **Continue**.

Load samples, controls, and nanoparticles

At the **Deck Setup 3 of 3** stage:

1. Prepare the Sample Tubes as follows:
   a. Ensure that you have planned an order for your samples, recording the correspondence between Seer Sample Tube numbers (01-16, listed on the barcode labels) and plasma sample numbers or other sample identifiers.
   b. Confirm that the centrifuge has reached 4 °C, and then centrifuge the samples at 5,000 × g for 2 minutes.
   c. For each sample, transfer 250–270 µL plasma into the appropriate Seer Sample Tube.

      NOTE

      Observe the following best practices when pipetting sample plasma:

      - Minimize aspiration of residues that might settle at the bottom of the tubes.
      - Avoid aspirating any lipids that collect at the surface of the samples.
      - Minimize bubbles forming at the surface of the samples.

   d. Cap the Sample Tubes, and then centrifuge them again at 5,000 × g for 1 minute.
   e. Set aside the Sample Tubes on ice or at 4 °C.
2. Remove both tube carriers from the autoload tray and place on a workbench.

3. Load tubes into Tube Carrier 1 as follows:
    a. Uncap the Sample Tubes and load them into positions T1–T16 of Tube Carrier 1.

    WARNING

    Be careful to load Sample Tubes in precise numerical order (01, 02, ...). Failure to load tubes in the correct order will result in an error during barcode scanning.

    b. Load the Plasma Control tube into position T17 of Tube Carrier 1.
    c. Load the MPE Control tube into position T18 of Tube Carrier 1.
    d. Confirm that all barcodes in Tube Carrier 1 are visible and facing to the right, and then return the carrier to the autoload tray.
4. Load nanoparticle tubes into Tube Carrier 2 as follows:
    a. Tap NP #1 on the custom table to ensure that the lyophilized beads are settled and none are stuck on the cap.
    b. Uncap NP #1 and load it into position T33 of Tube Carrier 2.
    c. Repeat the steps above to load the remaining tubes into positions T34–T37.
    d. Confirm that all barcodes in Tube Carrier 2 are visible and facing to the right, and then return the carrier to the autoload tray.
5. Select the checkbox for each item.
6. Select **Continue**.

Check labware

1. At the **Labware Check** stage:
    a. Confirm proper loading of the carriers. For a schematic diagram of the loaded work deck, see Work deck quick reference (page 40).
    b. For each of the covered reservoirs, remove its lid, confirm it contains liquid, and replace the lid.
    c. Select the checkbox for each item.

    TIP

    You must select every checkbox before continuing.

    d. Select **Continue**.

2. At the **Sample Name** stage:
    a. Enter the name or identification number of each sample. You may enter sample names manually, or populate all Sample Name fields automatically as follows:
        - To populate all Sample Name fields with values from a .csv file, select **Load Sample Names**.

        NOTE
        The first column in the file should list the Sample Tube numbers, while the second column lists user-defined sample names. For example: `1S01, <SampleName1> … 1S16,<SampleName16>`.

        - To populate all Sample Name fields with default values, select **Default Sample Names**.

        NOTE
        Default values follow the format `<YYYYMMDD>_<S#>` with sample numbers in ascending order.

    WARNING
    If you accidentally transferred any plasma samples into the wrong Sample Tubes during deck setup, make sure to adjust your sample names accordingly during this step to avoid sample mis-identification.

    b. Select **Continue**.
3. Select **Continue**.

Scan barcodes and start the method

The barcode reader scans each barcode as a final check to confirm proper work deck setup. During the method, ICS displays each stage with time estimates. For information about time estimates, see Proteograph Assay steps (page 9).

WARNING
Do not attempt to open the front protective cover after the method starts. Doing so ***automatically aborts the method***. If you must open the front protective cover, pause the method.

1. At the **Begin Labware Barcoding** stage:
    a. Confirm proper placements:
        - All barcodes face the barcode reader.
        - All carriers are pushed to the stop hooks at the back of the autoload tray.
    b. Close the front protective cover.
    c. Select **Continue** to scan the barcodes.
2. At the **Setup Complete** stage:
    a. Close the front protective cover.
    b. Select **Run** to start the method.

    ICS locks the front protective cover.

Clean the instrument

1. When the method is complete, ensure that the autoload tray is free from objects and then choose one of the following options:
   - **Unload Carriers and Run MPE Flush** (recommended) — Unload the carriers and automatically start the MPE Flush method, which takes approximately 10 minutes.
   - **Unload Carriers Only** — Unload the carriers without automatically running the MPE Flush method. For instructions on manually starting the method, see Run the MPE Flush method (page 77).

   The carriers automatically move from the work deck to the autoload tray.
2. Immediately retrieve the Peptide Collection Plate, which contains the peptides.
3. Seal the plate with an adhesive mat, and then centrifuge at 2000 × g for 30 seconds.
4. Set aside the plate at room temperature. Start peptide quantification within ***1 hour***. For instructions, see Peptide Quantification method (page 52).
5. Remove labware from the carriers:
   a. Remove all plastic labware from the tube, plate, tip, and reservoir carriers.
   b. Remove the Peptide Cleanup Plate from the MPE module.
   c. Remove the black CO-RE lids and store for future use.
   d. Leave the magnetic plate on the instrument.
6. Dispose of all labware and leftover reagents in the appropriate waste containers per laboratory policy.
7. Proceed to Peptide Quantification method (page 52). Otherwise, if you will not be running the Peptide Quantification method immediately, continue to the next step.
8. Turn off the instrument and peripherals.

   NOTE

   Seer recommends shutting down the hardware when it is not in use.

   - **SP100** — Press the green switch on the front of the instrument.
   - **MPE power unit** — Press the power button on the front of the MPE power unit.
   - **Chiller power unit** — Press the power switch on the back panel of the chiller power unit.
   - **Instrument computer** — Press the power button on the front of the instrument computer.

Chapter 5

Peptide Quantification

This chapter provides instructions on how to prepare for and run the Peptide Quantification method on the Seer SP100 Automation Instrument.

Peptide Quantification method

After you run the Peptide Quantification method, Seer recommends that you quantify the concentration of the peptides in each well of the Peptide Collection Plate. Quantification includes running the Peptide Quantification method and drying the quantified peptides.

NOTE

Start quantification within ***one hour*** of removing the Peptide Collection Plate from the SP100 Automation Instrument. After quantification, you can store the plate for up to one year before reconstituting.

To run the Peptide Quantification method, you first prepare materials (see next) and then follow the ICS prompts to set up the work deck. Once the method starts, the instrument automatically performs the steps to convert proteins into peptides.

