USER GUIDE

Proteograph™ Product Suite

Proteograph™ XT Assay

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

seer™

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Revision history

DOCUMENT	DATE	DESCRIPTION OF CHANGE
CF-1053 B	June 2023	Updated references to external documents.
CF-1053 A	June 2023	Initial release.

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Figures

Tables

Chapter 1

Proteograph Overview

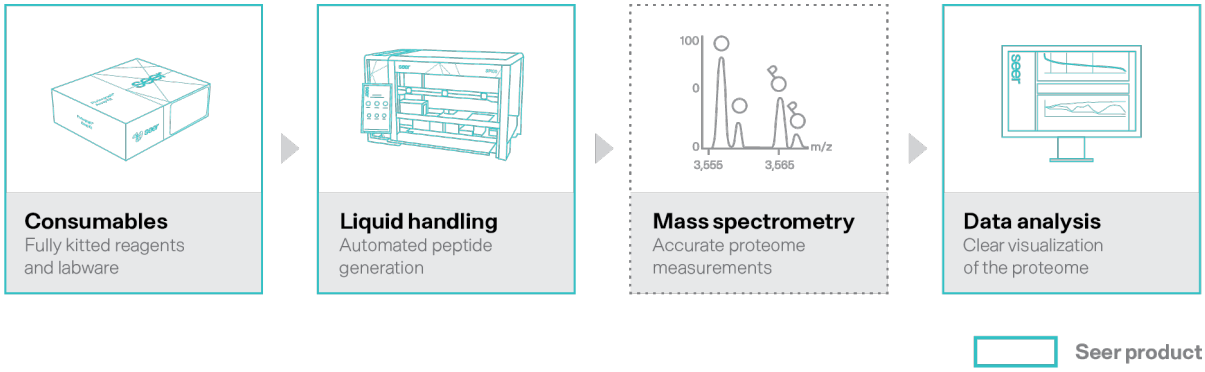
This chapter introduces you to the main components of the Seer Proteograph™ Product Suite.

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 XT workflow*



Proteograph Product Suite

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 XT Assay Kit**
  - Proprietary panel of two engineered nanoparticles for processing up to 20 or 40 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 troubleshoot 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. Cumulative times are rounded up to the nearest tenth of an hour.

NOTE

Where applicable, differences in step durations between the 20- and 40-sample Proteograph XT Assay Kits are noted below. The cumulative durations listed reflect the (slightly longer) step durations associated with the 40-sample kit.

Table 1. Summary of the Proteograph steps

STEP	APPROXIMATE TIME		CUMULATIVE TIME (HOURS)
	20 SAMPLES	40 SAMPLES	
Proteograph Assay method setup	30 minutes*		0.5
Sample dispensing and nanoparticle transfer	20 minutes	24 minutes	0.9
Protein corona formation	61 minutes		1.9
Protein corona wash	33 minutes		2.5
Protein corona denaturation	42 minutes		3.2
Protein digestion	3 hours and 08 minutes		6.3
Peptide cleanup, wash, and elution	53 minutes		7.2
Deck cleanup	10 minutes*		7.4
Peptide quantification	30 minutes**		7.9
Dry peptides	15 minutes* *THEN DRY OVERNIGHT*		8.1
STOPPING POINT			
Peptide reconstitution	25 minutes		8.5

* All hands-on.

** Includes approximately 10 minutes hands-on.

SP100 Automation Instrument

The SP100 Automation Instrument ("SP100") automates the pipetting of liquids to prepare peptides for downstream analysis. A custom table holds the instrument and stores the multi-flow positive pressure evaporative extraction ("[MPE]²") power unit, chiller unit, and instrument computer.

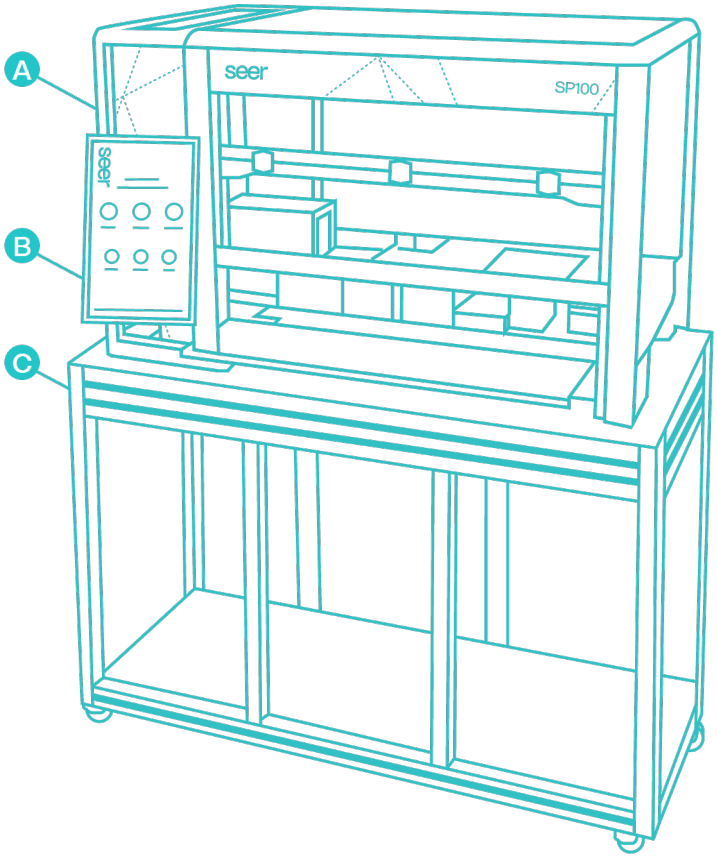
An adjustable arm affixed to the custom table presents the touchscreen monitor. The touchscreen is glove-compatible and displays the ICS user interface.

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 SP100 includes dedicated Proteograph Instrument Control Software (ICS) that controls instrument operations, providing an interface for running methods 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]² Flush	Instrument maintenance
	Water Run	Training and troubleshooting

Required Seer kits

Use of the SP100 requires the following Seer kits:

- **Proteograph XT Assay Kit** — Includes all consumables for assay runs (20 or 40 samples) on the SP100.
- **Proteograph XT PQR 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 28).

Chapter 2

SP100 Automation Instrument Overview

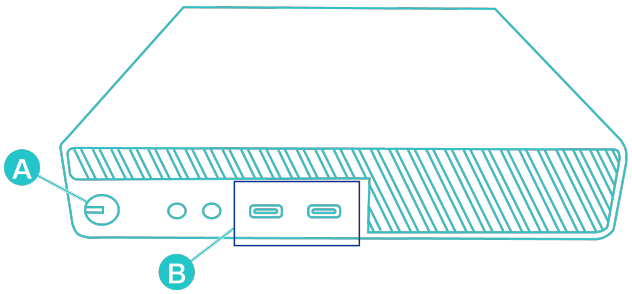
This chapter provides a detailed overview of the SP100 Automation Instrument, including the instrument computer, the work deck and autoload tray, and other hardware components.

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.

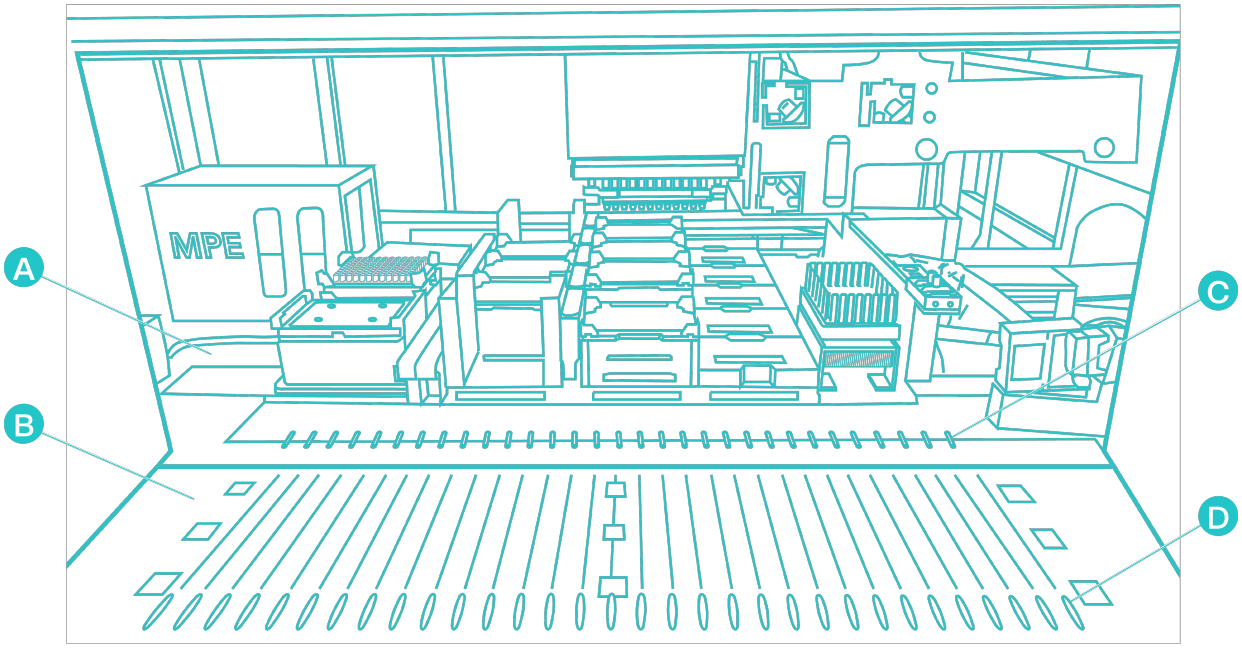
- For end-user use:
  - Proteograph™ Instrument Control Software (ICS)
  - Proteograph Support Tool (generates files used for troubleshooting)
- For Seer support personnel use:
  - Microlab Star Maintenance & Verification
  - Microlab STAR Verification 2
  - Hamilton Method Editor
  - Hamilton Run Control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

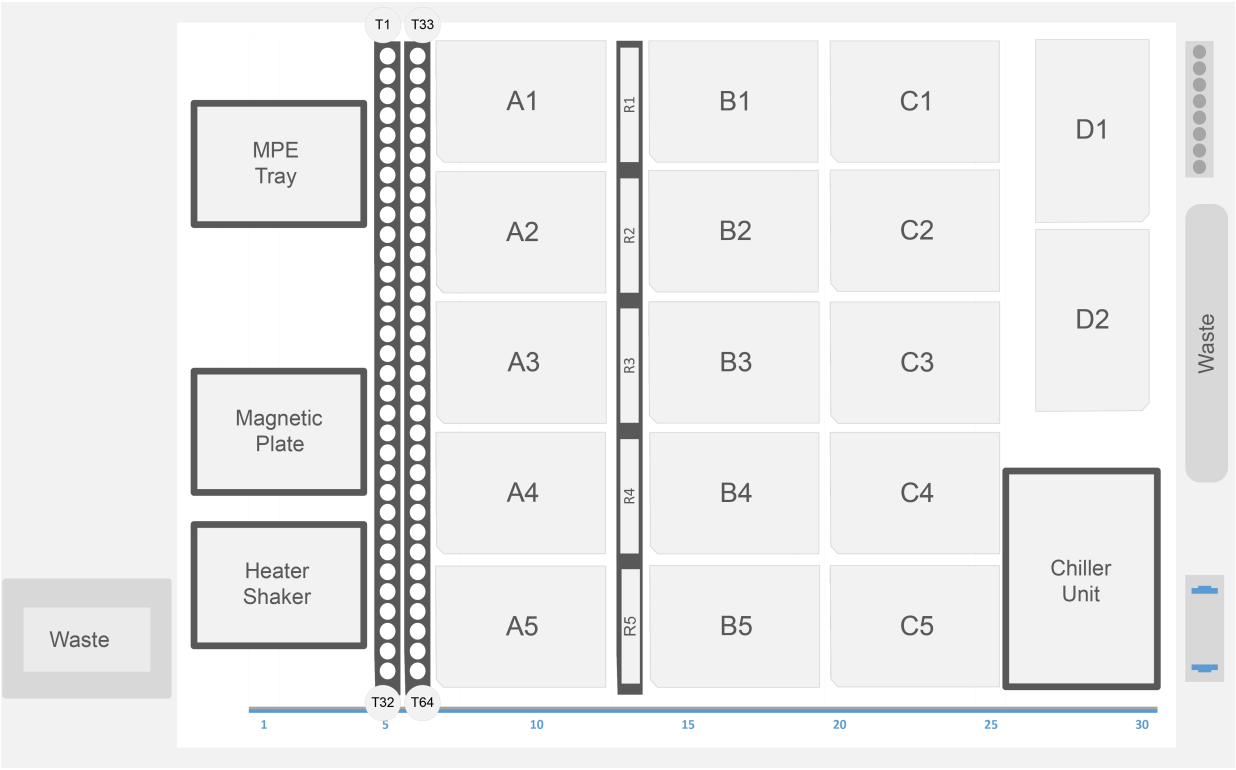


Table 2. Labware positions for the Proteograph Assay method (40 samples)

NOTE

The table below indicates the placement of labware for the 40-sample Proteograph Assay method. The 20-sample method uses a slightly different work deck layout, with one fewer stack of 300 µL NCTs in Tip Carrier C and a different arrangement of tubes in the tube carriers.

CARRIER	POSITION	ITEM	NOTES
Tube Carrier 1	T1–T32	Sample Tubes 01–32	
Tube Carrier 2	T33–T40	Sample Tubes 33-40	
	T41	Plasma Control	
	T42	MPE Control	
	T43–T46	Nanoparticle Tubes (XT NP A Tube 1 and Tube 2, XT NP B Tube 1 and Tube 2)	
	T47–T64	Empty	
Plate Carrier A	A1	Peptide Clean Up Plate (with microplate holder underneath)	a
	A2	Corona Wash Reservoir with Black Plate Lid	
	A3	6-Well Digestion Reservoir with Black Plate Lid	

CARRIER	POSITION	ITEM	NOTES
	A4	Sample Prep Plate with Clear Plate Lid	a
	A5	Intermediate Plate	a, b
Reservoir Carrier R	R1–R2	Empty Reservoirs	
	R3	Wash A Reservoir	
	R4	Wash B Reservoir	
	R5	Elution Reservoir	
Plate Carrier B	B1	Empty	
	B2	Nanoparticle Plate	a
	B3	Intermediate Plate	a
	B4	Intermediate Plate	a
	B5	Collection Plate	a
Tip Carrier C	C1	1 Stack of 300 µL Nested Conductive Tips (NCTs)	c
	C2	1 Stack of 300 µL 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–D2	Empty	
	Chiller unit	Trypsin/LysC 8-Well Reservoir with Black Plate Lid	

a This labware is loaded empty.
b One stack of two plates.
c One stack of four NCT 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

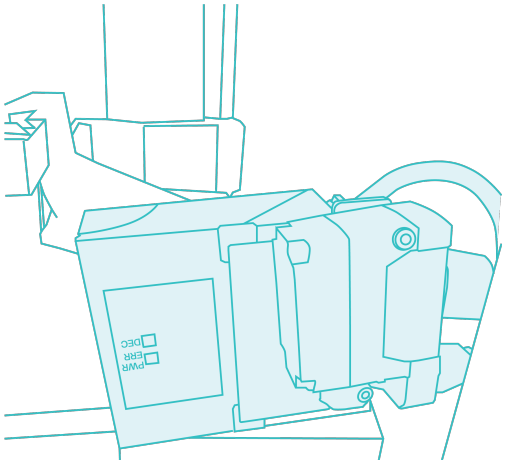
The SP100 is equipped with the following hardware, which pipettes liquids in labware and transports plates. All hardware components reside inside the instrument, except for the external waste containers (which are located below the instrument on the custom table) and carriers (which move in and out of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

Labels affixed to the instrument hardware identify safety considerations and hazards. For a list of labels with descriptions, see *Safety hazards* (page 86).

Barcode reader

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

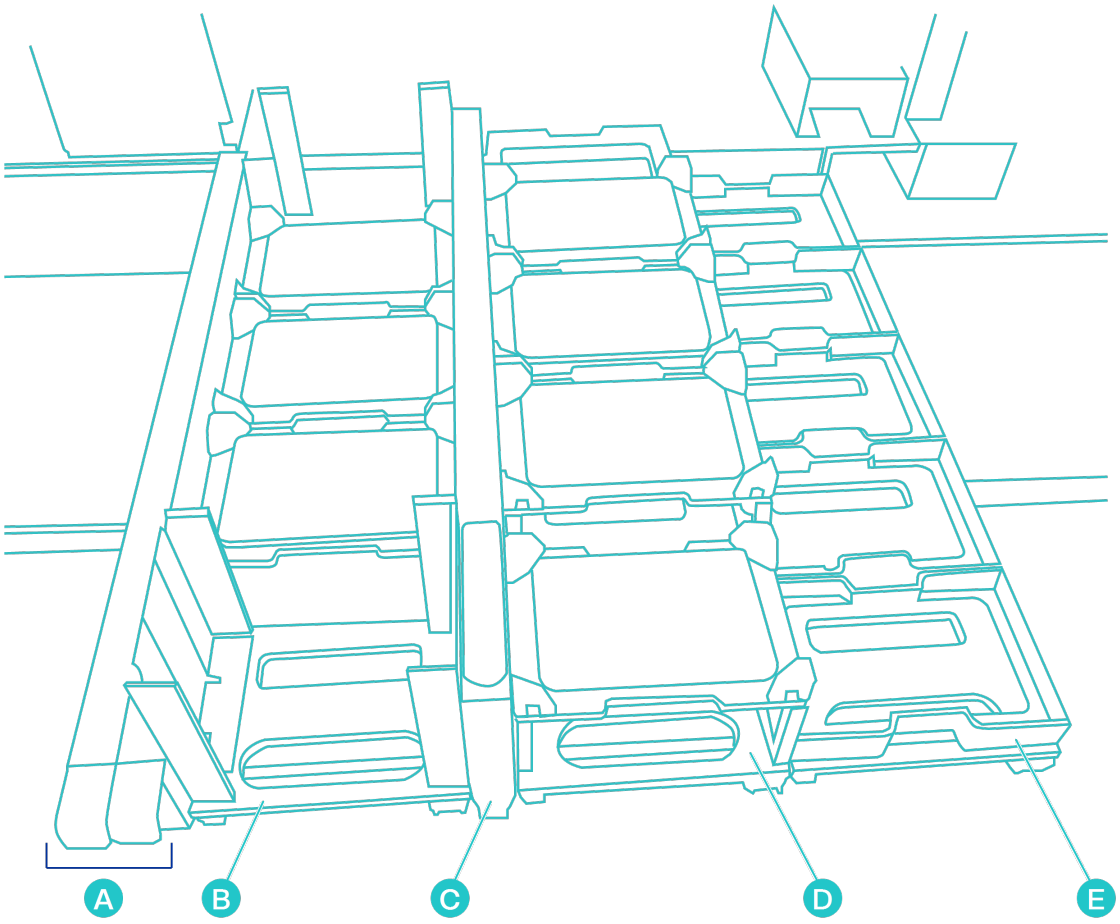
Figure 7. *Barcode reader*



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

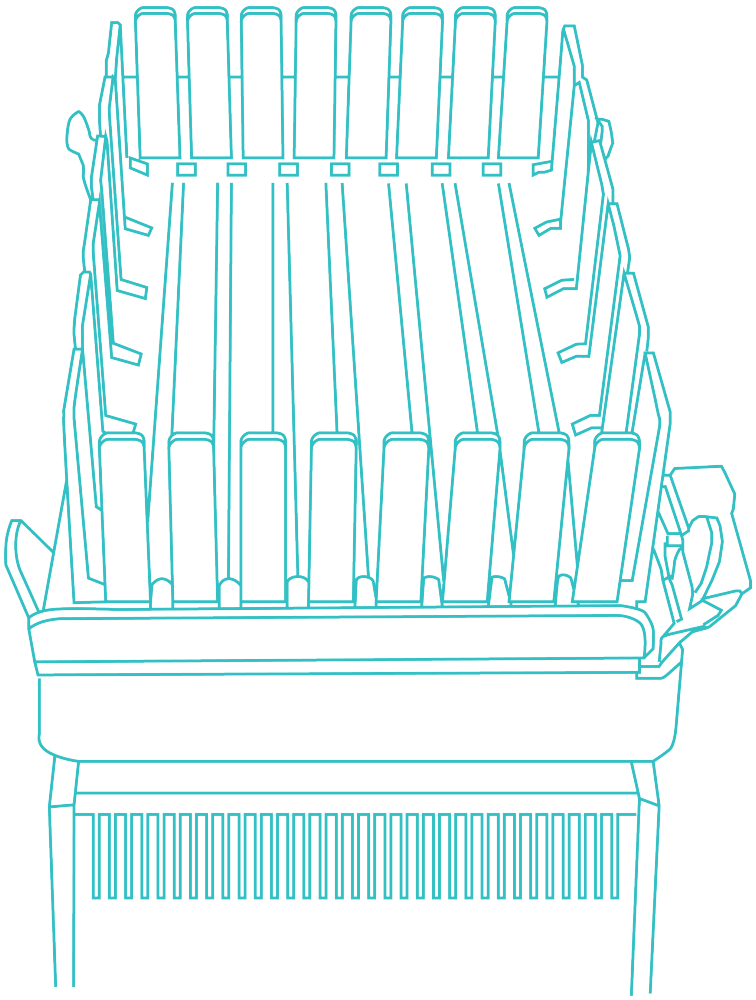
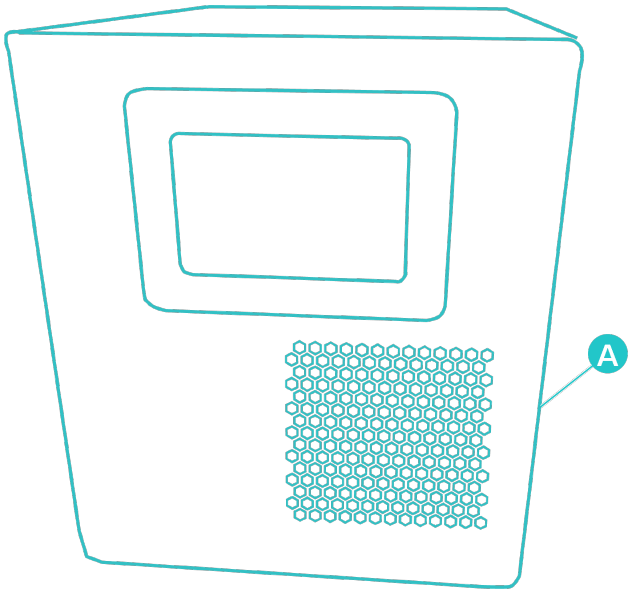


Figure 10. Chiller power unit



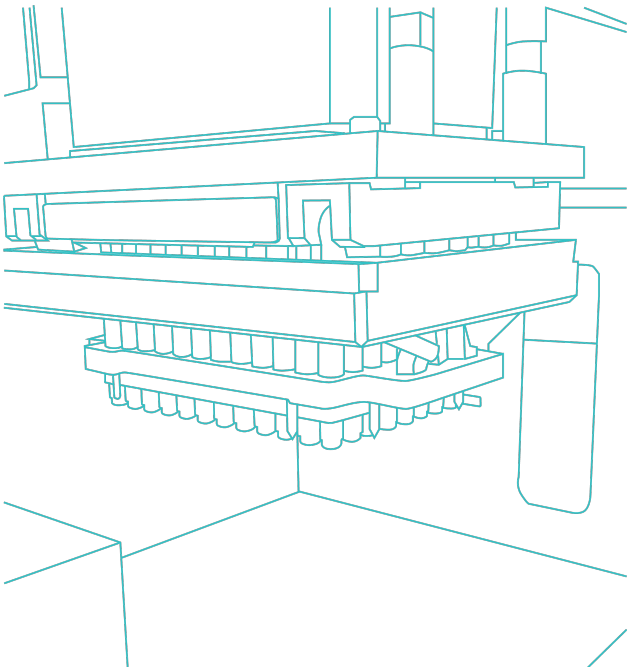
A Power switch (not shown; located in the upper-left corner of the back panel)

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



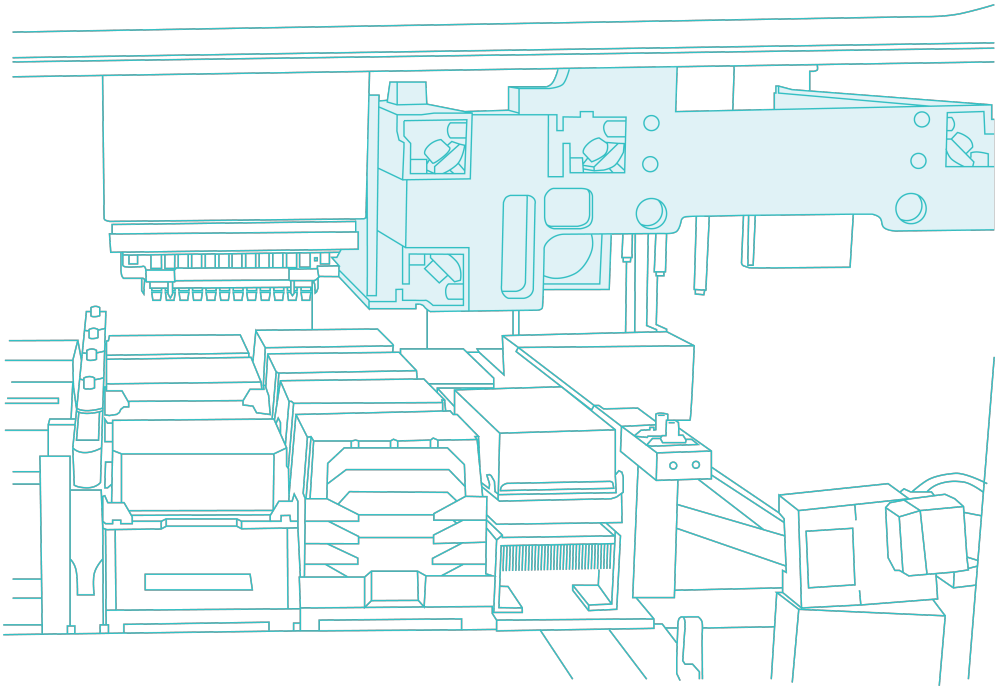
Gantry

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

WARNING

Do not touch the gantry during operation.