Required materials

The Peptide Quantification method is designed to work with the Seer Proteograph PQR Labware Kit (page 28) (PN S55R1108).

Additional required equipment and materials are listed below. (For supplier information, see Required equipment (page 29) and Additional required materials (page 30).)

Required equipment

- Acid-Resistant CentriVap Centrifugal Vacuum Concentrator
- Fluorescence intensity microplate reader

Additional required materials

- 300 µL Nested Conductive Tips (NCTs)
- Aluminum Sealing Foil 5 × 3 Inch
- Personal protective equipment:
  - Disposable latex gloves
  - Lab coats
  - Protective goggles
- Pierce Quantitative Fluorometric Peptide Assay
- Pipettes with tips:
  - 1–10 mL pipette with tips
  - 20–200 µL pipette with tips
  - 20–200 µL multichannel pipette with tips
  - 100–1000 µL pipette with tips

Turn on the instrument

If the SP100 is not already on, use the following instructions to initialize the system and ensure proper communication between the instrument and peripherals.

1. Turn on the following hardware:
   - **SP100** — Press the green switch on the front of the instrument.
   - **MPE power unit** — Press the power button on the front of the MPE power unit.
   - **Chiller power unit** — Press the power switch on the back panel of the chiller power unit.
   - **Instrument computer** — Press the power button on the front of the instrument computer.
2. Check the gas supply for appropriate pressure (≥110 psi).
3. Confirm that the MPE regulator (located near the MPE waste container) is set between 105–110 psi.
4. After the computer has initialized, select the **Proteograph ICS** desktop shortcut to launch ICS.

Prepare the quantification consumables

1. Remove the following reagents from 4 °C storage:
   - Elution Solution (1 bottle)
   - Pierce Quantitative Fluorometric Peptide Assay (sold separately)

   NOTE

   These materials must be removed from 4 °C storage 30 minutes before loading the work deck.

2. Confirm that the Pierce Quantitative Fluorometric Peptide Assay includes:
   - Fluorometric Peptide Assay Buffer
   - Fluorometric Peptide Assay Reagent (4 vials)
   - Peptide Digest Assay Standard (1 mg/mL)
3. Remove the following room-temperature consumables from the Proteograph PQR Labware Kit:

DESCRIPTION	QUANTITY
Black Plate Lid	1
Cleanup Reagents 4-Well Reservoir	1
Empty 2 mL Tube	8
Intermediate Plate	2
Microplate, 96 well, Black	1
Peptide Assay Buffer Reservoir	1
Peptide Assay Reagent Tube	8
Peptide Digest Assay Standard Tube	1

Run the Peptide Quantification method

1. On the ICS main menu, select **Peptide Quantification**.

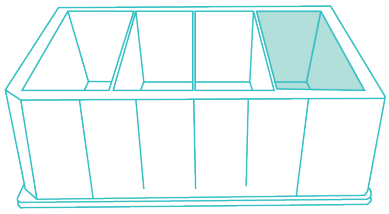
Onscreen prompts guide you through the remaining steps.

2. At the **Sign In** stage:
   a. Enter your username (up to 20 alphanumeric characters, including commas, dashes, periods, spaces, and underscores).
   b. Select **Continue**.
3. At the **Tip Counter** stage:
   a. Confirm that each of positions D1 and D2 has a stack with at least one full rack of 300 µL Nested Conductive Tips (NCTs) (two full racks, minimally).

> **NOTE**
> If ICS shows an incorrect arrangement or insufficient number of 300 µL NCTs needed for the method, perform a refill operation before continuing. See Refill the 300 µL NCTs (page 58)Refill the 300 µL NCTs (page 69).

   b. Having confirmed the 300 µL NCTs (and refilled, if necessary), select **Continue**.
4. At the **Deck Setup** stage, prepare reagents as follows and place labware into the indicated positions.

> **CAUTION**
> Ensure correct orientation of the Cleanup Reagents 4-Well Reservoir on the work deck before adding reagents to the reservoir. The barcode should be facing to the right (toward the barcode reader).

INSTRUCTION	CARRIER	POSITION
Add 100 µL Peptide Digest Assay Standard to the Peptide Digest Standard tube. Leave the tube uncapped.	Tube Carrier 1	T1
Uncap eight Empty Tubes. Leave the tubes empty.	Tube Carrier 1	T2–T9
Uncap eight Pep Assay Reagent Tubes and add 310 µL Fluorometric Peptide Assay Reagent to each tube. Leave the tubes uncapped.	Tube Carrier 1	T11–T18
Using a pipette, add 10 mL of Elution Solution into the 4th well. Do not pour the entire bottle. Return the remainder (if any) to the refrigerator. 	Plate Carrier A	A3
Place one Black Quantitation Plate into position A4.	Plate Carrier A	A4

INSTRUCTION	CARRIER	POSITION
Add 12 mL Fluorometric Peptide Assay Buffer to the Peptide Assay Buffer Reservoir.	Reservoir Carrier R	R4
Cover one Intermediate Plate with a Black Plate Lid.	Plate Carrier B	B3
Place one Intermediate Plate uncovered in position B4.	Plate Carrier B	B4
If necessary, unseal the Peptide Collection Plate.	Plate Carrier B	B5
Place one stack of NCT racks into position C2.	Tip Carrier C	C2

5. At the **Deck Setup Check** stage:
    a. Verify that all labware is properly loaded and all carriers are on the autoload tray.
    b. Select the checkbox for each item.

    TIP

    You must select every checkbox before continuing.

    c. Close the front protective cover.
    d. Select **Continue**.

    ICS moves the carriers from the autoload tray to the work deck.

    The method takes approximately 25–30 minutes to complete.

    WARNING

    Do not attempt to open the front protective cover after the method starts. Doing so ***automatically aborts the method***. If you must open the front protective cover, pause the method.

6. When the method is complete, do the following:
    a. Open the front protective cover.
    b. Immediately remove the Peptide Collection Plate from position B5 of Plate Carrier B.

    The plate contains the quantified peptides.

    c. Seal the Peptide Collection Plate with the Aluminum Sealing Foil 5 × 3 Inch.
    d. Remove the Black Quantitation Plate and read fluorescence on a microplate reader.

    NOTE

    The raw data should be added to the peptide quant data file (a Microsoft Excel (.xlsx) file, based on a template file you obtain from your Seer FAS) for use with the Peptide Reconstitution method. For detailed instructions on preparing that file, see Prepare the files needed for the Peptide Reconstitution method (page 64).

7. Dry the peptides:
    a. (Optional) Freeze the sealed Peptide Collection Plate at -80 °C for approximately 10 minutes. Then remove it from the freezer.