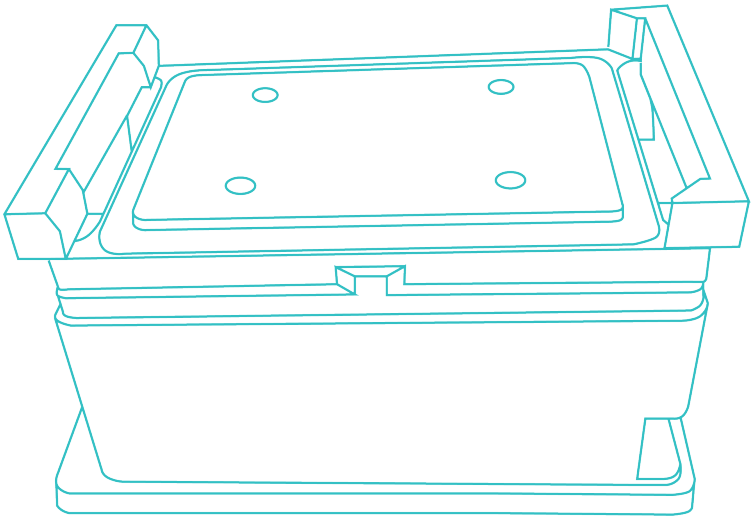
Figure 12. Gantry



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



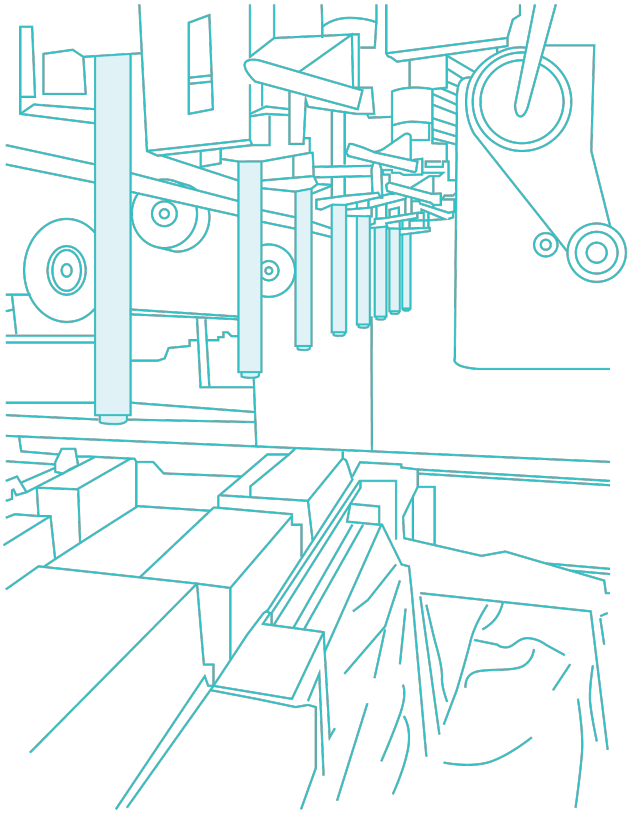
Independent 8-channel CO-RE pipette heads

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.

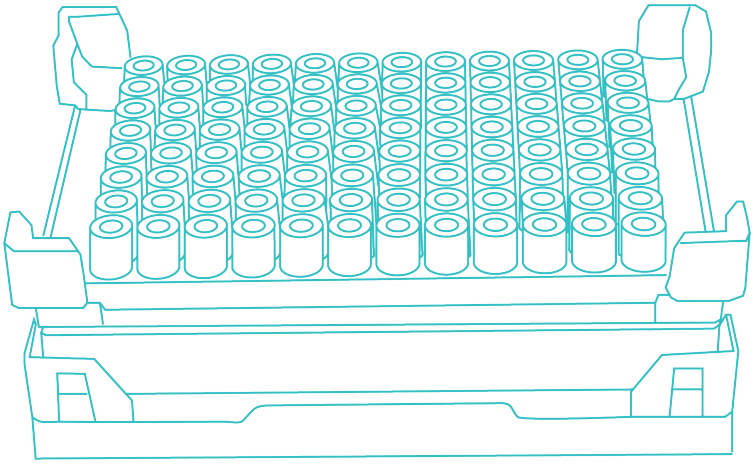
Figure 14. Independent 8-channel CO-RE pipette heads



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.

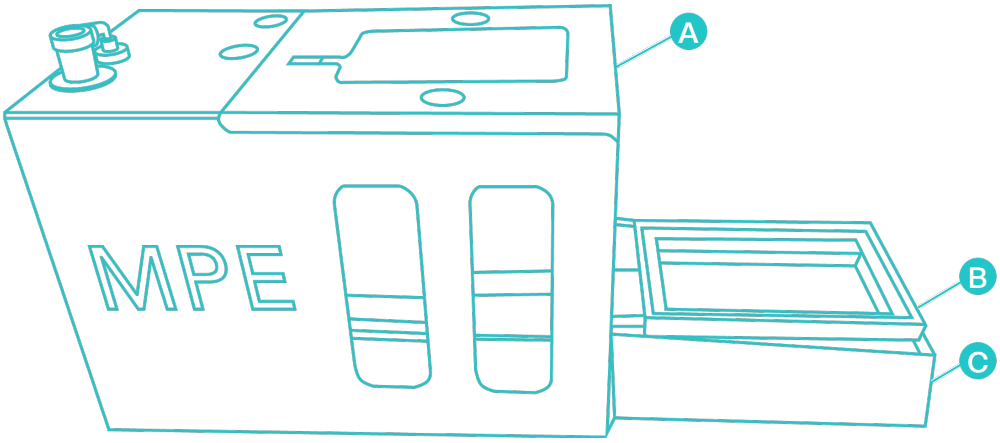
Figure 15. Magnetic plate



[MPE]² module and [MPE]² power unit

The [MPE]² module uses positive pressure to collect and purify peptides from the Peptide Clean Up Plate. During wash steps, the [MPE]² module sends liquid waste to an external container. The [MPE]² power unit (an instrument peripheral) controls power to the [MPE]² module.

Figure 16. [MPE]² module

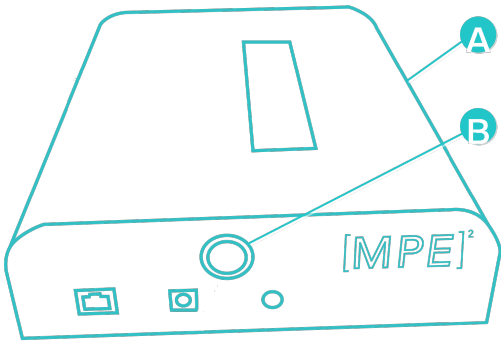


A [MPE]² module

B Filter Plate Adapter

C Waste Tray (affixed to shuttle)

Figure 17. [MPE]² power unit



A [MPE]² power unit

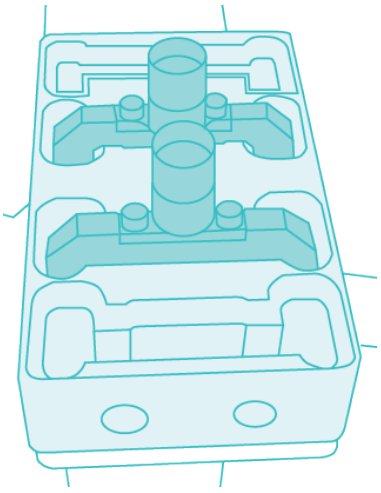
B Power button (which glows green when on)

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

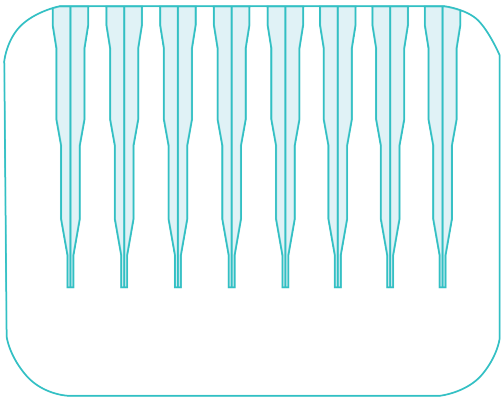


Teaching needles

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 empty NCT racks.
- **CO-RE head waste** — Waste bin for 300 µL NCTs from the CO-RE head.
- **[MPE]² waste** — Liquid waste container for all liquid waste, the majority being the [MPE]² 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 materials.

Kit contents and storage requirements

The Proteograph Assay method requires a Seer Proteograph XT Assay Kit. Additionally, the Peptide Quantification and Peptide Reconstitution methods require a Seer Proteograph XT PQR Kit.

The table below summarizes the attributes of these kits. (For the Proteograph XT Assay Kit, 20- and 40-sample versions are available.)

For detailed information about the contents of each kit, see *Proteograph XT Assay Kit* (below) and *Proteograph XT PQR Kit* (page 31). Additional third-party equipment and materials required for peptide preparation on the SP100 are described in *Required equipment* (page 32) and *Additional required materials* (page 32).

Table 3. *Seer Proteograph Kits*

KIT	SAMPLES PER RUN	NUMBER OF BOXES	CONTAINS	PART NUMBER	NOTES
Proteograph XT Assay Kit 20 Samples	20	1	All consumables for one 20-sample assay run on the SP100.	S55R4003	
Proteograph XT Assay Kit 40 Samples	40	1	All consumables for one 40-sample assay run on the SP100.	S55R4004	
Proteograph XT Assay Kit 80 Samples	20	4	All consumables for four 20-sample assay runs on the SP100.	S55R4001	
Proteograph XT Assay Kit 160 Samples	40	4	All consumables for four 40-sample assay runs on the SP100.	S55R4002	
Proteograph XT PQR Kit	N/A	1	Consumables for four peptide quantification and reconstitution (PQR) runs on the SP100.	S55R4007	a

a In addition to the consumables in the Proteograph XT PQR Kit, peptide reconstitution runs require a user-prepared Peptide Reconstitution Buffer. See *Peptide Reconstitution Buffer preparation* (page 62).

Proteograph XT Assay Kit

The Seer Proteograph XT Assay Kit provides the labware and reagents needed to run the Proteograph Assay method on the SP100. The base kit provides materials for one 20- or 40-sample assay run; Seer also provides each of these kits in multiples of four. (See *Kit contents and storage requirements* (above).)

Each 20- or 40-sample box within the Proteograph XT Assay Kit includes two components: a refrigerated box of reagents, and a room-temperature box of labware. When you receive a Proteograph XT Assay Kit, ***promptly store*** the refrigerated box at 4 °C.

Refrigerated box (reagents)

The refrigerated box includes two separately packaged pouches:

- Proteograph XT Control Panel
- Proteograph XT Nanoparticle Tubes

All contents of the refrigerated box should be stored at 4 °C.

Table 4. Contents of the Proteograph XT Assay Kit refrigerated box

DESCRIPTION	QUANTITY	CAP COLOR / LABEL	VOLUME	NOTES
Alkylation Solution	1	Green	7 mL	
Corona Wash Buffer	1 or 2	White	100 mL	a
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	b
MS Peptide Control	1	Clear with black ring	n/a	b
Peptide Wash A Solution	1	Wash A	40 mL	
Peptide Wash B Solution	2	Wash B	27 mL	
Plasma Control	1	Clear with black ring	n/a	b
Reconstitution Buffer A	1	Clear	16 mL	
Reconstitution Buffer B	1	Clear	22 mL	
Reduction Solution	1	Blue	7 mL	
Trypsin/LysC Protease MS Grade	5 or 6	Gray	n/a	c
XT Nanoparticle A (XT NP A), Tube 1	1	Clear with black ring	n/a	d
XT Nanoparticle A (XT NP A), Tube 2	1	Clear with black ring	n/a	d
XT Nanoparticle B (XT NP B), Tube 1	1	Clear with black ring	n/a	d
XT Nanoparticle B (XT NP B), Tube 2	1	Clear with black ring	n/a	d

a The 20-sample kit includes one bottle, while the 40-sample kit includes two.
b Packaged in the Proteograph XT Control Panel pouch.
c The 20-sample kit includes five vials, while the 40-sample kit includes six.
d Packaged in the Proteograph XT Nanoparticle Tubes pouch.

Room-temperature box (labware)

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

DESCRIPTION	QUANTITY
6-Well Digestion Reservoir	1
Black Plate Lid	3
Clear Plate Lid	1
Collection Plate	1
Corona Wash Reservoir	1
Elution Reservoir	1
Empty Reservoir	2
Intermediate Plate	4
Nanoparticle Plate	1
Peptide Clean Up Plate (with plate holder)	1

DESCRIPTION	QUANTITY
Sample Prep Plate	1
Sample Tubes (01-20 or 01-40)	20 or 40
Trypsin/LysC 8-Well Reservoir	1
Wash A Reservoir	1
Wash B Reservoir	1

Proteograph XT PQR Kit

The Seer Proteograph XT PQR Kit (PN S55R4007) 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 XT PQR Kit

DESCRIPTION	QUANTITY	NOTES
4-Well Reservoir	4	a
Black Plate Lid	4	a
Black Quantitation Plate ("Black Quant. Plate")	4	a
Empty Tubes (2 mL)	32	a
Intermediate Plate	4	a
Peptide Assay Reagent Tubes	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 following third-party consumables and other materials.

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 pipette with tips	Rainin, material # 17014391		a
20–200 µL multichannel pipette with tips	Rainin, material # 17013810		a
100–1000 µL pipette with tips	Rainin, material # 17014382		a
300 µL Nested Conductive Tips (NCTs)	Hamilton, part # 235950		
70% isopropyl alcohol or 70% ethanol	General lab supplier		
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		

DESCRIPTION	SUPPLIER	REQUIREMENTS	NOTES
Disposable latex gloves	General lab supplier		
Eppendorf twin.tec PCR Plates 96 LoBind, semi-skirted, 250 µL, PCR clean, colorless	Thermo Fisher Scientific, catalog # 0030129504		b
Kimwipes or similar lint-free tissues	General lab supplier		
Lab coats	General lab supplier		
Peptide Reconstitution Buffer	Laboratory-prepared		c
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 62).
d Must be stored at 4 °C.

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 describes 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 methods 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 84).
- Confirm you have all *Proteograph XT Assay Kit* (page 29) items and other materials. See *Required equipment* (page 32) and *Additional required materials* (page 32).
- Review best practices to help load the work deck correctly and efficiently. See *Best practices* (page 37).

Required materials

The Proteograph Assay method is designed to work with the Seer *Proteograph XT Assay Kit* (page 29).

Additional required equipment and materials are listed below. (For supplier information, see *Required equipment* (page 32) and *Additional required materials* (page 32).)

Required equipment

- Refrigerated Microcentrifuge

Additional required materials

- 300 µL Nested Conductive Tips (NCTs)
- 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 either 20 or 40 plasma samples. During the assay, each sample is partitioned, and each half incubates (simultaneously) with either XT Nanoparticle A (XT NP A) or XT Nanoparticle B (XT NP B). This process produces an output of 40 (for 20 samples) or 80 (for 40 samples) wells of peptides in a 96-well plate.

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

- **Process Control 1** — A mixture of pooled plasma samples that is subsequently mixed with XT NP A. It is exposed to every step, including corona formation, trypsin digestion, and peptide cleanup.
- **Digestion Control** — A mixture of the plasma control diluted with Reconstitution Buffer B. 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 trypsin digestion and exposed to the peptide cleanup step.
- **Process Control 2** — A mixture of pooled plasma samples that is subsequently mixed with XT NP B. It is exposed to every step, including corona formation, trypsin digestion, and peptide cleanup.

Collection Plate

The output of the Proteograph Assay method is the Collection Plate containing the purified peptides. Samples are incubated with either XT NP A or XT NP B in columns 1-10 (for the 40-sample assay) or columns 6-11 (for the 20-sample assay); the output of this incubation is XT NP Suspension A or XT NP Suspension B. Controls occupy wells A11, B11, C11, and D11.

Figure 20. Layout of the post-assay Collection Plate (20 samples)

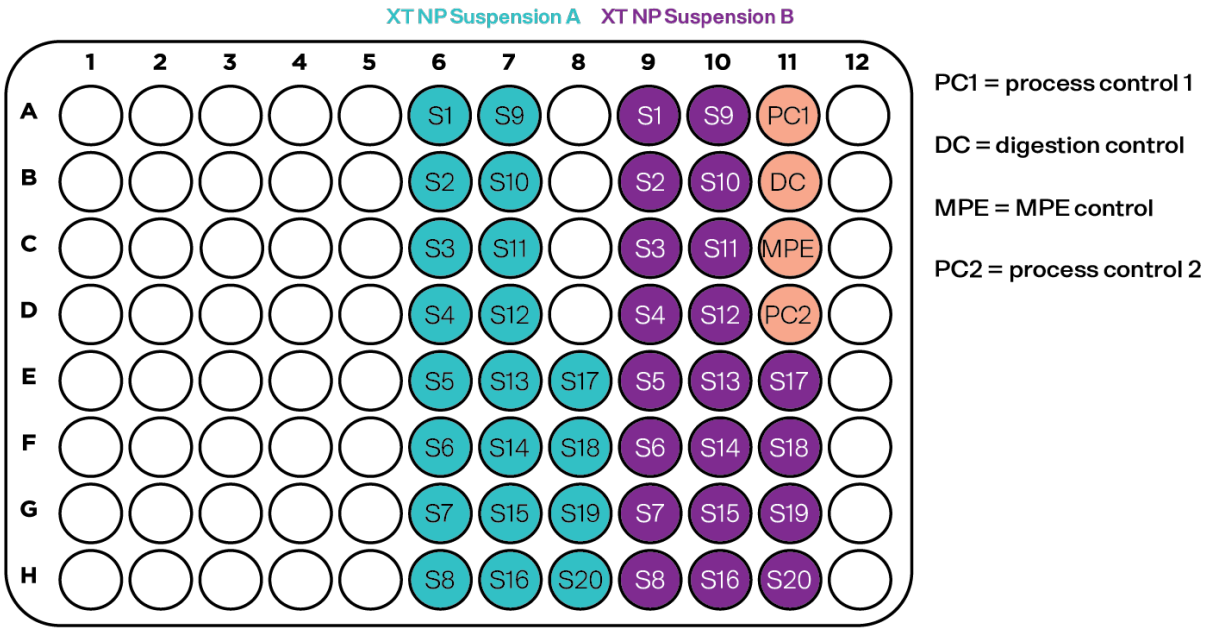
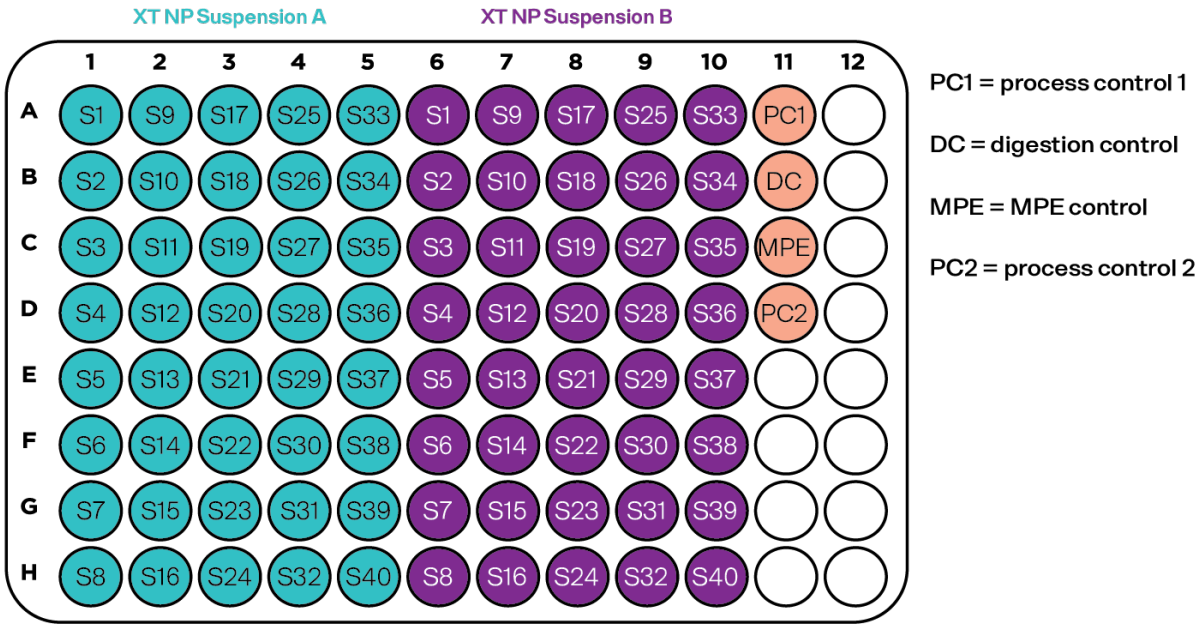


Figure 21. Layout of the post-assay Collection Plate (40 samples)



Best practices

When setting up the work deck, observe 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 *Chapter 8. Troubleshooting* (page 81).

Pipetting samples

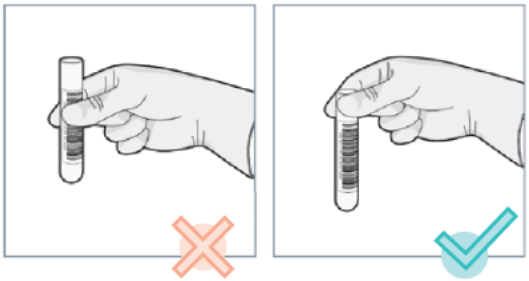
When pipetting samples into Proteograph sample tubes, observe the following best practices to avoid the formation of bubbles.

- After spinning down the tube, carefully aspirate 240 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.

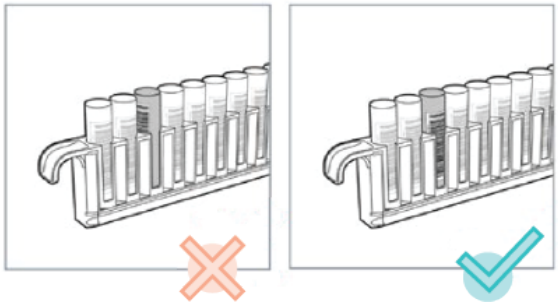
Loading labware

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.

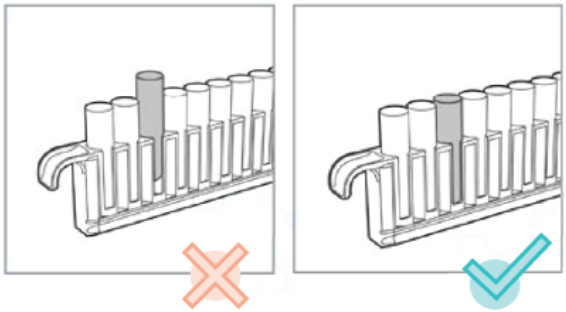
- **Tubes** — Hold tubes at the top to avoid warming samples in your hand. Avoid placing your fingers over barcodes.



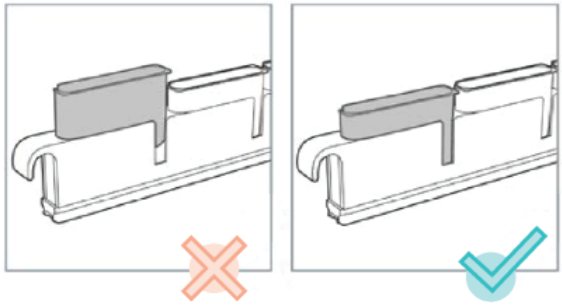
Face all tube barcodes to the right so the barcode reader can scan them. Incorrect orientation makes the barcodes unreadable.



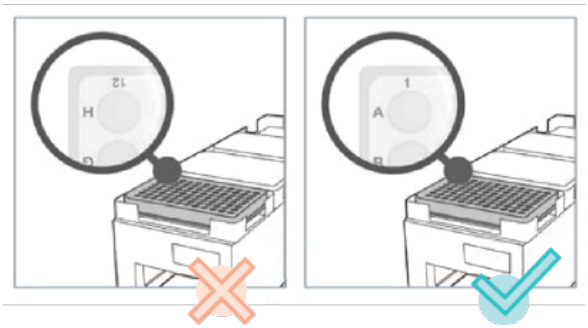
Fully insert tubes into the tube carrier. Improper seating can cause malfunction.



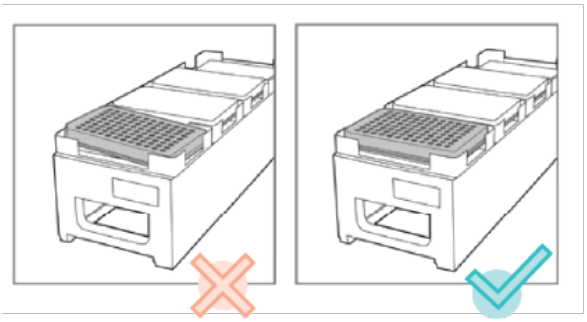
- **Reservoirs** — Fully insert reservoirs into the Reservoir Carrier R.



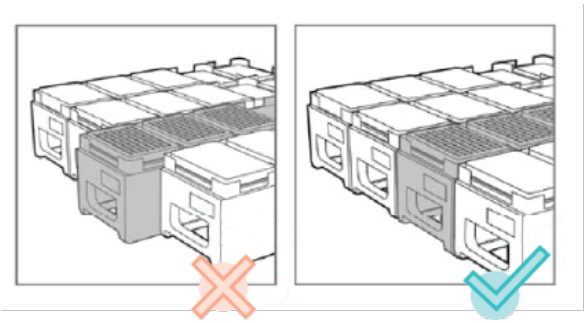
- **Wells** — Position wells on plate carriers so that well A1 is in the upper-left corner.



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



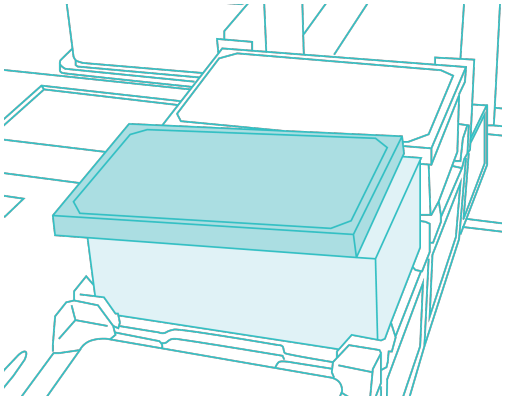
- **Carriers** — Push all carriers to the stop hooks at the back of the autoload tray. Incorrect positioning causes collision or malfunction. Do not pull carriers out during method runs.



Placing lids

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

Figure 22. Misaligned lid



Work deck quick reference

The following figures show 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 figures below to confirm proper work deck layout before starting the method.
- An experienced user, use the figures below as a quick reference when loading the work deck.