    > **TIP**
    > Seer recommends freezing the Peptide Collection Plate to prevent accidental spills while transferring the plate to a vacuum concentrator.

    b. Unseal the plate.
    c. Transfer the plate to a vacuum concentrator.
    d. Confirm the vacuum concentrator is balanced appropriately.
    e. Set the temperature to ≤ 20 °C.
    f. Run the vacuum concentrator until the peptides are fully dried, which can take several hours.

    > **TIP**
    > Run the vacuum concentrator overnight.

    g. Visually confirm the peptides are fully dried.
8. Clean the work deck and appropriately discard reagents and labware.
9. Proceed depending on when you are reconstituting the peptides:
    - If you are reconstituting immediately, proceed to Peptide Reconstitution (page 59).
    - If you will reconstitute later, seal the Peptide Collection Plate and store at -80 °C for up to one year.
10. Turn off the instrument and peripherals.

    > **NOTE**
    > Seer recommends shutting down the hardware when it is not in use.

    - **SP100** — Press the green switch on the front of the instrument.
    - **MPE power unit** — Press the power button on the front of the MPE power unit.
    - **Chiller power unit** — Press the power switch on the back panel of the chiller power unit.
    - **Instrument computer** — Press the power button on the front of the instrument computer.

Refill the 300 µL NCTs

During the method's **Tip Counter** stage, ICS may show an incorrect arrangement or insufficient number of 300 µL Nested Conductive Tips (NCTs). When this happens, you will need to perform a refill operation.

NOTE

At any time, you can discontinue the refill operation by selecting **Reset** at the top of the **Tip Counter** screen. That will return the **NTR Details** and **Top Stack Details** to their original configuration.

1. Replace each of the stacks in positions D1 and D2 with a stack of four full racks of 300 µL NCTs (eight racks in all).
2. Select the **Refill All Eight (8) Nested Tip Racks (NTR)** checkbox.
3. Select **Refill** at the top of the **Tip Counter** screen.

   When the refill operation is finished, ICS updates the **NTR Details**.
4. Return to the **Tip Counter** stage step in Run the Peptide Quantification method (page 55).

Chapter 6

Peptide Reconstitution

This chapter provides instructions on how to prepare for and run the Peptide Reconstitution method on the Seer SP100 Automation Instrument.

Peptide Reconstitution method

After the Peptide Quantification method, reconstitute the dried peptides to the concentration and volume needed for MS. Reconstitution includes running the Peptide Reconstitution method to produce MS-ready peptides.

To run the Peptide Reconstitution method, you will prepare materials (see next) and then follow the ICS prompts to set up the work deck. Once the method starts, the instrument automatically performs the steps to reconstitute the peptides.

Required materials

The Peptide Reconstitution method is designed to work with the Seer Proteograph PQR Labware Kit (page 28) (PN S55R1108).

Additional required materials are listed below. (For supplier information, see Additional required materials (page 30).)

- 300 µL Nested Conductive Tips (NCTs)
- Aluminum Sealing Foil 5 × 3 Inch
- Axygen AxyMats 96 Round Well Sealing Mat for PCR Microplates
- Peptide Reconstitution Buffer (see Peptide Reconstitution Buffer preparation (next page))
- Personal protective equipment:
  - Disposable latex gloves
  - Lab coats
  - Protective goggles
- Pipettes with tips:
  - 1–10 mL pipette with tips
  - 20–200 µL multichannel pipette with tips
  - 20–200 µL pipette with tips
  - 100–1000 µL pipette with tips

Peptide Reconstitution Buffer preparation

Peptides should be reconstituted with a buffer compatible with the LC-MS system that will be used for analysis.

The following information offers Seer's recommended preparation, which yields 100 mL of reconstitution buffer. Alternatively, you can use any "recipe" that produces an equivalent buffer.

Equipment

PRODUCT NAME / DESCRIPTION	SUPPLIER / MANUFACTURER	CATALOG / PART NUMBER
20-200 µL pipette	Rainin or equivalent	17014392 or equivalent
100-1000 µL pipette	Rainin or equivalent	17014382 or equivalent
Analog vortex mixer	VWR	444-2791 or equivalent
Microcentrifuge	Thermo Fisher Scientific	75002451 or equivalent

Reagents and materials

PRODUCT NAME / DESCRIPTION	SUPPLIER	ORDER NUMBER	STORAGE TEMPERATURE
100-1000 µL pipette tips	Rainin or equivalent	GPS-L1000 or equivalent	Room temperature
20-200 µL pipette tips	Rainin or equivalent	GPS-L250 or equivalent	Room temperature
150 mL Polypropylene Storage Bottles, Sterile	Corning	430281 or equivalent	Room temperature
Acetonitrile (ACN), Mass Spec-grade organic solvent	JT Baker / Thermo Fisher Scientific	9829-03 / 02-002-174 or equivalent	Room temperature
Eppendorf Tubes 5.0 mL with snap cap	Eppendorf	0030119401 or equivalent	Room temperature
Formic acid (FA) 98% - 100%	EMD Millipore	1.00264.0100	Room temperature
MS Synthetic Peptide Calibration Kit PepCalMix*	SCIEX	5045759	-20 °C
Water, HPLC-grade	Fisher Chemical or equivalent	W5-4 or equivalent	Room temperature

*A commercially available product containing 20 peptides in solution with known mass-to-charge ratios.

Preparation steps

CAUTION

Follow all safety, labeling, recordkeeping, and other trained laboratory practices when performing the following procedure. Wear appropriate personal protective equipment (PPE) when operating equipment and handling reagents.

1. Determine the amount of each reagent needed to prepare 100 mL of Peptide Reconstitution Buffer.

WATER	ACN	FA	PEPCALMIX	TOTAL
96.5 mL	3 mL	100 µL	10 vials	100 mL

2. Prepare the ***PepCal Diluent*** as follows:
    a. Obtain a 150 mL or appropriate size storage bottle and label with the following information:
        - 3% ACN, 0.1% FA, spiked with 5 pmol/mL PepCalMix
        - Preparation date
        - Operator name / initials
    b. Add 96.5 mL of HPLC-grade water, 3 mL of ACN and 100 µL of FA to the bottle.
    c. Vortex the bottle for 30 seconds to mix.
3. Prepare the ***PepCalMix*** as follows:
    a. Remove the 10 PepCalMix vials from -20 °C storage. Thaw the vials at room temperature.
    b. Add 1 mL of PepCal Diluent in each PepCalMix vial to dilute the peptides.
    c. Vortex each PepCalMix vial for 10 to 15 seconds.
    d. Place the PepCalMix vials in the microcentrifuge and spin down at 16,000 x g for 1 minute.
    e. Remove the PepCalMix vials from the microcentrifuge and spike their entire contents into the bottle of PepCal Diluent.
    f. Vortex the bottle for 30 seconds to mix.
4. Prepare the ***PepCalMix aliquots*** as follows:
    a. Obtain a sufficient number (approximately 20) of the 5 mL Eppendorf Tubes and label each with the following information:
        - 3% ACN, 0.1% FA, spiked with 5 pmol/mL PepCalMix
        - Preparation date
        - Expiration date (1 year after preparation date)
        - Operator name / initials
    b. After mixing the solution thoroughly, aliquot the appropriate amount of Peptide Reconstitution Buffer into each 5 mL Eppendorf Tube.
    c. Store the prepared aliquots at -80 °C.