Figure 23. Work deck loaded for the Proteograph Assay method (20 samples)

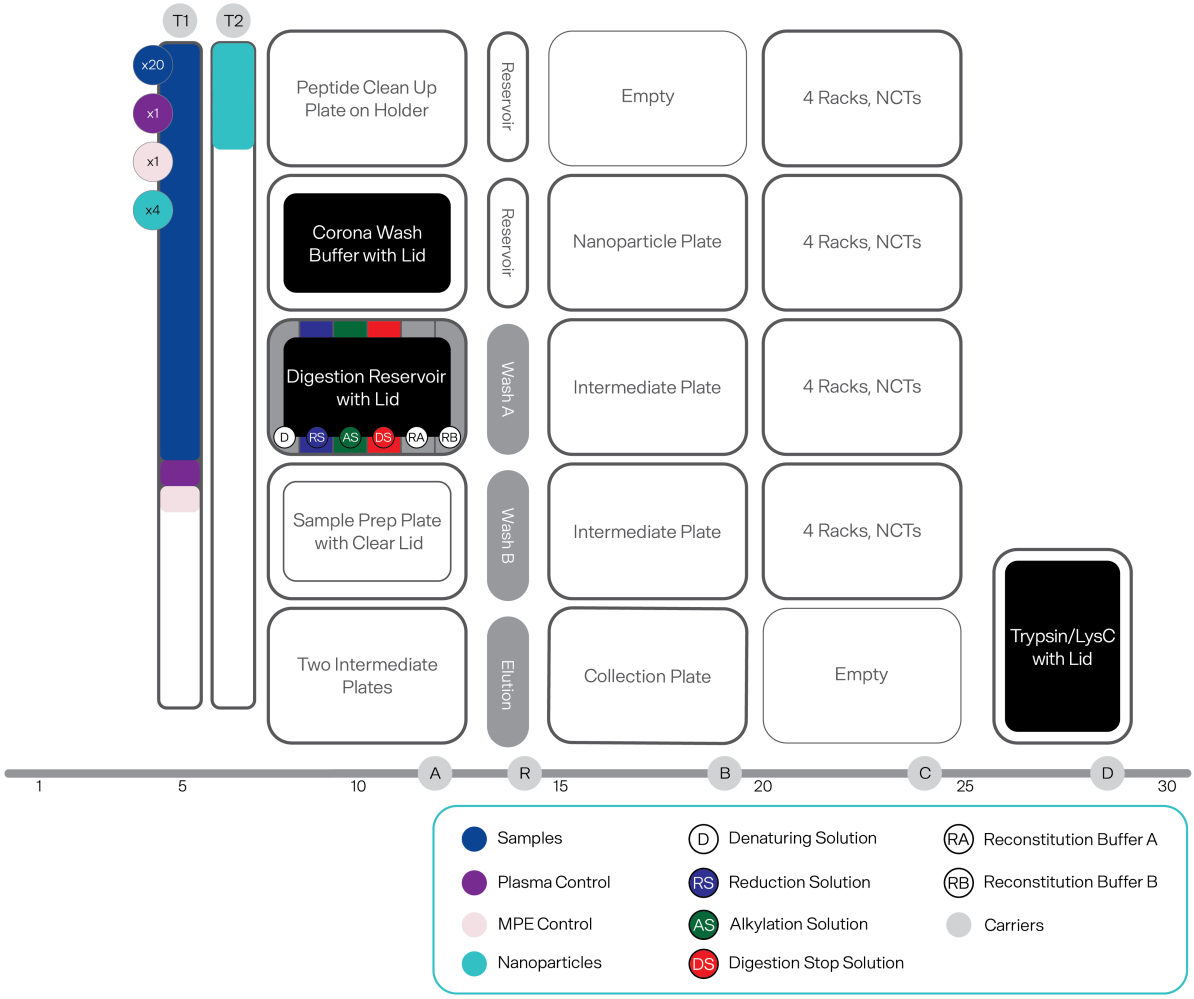
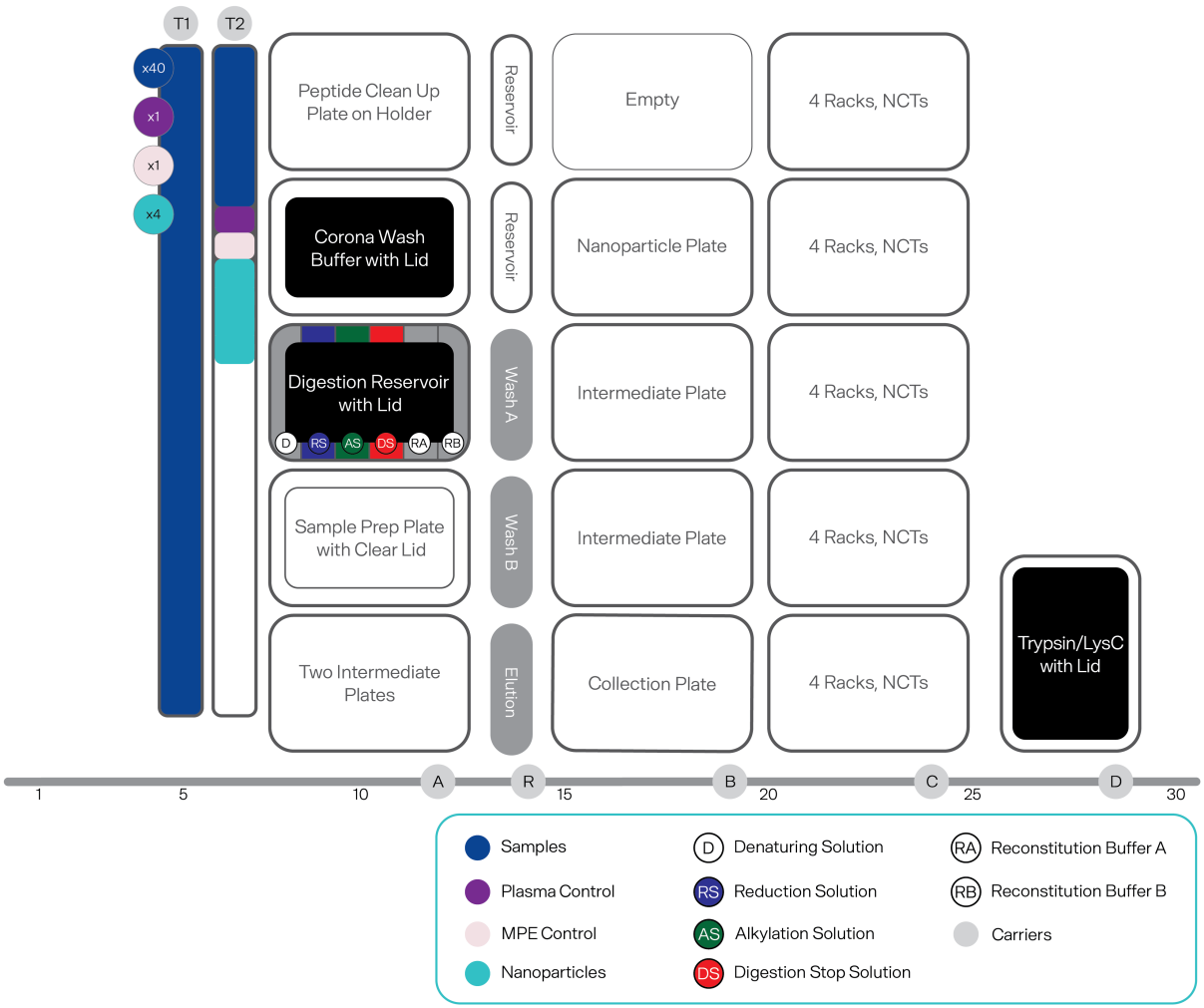


Figure 24. Work deck loaded for the Proteograph Assay method (40 samples)



Protocol steps

The Proteograph Assay protocol consists of the following steps:

1. *Prepare the instrument* (below)
2. *Prepare the assay materials* (next page)
3. *Set up and run the Proteograph Assay method* (next page)
    a. *Load the work deck* (page 45)
        i. *Deck Setup 1 of 4: Load Plate Carrier A* (page 46)
        ii. *Deck Setup 2 of 4: Load Reservoir Carrier R* (page 47)
        iii. *Deck Setup 3 of 4: Load Plate Carrier B, Tip Carrier C, and chiller unit* (page 47)
        iv. *Deck Setup 4 of 4: Load tube carriers* (page 48)
    b. *Complete setup and start the method* (page 49)
4. *Unload the work deck* (page 50)

NOTE
You must begin peptide quantification within ***1 hour*** after completing the Proteograph Assay method.

Prepare the instrument

1. Turn on the instrument:

NOTE
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.

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

2. Run the Daily Maintenance method. (See *Run the Daily Maintenance method* (page 72).)

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.

   NOTE

   Do not allow plasma samples to warm to room temperature. Keep samples on ice or at 4 °C until they are loaded into Sample Tubes and onto the work deck.

4. Remove labware from room-temperature storage and arrange as follows:
   - Place the Trypsin/LysC 8-Well Reservoir on ice, covered with a Black Plate Lid.
   - Check all labware for appropriate barcode labels.

     NOTE

     The Peptide Clean Up Plate does not have a barcode label.

5. Remove the refrigerated box from 4 °C storage and arrange reagents as follows:
   - Place the Enzyme Reconstitution Solution on ice.
   - Place the five or six vials (depending on your Proteograph XT Assay Kit version) of Trypsin/LysC Protease MS Grade on ice.
   - Set aside the remaining reagents at room temperature.

Set up and run the Proteograph Assay method

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

   On-screen prompts guide you through the remaining steps.
2. At **Assay Kit Selection**, select the number of samples corresponding to your Proteograph XT Assay Kit.
3. At **Sign In**, enter your username, and then select **Continue** to advance.

   NOTE

   Usernames can include up to 200 alphanumeric characters, including commas, dashes, periods, spaces, and underscores.

4. At **Registration**, do the following:
   a. In the **Project Name** field, enter a name* for your experiment.

      TIP

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

b. In the **Plate Name** field, enter a unique name* for the run.

c. Select **Continue** to advance.

*Each name can contain up to 50 alphanumeric characters, including dashes and underscores.

5. At **Waste Check**, do the following:

> **CAUTION**
>
> Failure to empty the liquid waste container can damage the [MPE]² module.

a. Inspect the liquid waste container to determine how full it is.

b. If the liquid level is at or above the indicated fill level, 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.

c. Select the **Completed Waste Check** checkbox.

d. Select **Continue** to advance.

6. At **Device Initialization**, do the following:

a. Verify that the SP100 instrument, the [MPE]² power unit, and the chiller power unit are turned on.

b. If necessary, empty tip and [MPE]² waste.

c. Pull all carriers out onto the autoload tray and ensure that the work deck is clear of any labware or spills.

d. 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.

e. Confirm that the [MPE]² regulator (located near the [MPE]² waste container) is set between 105–110 psi.

f. Select **Continue** to advance.

7. At **[MPE]² Initialization**, do the following:

a. Verify there is no plate in the [MPE]² module's Filter Plate Adapter.

b. Select **Initialize**.

The [MPE]² module will test that the vacuum and shuttle are functioning correctly.

Load the work deck

A series of Deck Setup screens guide you through loading the work deck.

Deck Setup 1 of 4: Load Plate Carrier A

At **Deck Setup 1 of 4**, do the following:

1. Place labware and reagents on the work deck according to the layout shown on-screen. Load items in the order listed. As you complete each step, select the corresponding checkbox on-screen.

> **NOTE**
>
> The table below lists labware positions for the 40-sample Proteograph Assay method. The work deck layout for the 20-sample method is slightly different; follow the on-screen instructions in ICS to load the work deck correctly.

> **CAUTION**
>
> Ensure correct orientation of the 6-Well Digestion Reservoir ***before adding reagents*** to the reservoir. The barcode should be facing to the right (toward the barcode reader).

POSITION	ITEM(S)	INSTRUCTION
A1	Peptide Clean Up Plate (including microplate holder underneath it)	Place on the work deck.
A2	Corona Wash Reservoir	Place on the work deck.
A2	◦ Corona Wash Buffer (1 or 2 bottles) ◦ Black Plate Lid	Add the reagent to the reservoir, and then cover with the lid. **NOTE** The 20-sample Proteograph XT Assay Kit includes one bottle of Corona Wash Buffer, while the 40-sample kit includes two bottles.
Off-Deck	◦ 6-Well Digestion Reservoir ◦ Well 1: Denaturing Solution ◦ Well 2: Reduction Solution ◦ Well 3: Alkylation Solution ◦ Well 4: Digestion Stop Solution ◦ Well 5: Reconstitution Buffer A ◦ Well 6: Reconstitution Buffer B	Add reagents to the 6-Well Digestion Reservoir in the specified wells. **TIP** Pour reagents from the front of the reservoir to prevent cross-contamination between wells.
A3	◦ 6-Well Digestion Reservoir ◦ Black Plate Lid	Place the reservoir on the work deck, and then cover the reservoir with the lid.
A4	◦ Sample Prep Plate ◦ Clear Plate Lid	Place the plate on the work deck, and then cover with the lid.
A5	Intermediate Plate (2)	Place a stack of 2 plates on the work deck.

2. Once you have selected all checkboxes on-screen, select **Continue** to advance.

Deck Setup 2 of 4: Load Reservoir Carrier R

At **Deck Setup 2 of 4**, do the following:

1. Place reservoirs and reagents into Reservoir Carrier R as shown in the work deck layout on-screen. Load reservoirs in the order listed. As you complete each step, select the corresponding checkbox on-screen.

   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.

   a. Place Empty Reservoirs in positions R1 and R2. (Leave these reservoirs uncovered.)

   b. For the remaining reservoirs, place each reservoir into its labeled position, fill it with its corresponding reagent, and cover it with a black CO-RE lid.

POSITION	RESERVOIR	REAGENT
R3	Wash A Reservoir	Peptide Wash A Solution (1 bottle)
R4	Wash B Reservoir	Peptide Wash B Solution (2 bottles)
R5	Elution Reservoir	Elution Solution (1 bottle)

2. Once you have selected all checkboxes on-screen, select **Continue** to advance.

Deck Setup 3 of 4: Load Plate Carrier B, Tip Carrier C, and chiller unit

At **Deck Setup 3 of 4**, do the following. As you complete each step, select the corresponding checkbox on-screen.

1. Place labware onto Plate Carrier B and Tip Carrier C according to the layout shown on-screen. Load items in the order listed.

   NOTE

   The table below lists labware positions for the 40-sample Proteograph Assay method. The work deck layout for the 20-sample method is slightly different; follow the on-screen instructions in ICS to load the work deck correctly.

POSITION(S)	ITEM	INSTRUCTION
B1	n/a	Leave empty.
B2	Nanoparticle Plate	Place on the work deck.
B3	Intermediate Plate	Place on the work deck.
B4	Intermediate Plate	Place on the work deck.
B5	Collection Plate	Place on the work deck.
C1-C5	300 µL Nested Conductive Tips (4 racks x 5)	Place a stack of 4 racks in each position.

2. Prepare the Trypsin/LysC 8-Well Reservoir and load it into the chiller unit as follows:

   a. On ice, perform the following steps:

      i. Add 500 µL Enzyme Reconstitution Solution to one vial of Trypsin/LysC Protease MS Grade for a final concentration of 0.2 ug/µL.

ii. Pipette 2–3 times to mix.

iii. Repeat the two steps above for the remaining Trypsin/LysC vials.

iv. Transfer 500 µL from each Trypsin/LysC vial 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.

v. Cover the reservoir with a Black Plate Lid.

b. 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 98) for contact information.

c. Place the reservoir (with lid) into the chiller unit. Ensure the barcode faces to the right.

3. Once you have selected all checkboxes on-screen, select **Continue** to advance.

Deck Setup 4 of 4: Load tube carriers

At **Deck Setup 4 of 4**, do the following:

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 (01-20 or 01-40, 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 240 µL of 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, then centrifuge them again at 5,000 x 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. Place tubes into the tube carriers according to the layout shown on-screen. Load tubes in the order listed. As you complete each step, select the corresponding checkbox on-screen.

> **NOTE**
> Remove caps from tubes prior to placing them.

a. Uncap the Samples Tubes and load them into the tube carriers according to the layout shown on-screen. Load tubes from back to front (beginning with Slot 1 in each carrier).

> **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. Place the Plasma Control and MPE Control tubes into the tube carriers according to the layout shown on-screen.

> **NOTE**
> Before placing the Plasma Control tube, inspect the tube and verify that the pellet of lyophilized plasma is at the bottom of the tube. If it is not, tap or briefly centrifuge the tube to settle the pellet to the bottom of the tube.

c. For each of the four nanoparticle tubes, do the following:

i. Tap the tube on the custom table to ensure that the lyophilized beads are settled and none are stuck on the cap.

ii. Uncap the tube, then place it into the tube carriers according to the layout shown on-screen.

4. Confirm that all barcodes in the tube carriers are visible and facing to the right, then return the carriers to the autoload tray.
5. Once you have selected all checkboxes on-screen, select **Continue** to advance.

Complete setup and start the method

1. At **Labware Check**, do the following:
   a. Verify the correct placement of each labware item on the work deck according to the layout shown on-screen. As you verify each item, select its corresponding checkbox on-screen.

   > **NOTE**
   > For a diagram of the loaded work deck, you may also refer to *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 **Continue** to advance.
2. At **Sample Names**, do the following:
   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, .tsv, or .xlsx file, select **Load Sample Names**.

NOTE

The first column in the file should list the sample tube numbers (1, 2, 3...), while the second column lists user-defined sample names. For example: `1,<SampleName1> … 40,<SampleName40>`

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

NOTE

Default values follow the format `Sample<##>`, with sample numbers in ascending order (`Sample01`, `Sample02`, etc.).

WARNING

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

b. Select **Continue** to advance.

3. At **Begin Labware Barcoding**, do the following:
   a. Ensure that barcodes are fully visible.
   b. Orient all labware with barcodes facing the barcode scanner.
   c. Ensure that each carrier is pushed forward against the stop hooks at the back of the autoload tray.
   d. Select **Continue** to advance.

      ICS moves the carriers from the autoload tray to the work deck and begins barcode scanning.

      NOTE

      The barcode reader scans each barcode as a final check to confirm proper work deck setup.

4. At **Setup Complete**, do the following:
   a. Close the front protective cover.
   b. Select **Run** to start the method.

      ICS locks the front protective cover.

The method takes approximately 6.5 hours to complete. During the method, ICS displays each stage with time estimates. For information about time estimates, see *Proteograph Assay steps* (page 10).

When the method has finished, proceed to *Unload the work deck* (below).

WARNING

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

NOTE

If you encounter an unrecoverable error during the Proteograph Assay method, contact your field service representative or Seer support. See *Technical Support* (page 98) for contact information.

Unload the work deck

The **Complete** stage indicates the successful completion of the method. Do the following:

1. Ensure that the autoload tray is clear of any obstructions.
2. (Optional) Select **Export Data** to save the plate map file.

   NOTE

   Plate map files are saved to the following directory on the instrument computer: `C:\Users\Public\Seer\Proteograh\Runs\YYYYMMDD_BARCODE\`, where YYYYMMDD is the date and BARCODE is the collection plate barcode.

   WARNING

   You may ***copy*** plate map files to other locations on the instrument computer, but ***do not directly move or modify any files in the above directory***.

3. Select one of the following options:
   - **Unload Carriers and Run [MPE]² Flush** — Unload the carriers and automatically start the [MPE]² Flush method, which takes approximately 10 minutes to run.
   - **Unload Carriers Only** — Unload the carriers without running the [MPE]² Flush method.

     NOTE

     Seer recommends running the [MPE]² Flush method before proceeding to peptide quantification. For instructions on manually starting the [MPE]² Flush method after exiting the Proteograph Assay method, see *Run the [MPE]² Flush method* (page 78).

   After you select either option, the carriers immediately move from the work deck to the autoload tray.

   If you selected **Unload Carriers Only**, ICS returns to the main menu. If you selected **Unload Carriers and Run [MPE]² Flush**, ICS initiates setup for the [MPE]² Flush method.

   NOTE

   If you chose to run the [MPE]² Flush method, complete the following steps to unload assay labware before proceeding with the on-screen instructions to set up that method.

4. Immediately retrieve the Collection Plate, which contains the peptides:
   a. Remove the Collection Plate from the work deck.
   b. Seal the plate with an Aluminum Sealing Foil 5 × 3 Inch.
   c. Centrifuge the sealed plate at 2000 × g for 30 seconds.
   d. Set the plate aside at room temperature.

      WARNING

      You must begin peptide quantification within ***1 hour***.

5. Remove all other labware from the work deck:

   NOTE

   Leave the magnetic plate on the instrument.

   a. Remove all plastic labware from the tube, plate, tip, and reservoir carriers.
   b. Remove the Peptide Clean Up Plate from the [MPE]² module.

c. Remove the black CO-RE lids and store for future use.

d. Dispose of other labware and leftover reagents in the appropriate waste containers per laboratory policy.

6. Proceed to *Peptide Quantification* (page 53).

Chapter 5

Peptide Quantification

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

Peptide Quantification method

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

NOTE
Start quantification within ***one hour*** of removing the Collection Plate from the SP100. After peptides have been dried, they can be stored at -80 ˚C 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 quantify peptides.

Required materials

The Peptide Quantification method is designed to work with the Seer *Proteograph XT PQR Kit* (page 31) (PN S55R4007).

Additional required equipment and materials are listed below. (For supplier information, see *Required equipment* (page 32) and *Additional required materials* (page 32).)

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

Protocol steps

The Peptide Quantification protocol consists of the following steps:

1. *Prepare the instrument* (below)
2. *Prepare the quantification consumables* (below)
3. *Set up and run the Peptide Quantification method* (next page)
4. *Unload the work deck and dry peptides* (page 58)

Prepare the instrument

1. If the SP100 is not already on, turn on the instrument:

   NOTE

   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.

   a. Turn on the following hardware:
      - **SP100** — Press the green switch on the front of the instrument.
      - **[MPE]² power unit** — Press the power button on the front of the [MPE]² 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.

   b. Check the gas supply for appropriate pressure (≥110 psi).

   c. Confirm that the [MPE]² regulator (located near the [MPE]² waste container) is set between 105–110 psi.

   d. After the computer has initialized, select the **Proteograph ICS** desktop shortcut to launch ICS.

2. If you have not already done so today, run the Daily Maintenance method. (See *Run the Daily Maintenance method* (page 72).)

Prepare the quantification consumables

1. Remove the following reagents from 4 °C storage:
   - Peptide 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
4-Well Reservoir	1
Black Plate Lid	1
Black Quantitation Plate	1
Empty Tubes (2 mL)	8
Intermediate Plate	1
Peptide Assay Reagent Tubes	8
Peptide Digest Assay Standard Tube	1

Set up and run the Peptide Quantification method

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

On-screen prompts guide you through the remaining steps.

2. At **Sign In**, enter your username, and then select **Continue** to advance.

NOTE

Usernames can include up to 200 alphanumeric characters, including commas, dashes, periods, spaces, and underscores.

3. At **Deck Setup**, do the following:

   a. Place labware and reagents on the work deck according to the layout shown on-screen. Load items in the order listed. As you complete each step, select the corresponding checkbox on-screen.

CAUTION

Ensure correct orientation of the 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).

NOTE

Remove caps from tubes prior to placing them.

POSITION(S)	ITEM(S)	INSTRUCTION
T1: Slot 1	○ Peptide Digest Assay Standard Tube ○ Peptide Digest Assay Standard (reagent)	Transfer 100 µL of reagent to the Seer-barcoded tube, and then place the tube into the tube carrier.
T1: Slots 2-9	Empty Tubes (8)	Place a tube into each slot.
T1: Slot 10	n/a	Leave empty.

POSITION(S)	ITEM(S)	INSTRUCTION
T1: Slots 11-18	◦ Peptide Assay Reagent Tubes (8) ◦ Fluorometric Peptide Assay Reagent	Transfer 310 µL of reagent to each tube, and then place tubes into the tube carrier.
A3	4-Well Reservoir	Place on the work deck.
A3: Well 1	Peptide Elution Solution (10 mL)	Using a pipette, add to well 1 of reservoir. **NOTE** Do not pour the entire bottle. Return remainder, if any, to refrigerator.
A3: Well 4	Fluorometric Peptide Assay Buffer (12 mL)	Using a pipette, add to well 4 of reservoir. **NOTE** Do not pour the entire bottle. Return remainder, if any, to refrigerator.
A3	Black Plate Lid	Place lid on the reservoir.
B3	Intermediate Plate	Place on the work deck.
B4	Black Quantitation Plate	Place on the work deck.
B5	Collection Plate	Place on the work deck. **NOTE** If necessary, unseal the plate and remove any lid.
C1	300 µL NCTs (4 racks)	Place on the work deck.
All other positions	n/a	Leave empty.

b. Once you have selected all checkboxes on-screen, select **Continue** to advance.

4. At **Labware Check**, do the following:
   a. Verify the correct placement of each labware item on the work deck according to the layout shown on-screen. As you verify each item, select its corresponding checkbox on-screen.
   b. Select **Continue** to advance.
5. At **Begin Labware Barcoding**, do the following:
   a. Ensure that barcodes are fully visible.
   b. Orient all labware with barcodes facing the barcode scanner.
   c. Ensure that each carrier is pushed forward against the stop hooks at the back of the autoload tray.
   d. Select **Continue** to advance.

   ICS moves the carriers from the autoload tray to the work deck and begins barcode scanning.

NOTE

The barcode reader scans each barcode as a final check to confirm proper work deck setup.

6. At **Peptide Quantification – Setup Complete**, do the following:
    a. Close the front protective cover.
    b. Select **Run** to start the method.

       ICS locks the front protective cover.

The method takes approximately 23 minutes to complete. When the method has finished, proceed to *Unload the work deck and dry peptides* (below).

WARNING

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

Unload the work deck and dry peptides

The **Incubation Complete** screen indicates the successful completion of the method.

1. At **Incubation Complete**, do the following:
    a. Open the front protective cover.
    b. Immediately remove the Collection Plate from the work deck. (This plate contains the quantified peptides.)
    c. Seal the Collection Plate with the Aluminum Sealing Foil 5 × 3 Inch.
    d. Set the Collection Plate aside at room temperature until you are ready to proceed with drying peptides (see below).
    e. Read fluorescence data from the Black Quantitation Plate:
        i. Remove the Black Quantitation Plate from the work deck and move it to a microplate reader for fluorescence analysis.
        ii. Save the resulting fluorescence data as a comma-separated values (.csv), tab-separated values (.tsv), or Microsoft Excel (.xlsx) file.

            NOTE

            The raw fluorescence values should be saved in a 96-well plate format (12 columns by 8 rows), without headers or any other text.

    f. Select **Continue** to advance.

       NOTE

       Do not dry peptides until you reach the next screen.

2. At **Peptide Quantification Complete**, do the following:
    a. Select **Calculate Concentrations**.

        The **Reconstitution Volume** screen appears. On this screen, do the following:

        i. In the **Input Fluorescence Data** section of the screen, input relative fluorescence unit (RFU) values using either of the following methods:

            - Transfer the .csv, .tsv, or .xlsx file with the raw fluorescence data to the instrument computer using a USB drive, and then upload the file by selecting **Load Fluorometer Data**.

                NOTE

                The name of the file must include a barcode number matching the Collection Plate.

            - Copy and paste RFU values into ICS (using keyboard commands `Ctrl-C` and `Ctrl-V`).

        Once you have added this fluorescence data, other sections of the **Reconstitution Volume** screen will populate with additional information.

        ii. Using the **Peptide Concentration**, **Peptide Recovered**, and **Outliers** sections of the screen, confirm that all wells have recorded concentrations and yields of peptides within expected ranges.

        For detailed information about the sections of the **Reconstitution Volume** screen and how to use them, see *Reconstitution Volume Screen* (page 89).

        NOTE

        If any wells fail to show peptides, contact your Seer field service representative or Seer support for assistance before proceeding to dry peptides.

        iii. Within the **Reconstitution** section of the screen, modify parameters as appropriate to suit your experimental criteria. Once you have done so, write down the resulting values for **Average Recon (µL)** that appear at the bottom of this section. (You will need these values during setup for the Peptide Reconstitution method.)

        iv. Select **Continue** to return to the **Peptide Quantification Complete** screen.

    b. Dry the quantified peptides:

        i. (Optional) Freeze the sealed Collection Plate at -80 °C for at least 15 minutes. Then remove it from the freezer.

            TIP

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

        ii. Unseal the plate.

        iii. Transfer the plate to a vacuum concentrator.

        iv. Confirm that the vacuum concentrator is balanced appropriately.

        v. Set the temperature to ≤ 20 °C.

vi. Run the vacuum concentrator until the peptides are fully dried, which can take several hours.