Turn on the instrument

If the SP100 is not already on, use the following instructions to initialize the system and ensure proper communication between the instrument and peripherals.

1. Turn on the following hardware:
   - **SP100** — Press the green switch on the front of the instrument.
   - **MPE power unit** — Press the power button on the front of the MPE power unit.
   - **Chiller power unit** — Press the power switch on the back panel of the chiller power unit.
   - **Instrument computer** — Press the power button on the front of the instrument computer.
2. Check the gas supply for appropriate pressure (≥110 psi).
3. Confirm that the MPE regulator (located near the MPE waste container) is set between 105–110 psi.
4. After the computer has initialized, select the **Proteograph ICS** desktop shortcut to launch ICS.

Prepare the files needed for the Peptide Reconstitution method

The Peptide Reconstitution method requires two files that you will need to prepare before running the method:

- Peptide quant data file – A Microsoft Excel (.xlsx) file, based on a template file you obtain from your Seer field application scientist (FAS) or Seer support. For contact information, see Technical Support (page 100).
- Partial plate map file – A comma-separated values (.csv) file generated by the Proteograph Assay method.

ICS will use data from the peptide quant data file to populate columns in the partial plate map file and will then generate a complete plate map file. The complete plate map file will be used as input for post-MS analysis in PAS.

Prepare the peptide quant data file

NOTE

Before you begin, obtain a copy of the peptide quant data template file from your Seer FAS or support@seer.bio.

On a computer other than the instrument computer, do the following. (You will need Microsoft Excel.)

1. Locate the peptide quant data template file. The file should have a name similar to the following:

   `PepQuantTemplate_v1p2_1LQWERTYU######_data.xlsx`

   Rename the file to replace `######` with the unique six-digit number of the Peptide Collection Plate barcode for the plate to be reconstituted.
2. Open the file in Excel and modify it as follows:
   a. In the Raw Data worksheet, copy and paste relative fluorescence unit (RFU) values from your fluorometer into the appropriate cells.
   b. Save the file and close Excel.
3. Copy the file to portable media (such as a USB drive) so that you can transfer it to the instrument computer.

On the instrument computer:

1. Navigate to `C:\Users\Public\Seer\Proteograph\DataFile\Peptide Quant Data File`.
2. Copy the peptide quant data file from your portable media to this folder.

Prepare the partial plate map file

On the instrument computer:

1. Navigate to `C:\Users\Public\Seer\Proteograph\DataFile\Partial Plate Map File`.
2. Locate the partial plate map file. The file should have a name in the following format:

   `PlateMapFile_<date>_<plate name>_1LQWERTYU<barcode number>_a.csv`
3. Verify that the Peptide Collection Plate barcode number in the name of the partial plate map file matches that in the name of the peptide quant data file, and that both correspond to the collection plate to be reconstituted.

Prepare the reconstitution consumables

1. If necessary, remove the Peptide Collection Plate containing the dried, quantified peptides from -80 °C storage and bring to room temperature.
2. Remove the Peptide Reconstitution Buffer from -80 °C and thaw in a room-temperature water bath.
3. Remove the following labware from room-temperature storage:
   - Reconstitution Buffer Reservoir
   - 300 µL NCTs
4. Prepare the thawed Peptide Reconstitution Buffer:
   a. (Optional) Sonicate for approximately 10 seconds in a room-temperature water bath.
   b. Vortex briefly.
5. Reconstitute the MS Control.
   a. Remove from 4 °C storage.
   b. Add 24 µl Peptide Reconstitution Buffer to the MS Control tube.
   c. Allow to stand for 1 minute.
   d. Gently pipette 10 times to mix.
   e. Place on ice and use within the day.

Run the Peptide Reconstitution method

1. On the ICS main menu, select **Peptide Reconstitution**.

   Onscreen prompts guide you through the remaining steps.

2. At the **Sign In** stage:
   a. Enter your username (up to 20 alphanumeric characters, including commas, dashes, periods, spaces, and underscores).
   b. Select **Continue**.

3. At the **Start** stage:
   a. Verify that the files you prepared earlier are in the expected directories on the instrument computer. (If you have not yet prepared the files, see Prepare the files needed for the Peptide Reconstitution method (page 64) before continuing.)
      - Partial plate map file (extension `_a.csv`) is in `C:\Users\Public\Seer\Proteograph\DataFile\Partial Plate Map File`.
      - Peptide quant data file (extension `_data.xlsx`) is in `C:\Users\Public\Seer\Proteograph\DataFile\Peptide Quant Data File`.
   b. Select **Continue**.

4. At the **Tip Counter** stage:
   a. Confirm that each of positions D1 and D2 has a stack with at least one full rack of 300 µL Nested Conductive Tips (NCTs) (two full racks, minimally).

      NOTE

      If ICS shows an incorrect arrangement or insufficient number of 300 µL NCTs needed for the method, perform a refill operation before continuing. See Refill the 300 µL NCTs (page 58)Refill the 300 µL NCTs (page 69).

   b. Having confirmed the 300 µL NCTs (and refilled, if necessary), select **Continue**.

5. At the **Deck Setup** stage, load reagents and labware:
   a. Add Peptide Reconstitution Buffer to the Reconstitution Buffer Reservoir.

      TIP

      For the correct quantify of buffer to add, refer to the peptide quant data file.

   b. Place the reservoir into position R1 of the Reservoir Carrier R.
   c. Place the Peptide Collection Plate into position B5 of Plate Carrier B.
   d. Select **Continue**.

6. At the **Deck Setup Check** stage:
    a. Verify that all labware is properly loaded and all carriers are on the autoload tray.
    b. Select the checkbox for each item.

    > **TIP**
    > You must select every checkbox before continuing.

    c. Select **Continue**.
7. At the **Begin Labware Barcoding** stage:
    a. Confirm proper placements:
        - All barcodes face the barcode reader.
        - All carriers are pushed to the stop hooks at the back of the autoload tray.
    b. Close the front protective cover.
    c. Select **Continue** to scan the barcodes.
8. At the **Volume Verification** stage:
    a. For each well location, verify that its value is as you expect. (The values shown are read in from the peptide quant file.) If a value is not as you expect, enter the correct value. Values must be positive integers or zero, within the range of 0–600 µL.
    b. Select **Continue**.

    The method now begins. It will take approximately 20 minutes to complete.

    > **WARNING**
    > Do not attempt to open the front protective cover after the method starts. Doing so ***automatically aborts the method***. If you must open the front protective cover, pause the method.

9. When the method is complete, select **End** and then open the front protective cover.
10. Remove the Peptide Collection Plate from position B5 of Plate Carrier B.
11. Add 20 µL reconstituted MS Control to an empty well.

    Wells D11–H11 and all wells in column 12 are empty.
12. Seal the Peptide Collection Plate with an MS-compatible seal and store at 4 °C for up to four days. For longer periods, store at -80 °C.

    > **NOTE**
    > If needed, transfer the reconstituted peptides with a multichannel pipette to a microplate that is compatible with your LC-MS system.

13. Turn off the instrument and peripherals.

    NOTE

    Seer recommends shutting down the hardware when it is not in use.

    - **SP100** — Press the green switch on the front of the instrument.
    - **MPE power unit** — Press the power button on the front of the MPE power unit.
    - **Chiller power unit** — Press the power switch on the back panel of the chiller power unit.
    - **Instrument computer** — Press the power button on the front of the instrument computer.

Refill the 300 µL NCTs

During the method's **Tip Counter** stage, ICS may show an incorrect arrangement or insufficient number of 300 µL Nested Conductive Tips (NCTs). When this happens, you will need to perform a refill operation.

NOTE

At any time, you can discontinue the refill operation by selecting **Reset** at the top of the **Tip Counter** screen. That will return the **NTR Details** and **Top Stack Details** to their original configuration.

1. Replace each of the stacks in positions D1 and D2 with a stack of four full racks of 300 µL NCTs (eight racks in all).
2. Select the **Refill All Eight (8) Nested Tip Racks (NTR)** checkbox.
3. Select **Refill** at the top of the **Tip Counter** screen.

   When the refill operation is finished, ICS updates the **NTR Details**.
4. Return to the **Tip Counter** stage step in Run the Peptide Reconstitution method (page 67).

Chapter 7

Instrument Maintenance

This chapter offers procedures and best practices for maintenance of the Seer SP100 Automation Instrument.

Maintenance methods

ICS guides you through the following maintenance methods. The Daily Maintenance, Weekly Maintenance, and MPE Flush methods are part of normal instrument operation. Perform the Water Run method as needed for training or troubleshooting.

ICON	METHOD	APPROXIMATE DURATION	FREQUENCY	NOTES
	Daily Maintenance	10 minutes	At the start of each day (typically, before running the Proteograph Assay or Peptide Reconstitution methods)	
	Weekly Maintenance	30 minutes	At the end of each week	
	MPE Flush	10 minutes	After running the Proteograph Assay method	
	Water Run	10 minutes	During training or troubleshooting with Seer support	a

[a]The Water Run method is accessible on ICS's Demo and Training Menu.

If an error occurs during a maintenance method, try to resolve the issue and repeat the method. If the issue persists, contact your field service representative or Seer support. See Technical Support (page 100) for contact information.

Preventative maintenance

Schedule preventative maintenance, including verification, with a Seer field service engineer (FSE) every six months. A service agreement ensures maintenance and verification at regular intervals.

Materials for maintenance methods

The maintenance methods require the following materials. (For supplier information, see Additional required materials (page 30). See also Hardware cleaning guidance (page 73).)

- 70% isopropyl alcohol or 70% ethanol
- Personal protective equipment
  - Disposable latex gloves
  - Lab coats
  - Protective goggles
- Waste Bags with Biohazard Labeling
- Waste Container Biohazard Box

Run the Daily Maintenance method

The Daily Maintenance method ensures that waste is emptied and that the pipette heads are holding pressure and pipetting the correct volumes. A series of onscreen prompts guides you through each step.

NOTE

Waste is typically located on the custom table under the instrument.

1. On the ICS main menu, select **Maintenance**, and then select **Daily Maintenance**.

2. At the **MPE Waste Check** stage, inspect the MPE waste container.

CAUTION

Failure to empty the MPE waste container can damage the MPE module.

   a. If the liquid level meets or exceeds the indicator line, empty the container as follows:
      i. Press the metal prongs to disconnect the two tubes from the cap.
      ii. Unscrew the cap from the container.
      iii. Dispose of the contents per your laboratory policy.

WARNING

Never use disinfectants containing hypochlorite or bleach to decontaminate liquid waste from the SP100. Doing so can cause toxic fumes and other adverse chemical reactions.

      iv. Screw the cap onto the container and reconnect the tubes.
   b. Select the **Completed MPE Waste Check** checkbox.
   c. Select **Continue**.
3. At the **deck and tip waste** stage, select **Yes** to execute.
   a. At **deck**, open the front protective cover and check if the deck is clean.
      - If the deck is clean, select **OK** to proceed with daily maintenance.
      - If the deck is not clean, select **Cancel** and perform weekly maintenance instead of daily maintenance. See Run the Weekly Maintenance method (page 75).
   b. At **tip waste**, empty the tip waste container. Select **OK** to continue.
4. At the **Tightness Check 1000µl Channel** stage, confirm that the teaching needles are on the work deck. If they are not, load them on the work deck, and then select **Yes** to execute the check.
5. At the **cLLD Check 1000µl Channel** stage, you are again prompted to confirm that the teaching needles are on the work deck. Select **Yes** to execute the check.
6. When the method is complete, select **OK**.

Hardware cleaning guidance

Observe the following guidance when cleaning the instrument hardware.

- For cleaning the pipetting channels and acrylic panels, use either deionized water or microcide (Hamilton catalog # 3896-02).

  CAUTION

  Do not use alcohol to clean the pipette O-rings or acrylic panels as alcohol can damage these components.

- For cleaning other hardware parts, use a cleaning solution of 70% isopropyl alcohol or 70% ethanol.

  CAUTION

  Do not use disinfectants that contain hypochlorite or bleach.

- Spray the cleaning solution onto a lint-free cloth or Kimwipe, and then wipe down any spills.