> **TIP**
> Seer recommends running the vacuum concentrator overnight.

vii. When the vacuum concentrator is finished, visually confirm that the peptides are fully dried.

c. Unload all other labware from the work deck and appropriately discard reagents and labware.

d. Select one of the following options:

i. Select **Main Menu** to return to the ICS main menu.

ii. Select **Flush [MPE]²** to run the [MPE]² Flush maintenance method. (See *Run the [MPE]² Flush method* (page 78).)

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

3. If you intend to reconstitute peptides immediately, proceed to *Peptide Reconstitution* (page 61).

Otherwise, if you intend to reconstitute peptides later:

a. Seal the Collection Plate with an Aluminum Sealing Foil 5 × 3 Inch and store at -80 °C.

> **TIP**
> You can store peptides this way for up to one year before reconstitution.

b. 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]² power unit** — Press the power button on the front of the [MPE]² 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 6

Peptide Reconstitution

This chapter describes 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 XT PQR Kit* (page 31) (PN S55R4007).

Additional required equipment and materials are listed below. (For supplier information, see *Required equipment* (page 32) and *Additional required materials* (page 32).)

Additional required materials

- 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* (below))
- 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

PRODUCT NAME / DESCRIPTION	SUPPLIER / MANUFACTURER	CATALOG / PART NUMBER
Microcentrifuge	Thermo Fisher Scientific	75002451 or equivalent

Reagents and materials

PRODUCT NAME / DESCRIPTION	SUPPLIER	ORDER NUMBER	STORAGE TEMPERATURE	NOTES
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	a
Water, HPLC-grade	Fisher Chemical or equivalent	W5-4 or equivalent	Room temperature	

a 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.

Protocol steps

The Peptide Reconstitution protocol consists of the following steps:

1. *Prepare the instrument* (below)
2. *Prepare the reconstitution consumables* (next page)
3. *Set up and run the Peptide Reconstitution method* (next page)
4. *Unload the work deck and export data* (page 69)

> NOTE
> Before beginning the reconstitution protocol, ensure that you have already prepared and stored the Peptide Reconstitution Buffer. See *Peptide Reconstitution Buffer preparation* (page 62).

Prepare the instrument

1. If the SP100 is not already on, turn on the instrument:

> NOTE
> 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.

    a. Turn on the following hardware:
        - **SP100** — Press the green switch on the front of the instrument.
        - **[MPE]² power unit** — Press the power button on the front of the [MPE]² 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.

b. Check the gas supply for appropriate pressure (≥110 psi).

c. Confirm that the [MPE]² regulator (located near the [MPE]² waste container) is set between 105–110 psi.

d. After the computer has initialized, select the **Proteograph ICS** desktop shortcut to launch ICS.

2. If you have not already done so today, run the Daily Maintenance method. (See *Run the Daily Maintenance method* (page 72).)

Prepare the reconstitution consumables

1. If necessary, remove the 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 Peptide Control:
   a. Remove from -20 °C storage.
   b. Add 24 µl Peptide Reconstitution Buffer to the MS Peptide 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.

Set up and run the Peptide Reconstitution method

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

On-screen prompts guide you through the remaining steps.

2. At **Sign In**, enter your username, and then select **Continue** to advance.

   NOTE

   Usernames can include up to 200 alphanumeric characters, including commas, dashes, periods, spaces, and underscores.

3. At **Deck Setup**, do the following:

   a. Calculate the total volume of Peptide Reconstitution Buffer to add to the Reconstitution Buffer Reservoir. ***Use the larger of the two following values:***

      - Retrieve the value for **Average Recon (µL)** that you recorded after completing the Peptide Quantification method. Multiply this value by the number of wells in the Collection Plate containing peptides (including controls), and then add 1 mL (for overage).

        NOTE

        If you did not record the above value after peptide quantification, contact Seer support for assistance.

      - Alternatively, use a minimum total volume of 2 mL.

        CAUTION

        If you add a total buffer volume of less than 2 mL, the instrument's pipettes may fail to pick up the liquid.

   b. Place labware and reagents on the work deck according to the layout shown on-screen. Load items in the order listed. As you complete each step, select the corresponding checkbox on-screen.

POSITION(S)	ITEM	INSTRUCTION
R5	Reconstitution Buffer Reservoir	Place on the work deck.
R5	Peptide Reconstitution Buffer	Using a pipette, add the buffer to the reservoir. (See volume calculations above.)
B1	300 µL NCTs (1 rack)	Place on the work deck.
B5	Collection Plate	Place on the work deck. NOTE If necessary, unseal the plate.
All other positions	n/a	Leave empty.

   c. Once you have selected all checkboxes on-screen, select **Continue** to advance.

4. At **Labware Check**, do the following:

   a. Verify the correct placement of each labware item on the work deck according to the layout shown on-screen. As you verify each item, select its corresponding checkbox on-screen.

   b. Select **Continue** to advance.

5. At **Begin Labware Barcoding**, do the following:

a. Ensure that barcodes are fully visible.

b. Orient all labware with barcodes facing the barcode scanner.

c. Ensure that each carrier is pushed forward against the stop hooks at the back of the autoload tray.

d. Select **Continue** to advance.

ICS moves the carriers from the autoload tray to the work deck and begins barcode scanning.

> NOTE
> The barcode reader scans each barcode as a final check to confirm proper work deck setup.

6. At **Reconstitution Volume**, do the following:

a. In the **Input Fluorescence Data** section, input the relative fluorescence unit (RFU) values you obtained after peptide quantification using either of the following methods:

- Transfer the .csv, .tsv, or .xlsx file with the raw fluorescence data to the instrument computer using a USB drive, and then upload the file by selecting **Load Fluorometer Data**.

  > NOTE
  > The name of the file must include a barcode number matching the Collection Plate.

- Copy and paste RFU values into ICS (using keyboard commands `Ctrl-C` and `Ctrl-V`).

Once you have added this fluorescence data, other sections of the **Reconstitution Volume** screen will populate with additional information.

b. Carefully review the information shown under all sections of the screen. Where applicable, modify parameters to suit your experimental needs.

> NOTE
> Expand and collapse a section by selecting its arrow icon (at right).

For detailed information about the different sections of this screen and how to use them, see the following in *Appendix B. Reconstitution Volume Screen* (page 89):

- *Input Fluorescence Data* (page 90)
- *Reconstitution* (page 90)
- *Standard Curve* (page 91)
- *Peptide Concentration* (page 91)
- *Peptide Recovered* (page 91)
- *Outliers* (page 91)

> WARNING
> You must set appropriate values for the **Min Recon Volume (µL)**, **On-column load (µg)**, and **Loading volume (µL)** parameters before proceeding to peptide reconstitution.

c. Once you have set all parameters to the desired values, select **Continue** to advance.

7. At **Peptide Reconstitution – Setup Complete**, do the following:

a. Close the front protective cover.

b. Select **Run** to start the method.

   ICS locks the front protective cover.

The method takes approximately 6 minutes to complete. When the method has finished, proceed to *Unload the work deck and export data* (next page).

WARNING

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

Unload the work deck and export data

The **Peptide Reconstitution Complete** screen indicates the successful completion of the method.

1. At **Peptide Reconstitution Complete**, do the following:
    a. Select **Export Data** to save the plate mapping file with reconstitution data.
    b. Unload labware from the work deck:
        i. Open the front protective cover.
        ii. Remove the Collection Plate from the work deck.
        iii. Add 24 µL reconstituted MS Peptide Control to an empty well.

        Wells E11–H11 and all wells in column 12 are empty.
        iv. Seal the 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.

        v. Discard all other used labware and reagents appropriately.
    c. Select **End Method** to return to the ICS main menu.
2. 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]² power unit** — Press the power button on the front of the [MPE]² 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 7

Instrument Maintenance

This chapter describes 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]² Flush methods are part of normal instrument operation. Run the Water Run method as needed for training or troubleshooting.

Table 9. Maintenance methods

ICON	METHOD	APPROXIMATE DURATION	FREQUENCY
	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]² Flush	10 minutes	After running the Proteograph Assay method
	Water Run*	10 minutes	During training or troubleshooting with Seer support

* The Water Run method is accessible from ICS's **Demo and Training Materials** menu, rather than the **Maintenance** menu.

TIP

If an error occurs during a maintenance method, try to resolve the issue and then repeat the method. If the issue persists, contact your field service representative or Seer support. See *Technical Support* (page 98) 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.

Required materials for maintenance

All maintenance methods require the following materials. (For supplier information, see *Additional required materials* (page 32).)

- Personal protective equipment:
  - Disposable latex gloves
  - Lab coats
  - Protective goggles
- Waste Bags with Biohazard Labeling
- Waste Container Biohazard Box

Weekly maintenance requires additional materials; see *Required materials for weekly maintenance* (next page).

Maintenance history

The ICS main menu indicates the most recent date each maintenance method was run at the bottom of the screen. (This information is also available on the **Support** screen, in the **Instrument** section.)

Daily maintenance

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

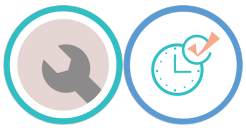
This maintenance method should be run at the start of each day, before running any peptide preparation methods on the instrument. (Typically, this will mean running the Daily Maintenance method before proceeding to either the Proteograph Assay or Peptide Reconstitution method.)

NOTE

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

Run the Daily Maintenance method

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



2. At **Waste Check**, do the following:

CAUTION

Failure to empty the liquid waste container can damage the [MPE]² module.

   a. Inspect the liquid waste container to determine how full it is.
   b. If the liquid level is at or above the indicated fill level, 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.
   c. Select the **Completed Waste Check** checkbox.
   d. Select **Continue** to advance.
3. At **Deck**, do the following:

a. Open the front protective cover and check whether the deck is clean.
    - If the deck is not clean, select **Abort** and perform weekly maintenance instead of daily maintenance. See *Run the Weekly Maintenance method* (next page).
    - If the deck is clean, select **Continue** to proceed with daily maintenance.

4. At **Tip Waste**, empty the tip waste container, and then select **Continue**.

> **WARNING**
> Failure to dispose of tip waste can cause damage to the instrument, assay failure, or contamination.

5. At **Close Cover**, close the front protective cover, and then select **Continue**.
6. At **Tightness Check 1000µl Channel**, do the following:
    a. Confirm that the teaching needles are on the work deck. If they are not, load them onto the work deck.
    b. Select **Yes** to execute the check.
7. At **cLLD Check 1000µl Channel**, do the following:
    a. Confirm that the teaching needles are on the work deck. If they are not, load them onto the work deck.
    b. Select **Yes** to execute the check.
8. The **Complete** screen indicates the successful completion of the method. Select **Continue** to return to the ICS main menu.

> **NOTE**
> The **Incomplete** screen will appear instead if you opted to skip either the **Tightness Check 1000µl Channel** or **cLLD Check 1000µl Channel** step. Do not proceed with any peptide preparation methods until the Daily Maintenance method has been completed in full.

Weekly maintenance

The Weekly Maintenance method performs the same checks as the Daily Maintenance method, plus additional steps to clean instrument hardware. A series of on-screen prompts guides you through each step.

Weekly maintenance should be performed every week, ***even if you did not otherwise operate the instrument during the preceding week***.

> **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 98) for contact information.

Required materials for weekly maintenance

In addition to the materials required for all maintenance methods (see *Required materials for maintenance* (page 71)), the Weekly Maintenance method requires the following materials. (For supplier information, see *Additional required materials* (page 32).)

- 70% isopropyl alcohol or 70% ethanol
- Deionized water

- Kimwipes or similar lint-free tissues

Hardware cleaning guidance

Observe the following guidance when cleaning the instrument hardware.

- For cleaning the pipetting channels and acrylic panels, use deionized water.

  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.

  WARNING

  Do not use disinfectants that contain hypochlorite or bleach.

- When cleaning any part of the instrument, spray the appropriate cleaning solution onto a Kimwipe or similar lint-free tissue, then wipe down the instrument surface.

  CAUTION

  Do not spray cleaning solutions directly inside the work deck.

Run the Weekly Maintenance method

Before proceeding, ensure you are familiar with *Hardware cleaning guidance* (above).

CAUTION

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

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 **Waste Check**, do the following:

   CAUTION

   Failure to empty the liquid waste container can damage the [MPE]² module.

   a. Inspect the liquid waste container to determine how full it is.

   b. If the liquid level is at or above the indicated fill level, 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.

c. Select the **Completed Waste Check** checkbox.

d. Select **Continue** to advance.

3. At **Prepare SP100**, do the following:

a. Clear any obstructions from the path of the barcode scanner.

b. Close the front protective cover.

c. Select **Run** to advance.

4. At **Turn off SP100**, do the following:

a. Turn off the instrument.

NOTE

A message may appear indicating that connection to the instrument has been lost.

b. Once the instrument is off, select **Continue** to advance.

5. At **Clean CO-RE Head O-rings**, do the following:

a. Open the front protective cover.

b. Prepare several lint-free tissues dampened with deionized water.

CAUTION

Never spray cleaning solutions directly onto the instrument; always spray directly onto Kimwipes or other lint-free tissues. Do not get liquid inside tip channels.

c. Floss each tip channel row and column back and forth 2 or 3 times. Change tissues frequently.

CAUTION

Dispose of used tissues in accordance with your institution's hazardous waste disposal practices.

d. Select **Continue** to advance.

6. At **Clean 8 channel O-rings**, do the following:

a. Prepare a lint-free tissue dampened with deionized water.

CAUTION

Never spray cleaning solutions directly onto the instrument; always spray directly onto Kimwipes or other lint-free tissues. Do not get liquid inside tip channels.

b. For each pipette, lift up its tip eject sleeve (the outer part of the pipetting channels) and gently clean the O-ring and stop disk.

> **CAUTION**
> Dispose of used tissues in accordance with your institution's hazardous waste disposal practices.

c. Select **Continue** to advance.