  CAUTION

  Do not spray cleaning solutions directly inside the work deck.

Weekly maintenance

Weekly maintenance consists of two procedures:

- Manually clean the instrument (below)
- Run the Weekly Maintenance method (next page)

NOTE

Your Seer FAS will have provided training on the weekly maintenance of the SP100. If you need assistance with maintenance, contact your field service representative or Seer support. See Technical Support (page 100) for contact information.

Manually clean the instrument

On a weekly basis with the instrument turned off, clean the pipette heads (96- and 8-channel), the eject plate, and the front protective cover. Do this prior to running the Weekly Maintenance method on the ICS. For best practices for cleaning these items, see Hardware cleaning guidance (previous page).

CAUTION

Never spray cleaning solution directly onto the instrument. Always spray directly onto a lint-free cloth or Kimwipe. Dispose of any used cloths or Kimwipes in accordance with your institution's hazardous waste disposal policies.

1. Ensure that the instrument is turned off.
2. Clean the pipetting channels as follows:
    a. On the 8-channel, clean the tip eject sleeves (the outer part of the pipetting channels) with a lint-free cloth or Kimwipe dampened with deionized water or microcide. Seer will provide training on how to correctly position the pipette channels for cleaning.
    b. Continue by cleaning the stop disk and O-rings of the pipette head. Do not get liquid inside the tip channel.
    c. Use the same technique to clean the pipette channels on the 96-channel CO-RE head. Seer will provide training on how to do this effectively.
3. Clean the eject plate as follows:
    a. Remove the eject plate from the instrument (the metal bracket where the pipette tips are ejected) and clean with a lint-free cloth or Kimwipe dampened with 70% isopropyl alcohol or 70% ethanol.
    b. Replace the eject plate.
4. Clean the inside and outside of the front protective cover with a lint-free cloth or Kimwipe dampened with deionized water. This will prevent dust or other foreign material from falling onto the work deck and contaminating an assay
5. When cleaning is complete, turn the instrument on and continue to Run the Weekly Maintenance method (next page).

Run the Weekly Maintenance method

A series of onscreen prompts guides you through the Weekly Maintenance method, which performs the same checks as the Daily Maintenance method plus hardware cleaning. For appropriate cleaning materials, see Hardware cleaning guidance (page 73).

NOTE

Waste is typically located on the custom table under the instrument.

1. On the ICS main menu, select **Maintenance**, and then select **Weekly Maintenance**.

2. At the **MPE Waste Check** stage, inspect the MPE waste container.

CAUTION

Failure to empty the MPE waste container can damage the MPE module.

   a. If the liquid level meets or exceeds the indicator line, empty the container as follows:
      i. Press the metal prongs to disconnect the two tubes from the cap.
      ii. Unscrew the cap from the container.
      iii. Dispose of the contents per your laboratory policy.

WARNING

Never use disinfectants containing hypochlorite or bleach to decontaminate liquid waste from the SP100. Doing so can cause toxic fumes and other adverse chemical reactions.

      iv. Screw the cap onto the container and reconnect the tubes.
   b. Select the **Completed MPE Waste Check** checkbox.
   c. Select **Continue**.

3. At the **deck and tip waste** stage, select **Yes** to execute.
   a. At **autoload tray**, confirm that there is no carrier on the autoload tray. Select **OK** to continue.
   b. At **carriers**, remove all carriers from the autoload tray and clean the carriers. Select **OK** to continue.
   c. At **deck**, open the front protective cover and clean the work deck. Select **OK** to continue.
   d. At **tip waste**, empty and clean the tip waste container. Select **OK** to continue.
   e. At **autoload**, clean the glass on the barcode reader with a lint-free cloth or cotton swabs lightly soaked in 70% ethanol. Select **OK** to continue.
4. At the **Tightness Check 1000µl Channel** stage, confirm that the teaching needles are on the work deck. If they are not, load them on the work deck, and then select **Yes** to execute the check.
5. At the **cLLD Check 1000µl Channel** stage, you are again prompted to confirm that the teaching needles are on the work deck. Select **Yes** to execute the check.

6. When the method is complete, return the carriers to the autoload tray:
    a. Load Tube Carrier 1 in the far-left position (track 5).
    b. Working left to right, successively load each of the remaining carriers.
    c. Select **OK**.

Run the MPE Flush method

After running the Proteograph Assay method, ICS prompts you to automatically run the MPE Flush method. Alternatively, you can initiate the method from the **Maintenance** menu.

1. On the ICS main menu, select **Maintenance**, and then select **MPE Flush**.

2. Confirm that all carriers are on the autoload tray.
3. Verify that the MPE filter tray is empty.
4. At the **Reagent Loading** stage:
    a. Add ~200 mL of 70% isopropyl alcohol to the Wash C Solution Single Reservoir.
    b. Place the reservoir into position A2.
    c. Place one rack of 300 µL NCTs into position B1.
    d. Select **Continue**.
5. At the **MPE Flush** stage, confirm the work deck setup.
    a. Leave all carriers on the autoload tray.
    b. Select **Continue** to start the method.
6. When the method is complete:
    a. Remove the reservoir and remaining 300 µL NCTs from the work deck.
    b. Select **Continue** to return to the ICS main menu.

Run the Water Run method

The Water Run method uses the same work deck layout as the Proteograph Assay method but replaces the reagents with water.

1. On the ICS main menu, select **Demo and Training Materials**, and then select **Water Run**.

2. Follow the onscreen prompts to load the work deck. Replace reagents with the minimum amounts of deionized water as shown below.

	DEIONIZED WATER VOLUME (MINIMUM)	POSITION
Sample Tubes 01-16	250 µL	T1-T16
Plasma Control	0 µL	T17
MPE Control	0 µL	T18
Nanoparticle Tubes	0 µL	T33 - T37
Wash C Solution Single Reservoir	200 mL	A2
Cleanup Reagents 4-Well Reservoir	Well 1: 7 mL Well 2: 40 mL Well 3: 54 mL Well 4: 20 mL	A3
Control Dilution Solution Reservoir	10 mL	R1
Denaturing Solution Reservoir	25 mL	R2
Reduction Solution Reservoir	7 mL	R3
Alkylation Solution Reservoir	7 mL	R4
Deionized Water Reservoir	15 mL	R5

3. When the method is complete, remove all labware from the work deck.

Chapter 8

Troubleshooting

This chapter offers troubleshooting guidance for the Seer SP100 Automation Instrument.

Resolve error messages

ICS guides you through any recoverable failures. If an error persists or the instrument experiences intermediate or critical failures, contact your field service representative or Seer support. See Technical Support (page 100) for contact information. Include any error code that ICS displays.