7. At **Clean Additional Items**, do the following:
   a. Clean the tip ejector plate:
      i. Remove the tip ejector plate (the metal bracket where the pipette tips are ejected).
      ii. Prepare a lint-free tissue dampened with 70% isopropyl alcohol.
      iii. Wipe the tip ejector plate.
      iv. Replace the tip ejector plate.
   b. Clean the front protective cover:
      i. Prepare a lint-free tissue dampened with deionized water.
      ii. Wipe the inside and outside of the cover.

      > **NOTE**
      > Cleaning the front protective cover prevents dust or other foreign material from falling onto the work deck and contaminating an assay.

   c. Select **Continue** to advance.
8. At **Turn on SP100**, do the following:
   a. Close the front protective cover.
   b. Turn on the instrument. (There will be a short delay after the instrument is turned on.)
   c. Select **Continue** to advance.
9. At **Autoload Tray**, do the following:
   a. Check to make sure that there are no carriers on the autoload tray.
   b. Select **Continue** to advance.
10. At **Carriers**, do the following:
    a. Pull all carriers out onto the autoload tray, and then remove them from the tray.
    b. Clean all carriers.
    c. Select **Continue** to advance.
11. At **Deck**, do the following:
    a. Open the front protective cover.
    b. Clean the work deck.
    c. Select **Continue** to advance.
12. At **Close Cover**, do the following:

a. Close the front protective cover.

b. Select **Continue** to advance.

13. At **Tip Waste**, do the following:

a. Open the front protective cover.

b. Empty the tip waste container.

> **WARNING**
>
> Failure to dispose of tip waste can cause damage to the instrument, assay failure, or contamination.

c. Clean the tip waste container.

d. Select **Continue** to advance.

14. At **Close Cover**, do the following:

a. Close the front protective cover.

b. Select **Continue** to advance.

15. At **Scanner**, do the following:

a. Prepare a lint-free tissue or cotton swab dampened with 70% isopropyl alcohol or 70% ethanol.

b. Clean the laser scanner window of the barcode reader.

c. Select **Continue** to advance.

16. At **Tightness Check 1000µl Channel**, do the following:

a. Confirm that the teaching needles are on the work deck. If they are not, load them onto the work deck.

b. Select **Yes** to execute the check.

17. At **cLLD Check 1000µl Channel**, do the following:

a. Confirm that the teaching needles are on the work deck. If they are not, load them onto the work deck.

b. Select **Yes** to execute the check.

18. The **Complete** screen indicates the successful completion of the method. Do the following:

a. Return all carriers to the autoload tray, beginning with Tube Carrier 1 and working from left to right.

b. Select **Continue** to return to the ICS main menu.

> **NOTE**
>
> The **Incomplete** screen will appear instead if you opted to skip either the **Tightness Check 1000µl Channel** or **cLLD Check 1000µl Channel** step. Do not proceed with normal instrument operation each week until the Weekly Maintenance method has been completed in full.

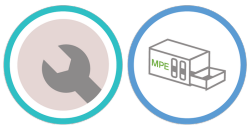
[MPE]² Flush

The [MPE]² Flush method cleans the [MPE]² waste tray and waste tubing, in order to prevent future clogging or other adverse effects.

This maintenance method should be performed following the Proteograph Assay method. At the end of the Proteograph Assay method, ICS prompts you to immediately run the [MPE]² Flush method. (Alternatively, you can initiate the method at any time from the **Maintenance** menu.)

Run the [MPE]² Flush method

1. On the ICS main menu, select **Maintenance**, and then select **[MPE]² Flush**.



2. At **Waste Check**, do the following:

> **CAUTION**
> Failure to empty the liquid waste container can damage the [MPE]² module.

   a. Inspect the liquid waste container to determine how full it is.
   b. If the liquid level is at or above the indicated fill level, 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.
   c. Select the **Completed Waste Check** checkbox.
   d. Select **Continue** to advance.

3. At **Load Reagents**, do the following:
   a. Place labware and reagents on the work deck according to the layout shown on-screen. Load items in the order listed. As you complete each step, select the corresponding checkbox on-screen.

POSITION	ITEM	INSTRUCTION
A2	Corona Wash Reservoir	Place on deck.
A2	70% isopropyl alcohol (~200 mL)	Add to reservoir.
B1	300 µL NCTs (1 rack)	Place on deck.
All other positions	n/a	Leave empty.

b. Once you have selected all checkboxes on-screen, select **Continue** to advance.

4. At **[MPE]² Initialization**, do the following:
   a. Verify there is no plate in the [MPE]² module's Filter Plate Adapter.
   b. Select **Initialize**.

      The [MPE]² module will test that the vacuum and shuttle are functioning correctly.

   The method begins to run.
5. The **[MPE]² Flush Complete** stage indicates the successful completion of the method. Do the following:
   a. Remove the reservoir and remaining 300 µL NCTs from the work deck.
   b. Select **OK** to return to the ICS main menu.

Water Run

The Water Run method may be run as needed for training or troubleshooting.

This maintenance method uses the same work deck layout as the Proteograph Assay method but directs you to use deionized water in place of reagents.

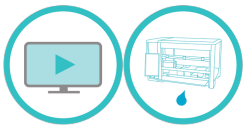
NOTE
Any laboratory-grade deionized water is sufficient for use with this method.

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.

NOTE
This method uses the 40-sample Proteograph Assay workflow, so you won't be prompted to select the number of samples. You also won't be prompted to sign in.

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



2. Follow the workflow for the Proteograph Assay method, starting at the **Registration** step. See *Set up and run the Proteograph Assay method* (page 44).

3. When ICS prompts you to load the work deck (beginning at **Deck Setup 1 of 4**), replace reagents with the minimum amounts of deionized water described below:

   a. At **Deck Setup 1 of 4**:

ITEM	DEIONIZED WATER VOLUME (MINIMUM)	POSITION
Corona Wash Reservoir	200 mL	A2
6-Well Digestion Reservoir	○ Well 1: 25 mL ○ Well 2: 7 mL ○ Well 3: 7 mL ○ Well 4: 7 mL ○ Well 5: 14 mL ○ Well 6: 22 mL	A3

   b. At **Deck Setup 2 of 4**:

ITEM	DEIONIZED WATER VOLUME (MINIMUM)	POSITION
Empty Reservoirs	0 mL	R1, R2
Wash A Reservoir	40 mL	R3
Wash B Reservoir	54 mL	R4
Elution Reservoir	20 mL	R5

   c. At **Deck Setup 3 of 4**, not applicable.

   d. At **Deck Setup 4 of 4**:

ITEM	DEIONIZED WATER VOLUME (MINIMUM)	POSITION
Sample Tubes (01–40)	240 µL	T1: Slots 1–32 T2: Slots 1–8
Plasma Control	0 µL	T2: Slot 9
MPE Control	0 µL	T2: Slot 10
Nanoparticle Tubes	0 µL	T2: Slots 11–14

4. 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.

See also ICS Error Reference in the ICS Help system.

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 on-screen 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.

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. At **Abort Completed**, do one of the following. If needed, contact your field service representative or Seer support. See *Technical Support* (page 98) for contact information.

- Select **Main Menu** to exit.
- ***Only when directed by your field service representative***, select **Advanced Recovery Mode**, and then follow the directions you are given.
- If directed by your field service representative, select **Export Support Package** to export machine information to a file, which you can then share with Seer support. Specify the start and end date of the data of interest, browse to a location to save the file, and select **Process**.

4. Dispose of all labware, plasma samples, and leftover reagents in the appropriate waste containers per laboratory policy.

Resolve error messages

As you run ICS methods, you may encounter recoverable errors. For information on specific error messages, see ICS Error Reference in the ICS Help system. For general advice on how to prevent errors, see *Best practices* (page 37).

If an error persists or the instrument experiences intermediate or critical failures, contact your field service representative or Seer support. See *Technical Support* (page 98) for contact information. Include any error code that ICS displays.

Package trace and log files

If you encounter a support issue and must send documentation to Seer, use the following instructions to package the necessary trace and log files. If you need assistance, contact Seer support. See *Technical Support* (page 98) for contact information.

1. Open a dialog box with options to export trace and log files from ICS using ***either*** of the following methods:
   - Within ICS, on the main menu, select **Support**, and then select **Export Support Package**.
   - On the desktop of the instrument computer, open the **Proteograph Support Tool**.
2. Choose a start and end date for the trace and log files you want to export.
3. Specify the location where you want to save the exported files, then select **Process**.

   The software places a .zip file in the specified location.
4. 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.

Table 10. Hazardous reagents

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

Safety hazards

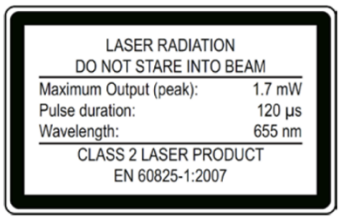
The following symbols identify safety hazards to consider when operating the instrument.

Table 11. 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]² 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.

Table 12. *Certification 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.

Appendix B

Reconstitution Volume Screen

This appendix provides detailed information about the contents of the **Reconstitution Volume** screen.

This screen appears at the end of the Peptide Quantification method (by selecting **Calculate Concentrations**) and during setup for the Peptide Reconstitution method.

Input Fluorescence Data

Use this section of the **Reconstitution Volume** screen to input relative fluorescence unit (RFU) values obtained after peptide quantification. Values are displayed in a table, showing each well of the 96-well Collection Plate.

- **Clear Data** — Clears the table of all previously input fluorescence data.
- **Load Fluorometer Data** — Select to load a .csv or .xlsx file with fluorescence values in a 96-well plate format (12 columns by 8 rows), without headers or any other text.

NOTE
The name of the file must include a barcode number matching the Collection Plate.

Alternatively, RFU values may be input by copying and pasting the values into ICS (using keyboard commands `ctrl-c` and `ctrl-v`), or by manually typing the values into each cell of the table.

Once you have input fluorescence data, other sections of the **Reconstitution Volume** screen will populate with additional information.

Reconstitution

This section displays modifiable parameters and other information pertaining to peptide reconstitution.

- **Title Bar** — If any wells have a reconstitution volume set below the user-defined minimum, a warning will appear in the title bar for this section indicating the number of wells below the threshold.
- **Final Assay Volume per Well (µL)** — This value should be `142`, as this is the volume of peptides collected at the end of the Proteograph Assay method.
- **Times Peptide Quantification Performed** — This value is set to `1` by default; if you have performed quantification on the same assay more than once, increment this value accordingly. This value is used to calculate the Remaining Total Volume per Well (each peptide quantification run consumes 10 µL of the original 142 µL).
- **Remaining Total Volume per Well (µL)** — This value is calculated by multiplying the Times Peptide Quantification Performed by 10 µL and subtracting that total from the original 142 µL. (For a typical assay with a single peptide quantification run performed, this should be 132 µL.)
- **Min Recon Volume (µL)** — This ***user-defined*** value sets the minimum reconstitution volume per well. If peptide yield is low, other user-defined values (described below) may result in a reconstitution volume below this threshold. Any wells with reconstitution volumes below this threshold will be highlighted red, and an error message will appear if the user attempts to proceed with reconstitution without having addressed the aberrant wells.
- **On-column load (µg)** — These ***user-defined*** values represent the mass of peptides you want to load onto your LC-MS system. The default value is 0.2 µg, but you may set values for each nanoparticle and each control well independently if desired.
- **Loading volume (µL)** — These ***user-defined*** values represent the volume of peptides you want to load onto your LC-MS system. The default value is 4 µL, but you may set values for each nanoparticle and each control well independently if desired.
- **Concentration (µg/µL)** — This value represents the final expected concentration of reconstituted peptides, calculated by dividing the on-column load by the loading volume.

> **TIP**
>
> Adjust the on-column load and the loading volume to achieve the desired concentration.

- **Expected Final Reconstitution Volume (µL)** — This table shows the expected reconstitution volume for each well based on the user-defined settings described above and the calculated peptide yields for each well. (Calculated peptide yields are in turn based on the RFU values input in the **Input Fluorescence Data** section.)
- **Average Recon (µL)** and **Median Recon (µL)** — These are the average and median reconstitution volumes (per well) for each of the two nanoparticles. These values can be used to determine the total volume of Peptide Reconstitution Buffer to add during setup for the Peptide Reconstitution method.

Standard Curve

This section provides information about the standard curve of the fluorescence data that was input above.

The standard curve is calculated as a linear fit and displayed as a graph on the righthand side. Data tables on the lefthand side list the concentrations of the 8 standards and their corresponding RFU values, as well as the slope, intercept, and R^2 values of the calculated curve. (The R^2 value is also displayed on the title bar of this section.)

Peptide Concentration

This section displays a table showing the final concentrations of peptides in each well recovered during the assay (in µg/µL), as calculated based on the resulting fluorescence data and the standard curve.

Below this table, the average and median concentrations of peptides obtained from samples incubated with each of the two nanoparticles are shown.

Peptide Recovered

This section displays a table showing the recovered final mass of peptides in each well (in µg).

Below this table, the average and median yields of peptides obtained from samples incubated with each of the two nanoparticles are shown.

> **NOTE**
>
> The yields shown in this table refer to the total yield of the assay, before any volume is removed for peptide quantification.

Outliers

This section provides a tool allowing you to quickly identify any wells that performed significantly differently from similar wells. Use this section to check for unexpected results that may require troubleshooting.

A table displays the ratio of the recovered peptide mass in each well to the median mass for all wells incubated with that nanoparticle (as displayed in the **Peptide Recovered** section above). Using the **Lower Threshold** and **Upper Threshold** parameters, you can set minimum and maximum acceptable ratios. Any wells below the minimum or above the maximum thresholds will be highlighted in red, and a warning indicating the number of outliers will appear in the title bar.

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
Proteograph Instrument Control Software.

L

LC
Liquid chromatography.

LC-MS
Liquid chromatography mass spectrometry.

M

materials
Consumables and equipment.

MPE Control
Reference peptides added before desalting cleanup.

MS
Mass spectrometry.

MS Peptide 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
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 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.

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

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.

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