The following sections describe potential error messages displayed during method setup or when a method is running. For help preventing many of these errors, see Best practices (page 37).

Carrier Scan Error

The expected carrier was not found on the autoload tray, and the method cannot continue.

1. Verify that the carrier is seated all the way into the stop hooks.
2. Select **Retry**.

Cognex Initialization Error

An initialization error appears when ICS cannot connect to the barcode reader. Follow the onscreen instructions listed under the work deck graphic to resolve the error.

If the error persists, restart the instrument computer and try the method again.

Misaligned labware

ICS automatically pauses the gantry above misaligned labware on the work deck, preventing the method from proceeding.

1. Open the front protective cover.

   NOTE

   If the front protective cover is locked, either reach under it or pull out the carriers.

2. Follow the onscreen prompts to adjust the misaligned labware.
3. Close the front protective cover.
4. Select **Repeat**.
5. If the error persists and relates to tip pickup, replace the entire nested tip rack.

Lack of pressurized gas or low pressure error

If the MPE power unit or MPE module is not receiving pressurized gas during operation, ICS pauses the method. This error typically occurs during peptide cleanup.

Confirm that the gas pressure of the main supply and the regulator connected to the MPE power unit both read in the range of 105–110 psi, and then follow the onscreen prompts.

If the error persists, immediately contact your field service representative or Seer support. See Technical Support (page 100) for contact information.

No Barcode Scanned

The barcode scanning error appears when the barcode reader cannot scan a barcode.

1. Select **Rescan**.
2. If the error persists, perform a manual scan:
    a. Open the front protective cover and remove the applicable labware.
    b. Select **Manual Scan**.
    c. Enter the barcode number printed on the labware.
    d. Return the labware to the work deck. Ensure proper placement.
    e. Close the front protective cover.
    f. Select **Manual Scan**.

TIP

If there is no barcode on the labware, contact your field service representative or Seer support. See Technical Support (page 100) for contact information.

NTR Scan Error

The NTR scan error appears when the barcode reader cannot scan an NTR barcode.

1. Adjust the NTR:
    a. Remove the NTR.
    b. Return the NTR to the work deck. Ensure proper alignment.
2. Select **Retry**.

Pause a method

Pausing a method allows you to resolve an error without aborting the method.

CAUTION

Resume a paused method as promptly as possible to avoid liquid loss.

1. On the Running page, select **Pause**.

   ICS pauses the method.

2. Open the front protective cover.

   NOTE

   If the front protective cover is locked, either reach under it or pull out the carriers.

3. Adjust labware as indicated in the error message.
4. Close the front protective cover.
5. Select **Continue** to resume the method.

Abort a method

When a method experiences an unresolvable error, you must abort the method.

CAUTION
Aborting a method is ***final***. ICS cannot resume the method and consumables cannot be reused.

1. From ICS, select **Abort**.
2. Select **Yes** to confirm.

   ICS aborts the method and unlocks the protective front cover.
3. Remove labware from the carriers:
   a. Remove all plastic labware from the tube, plate, tip, and reservoir carriers.
   b. Remove the Peptide Cleanup Plate from the MPE module.
   c. Remove the black CO-RE lids and store for future use.
   d. Leave the magnetic plate on the instrument.
4. Dispose of all labware, plasma samples, and leftover reagents in the appropriate waste containers per laboratory policy.

Package trace files

If you encounter a support issue and must send documentation to Seer, use the following instructions to package the necessary trace files. If you need assistance, contact Seer support. See Technical Support (page 100) for contact information.

On the desktop of the instrument computer, double-click **Seer Support Package Creator**.

1. In the dialog, specify an output location for the trace files.
2. The software places a .zip file in the specified location.
3. Send the .zip file to Seer support.

Appendix A

Safety and Compliance

This appendix provides safety and regulatory compliance information for the proper use of the Seer SP100 Automation Instrument.

Safety considerations and markings

This appendix describes the primary safety hazards, including the safety symbols affixed to the instrument, and regulatory and compliance information. To ensure safe and correct instrument operation, review this information before operating or maintaining the SP100 Automation Instrument.

- For complete environmental, health, and safety information, refer to the safety data sheets (SDS) provided in the Training Kit.
- For instrument specifications and laboratory requirements, including installation information, refer to the *Site Preparation Guide* (CF-1017 or CF-1015 International).

Intended use

The Seer SP100 Automation Instrument is a robotic liquid handling workstation classified as a general laboratory instrument for research use only (RUO), and not as an *in vitro* diagnostic (IVD) device. The SP100 is intended to automate routine pipetting tasks and the transportation of plates, tips, and other labware.

Instrument operation

Only trained service personnel can install the instrument, and the instrument operator must attend Seer training. The procedures described in this guide are tested and optimized, so any deviation can compromise results or cause malfunction.

CAUTION
To avoid personal injury and/or equipment damage, never attempt to lift or move an installed instrument.

Always wear appropriate protective clothing, goggles, and gloves when operating the instrument or conducting maintenance. During routine operation, shield the instrument from direct sunlight and intense artificial light. Stand clear of all moving parts and the work deck. Do not lean over or into the instrument.

Emergency shutoff

Press the green power switch on the front of the instrument to turn off the SP100. If at risk of electric shock, also unplug the instrument.

Hazardous waste disposal

The Proteograph Assay produces liquid and solid waste that may be classified as biohazard (e.g., human plasma), chemical hazard, and flammable hazard. Always wear appropriate PPE and dispose of all waste in accordance with your local policies and regulations. Seer recommends that you generate an accurate waste profile.

WARNING

Never use disinfectants containing hypochlorite or bleach to decontaminate liquid waste from the SP100. Doing so can cause toxic fumes and other adverse chemical reactions.

The following table lists the hazardous reagents that will end up in both the liquid waste as well as residual in the labware after the assay is complete.

PROTEOGRAPH ASSAY KIT COMPONENT	REAGENT	HAZARD CLASS
Alkylation Solution	2-chloroacetamide	Chemical
Digestion Stop Solution	Formic acid	Chemical
MPE Control, MS Control, and Plasma Control	Biological sample/peptides	Biohazard
Peptide Wash Solution B, Elution Solution	Acetonitrile (ACN)	Chemical, Flammable
Reduction Solution	Tris-(2-carboxyethyl)phosphine, HCl	Chemical

Safety hazards

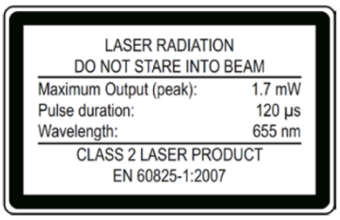
The following symbols identify safety hazards to consider when operating the instrument.

Table 9. Safety hazard symbols

SYMBOL	DESCRIPTION	LOCATION ON INSTRUMENT
	**Power Connection** – Connect only to an earth-grounded outlet. **Laser Beam (Autoload)** – Do not stare into the beam of the class 2 laser of the barcode reader.	Left side of exterior
	**Connection to PC** – Use only the appropriate shielded cables. **USB Connection** – Exceeding a cable distance of 5 m can cause signal loss. **Pipetting Arm** – Do not manually move the pipetting arm. **Moving Parts** – Do not open the protective front cover during a method. A moving arm resides in the instrument so opening the cover aborts the method.	• CO-RE head • Gantry • Stop hooks
	**Biohazard Warning** – The deck and waste might contain biohazardous chemicals. Do not touch biohazardous materials. The instrument drops used tips into a waste container emptied during daily maintenance or when full.	• Chiller • CO-RE head • Work deck • Heater shaker • Magnetic plate • MPE Waste
	**Hot Surface** – Avoid contact with the heater shaker, which has hot surfaces that can cause injury if touched.	Heater shaker
	**Pinch Point** – Keep fingers and hands clear of the area. Mechanical moving parts can injure fingers and hands.	• CO-RE head • Gantry
	**Magnetic Field** – Note that the magnetic plate generates a magnetic field. Incorrect use can harm the operator.	Magnetic plate

Laser beam

The barcode reader has a Class II Laser Diode. Do not stare into the beam.



Electromagnetic radio frequency

The SP100 conforms to European norms for interference immunity. However, exposure to electromagnetic radio frequency (RF) fields or the discharge of static electricity directly onto the instrument can negatively impact function. Keep the instrument away from equipment that emits electromagnetic RF fields and minimize static electricity in the immediate vicinity.

Electrostatic charge

When handling labware and tips, avoid any electrostatic charge. Electrostatic charge can damage the instrument and impact labware stability.

Regulatory compliance

The SP100 is designed, tested, and certified for compliance with the standards listed in the following table.

Product certification

The instrument is certified to the following standards.

STANDARD	DESCRIPTION
IEC/EN 61010-1:2010 (3rd Edition)	Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory use.
EN 61326-1:2013	Electrical Equipment for Measurement, Control and Laboratory Use. EMC Requirements.
EN 61326-2-6	Specifies minimum requirements for immunity and emissions regarding electromagnetic compatibility for *in vitro* diagnostic medical equipment, taking into account the particularities and specific aspects of this electrical equipment and their electromagnetic environment.
EN 61000-3-2	Electromagnetic compatibility (EMC) - Part 3-2: Limits - Limits for harmonic current emissions (equipment input current ≤ 16 A per phase).
EN 61000-3-3	Electromagnetic compatibility (EMC) - Part 3-3: Limits - Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current ≤ 16 A per phase and not subject to conditional connection.

CSA C/US mark

The CSA C/US mark signifies that the product is certified for both US and Canadian markets to the applicable US and Canadian standards.

CE Mark

The CE Mark indicates that assembly is covered by a Declaration of Conformity and has been declared in conformity with the provisions of all applicable directives in the European Economic Area (EEA).

RoHS directive

RoHS Directive (2011/65/EU): Restriction of the use of certain hazardous substances in electrical and electronic equipment

WEEE Directive (2012/19/EU): Waste Electrical and Electronic Equipment

FCC compliance

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

NOTE

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Conformité IC

ICES-003 (Canada): This Class A digital apparatus complies with Canadian ICES-003. Cet appareil numérique de la classe A est conforme à la norme NMB-003 du Canada.

Glossary

A

ACN
Acetonitrile.

C

case
Holds six sleeves of stacks, for a total of 120 NTRs holding 11,520 NCTs.

CO-RE
Compressed O-ring expansion.

consumables
Reagents and plasticware.

custom file
Optional sample information in a .csv, .tsv, .xls, or .xlsx file.

D

Digestion Control
A reference sample added before nanoparticle incubation.

E

equipment
Reusable laboratory equipment.

F

FA
Formic acid.

FAS
Field application scientist.

I

ICS
Abbreviation of Proteograph Instrument Control Software.

L

LC
Liquid chromatography.

LC-MS
Liquid chromatography mass spectrometry.

M

materials
Consumables and equipment.

MPE
Monitored multi-flow positive pressure evaporative extraction.

MPE Control
Reference peptides added before desalting cleanup.

MS
Mass spectrometry.

MS data file
The results of MS analysis for each sample or control in a plate in a .raw, .wiff, .wiff.scan file.

MS Control
Reference peptides added before LC-MS analysis.

N

NCT
Nested conductive tips.

NP
Nanoparticle.

NTR
Nested tip rack. See also rack.

P

pallet
Holds 32 cases of sleeves, for a total of 3,360 NTRs holding 322,560 NCTs.

partial plate map file
A comma-separated values (.csv) file, based on the file generated by the Proteograph Assay method.

PAS
Short name of Proteograph Analysis Suite.

peptide quant data file
A Microsoft Excel (.xlsx) file, based on a file you obtain from your Seer FAS or support@seer.com.

plate (labware)
A piece of labware containing 96 wells where various steps of the assay are performed. Types of plates used in the Proteograph Assay include intermediate (Nunc) plates, sample prep plate, peptide cleanup plate, and peptide collection plate.

plate map file
The location of each sample in a plate in a .csv file. Used when analyzing MS data in PAS and for automated peptide reconstitution on the SP100.

PQR
Peptide quantification and reconstitution.

Process Control
A reference sample added before nanoparticle incubation.

Proteograph Analysis Suite
Seer software used to process, analyze, and visualize LC-MS data.

Proteograph Assay Kit
A Seer kit containing the reagents and labware for preparing samples on the SP100.

Proteograph Instrument Control Software
Software onboard the SP100 used to operate the instrument.

Proteograph PQR Labware Kit
A Seer kit containing labware and reagents needed for four Peptide Quantification runs and four Peptide Reconstitution runs on the SP100.

Proteograph Product Suite
The bundle of Seer kits, instrument, and analysis software.

Q

QC
Quality control.

R

rack
Holds 96 NCTs. Also called a nested tip rack (NTR).

S

sleeve
Holds five stacks of NTR racks, for a total of 20 racks holding 1,920 NCTs.

SP100 Automation Instrument
The Seer liquid handling instrument.

stack
Holds four NTRs, for a total of 384 NCTs.

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Technical Support

For technical assistance, contact your field service representative or Seer support.

Contact Information

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