

USER GUIDE

Proteograph® Product Suite

FOR USE WITH
SP200 Automation Instrument
Proteograph® ONE PQR Kit
Proteograph® ONE Assay Kit (40 Samples)
Proteograph® ONE Assay Kit (80 Samples)
Proteograph® DIRECT Assay Kit (80 Samples)

Notice

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Revision history

Proteograph® Product Suite

DOCUMENT	DATE	DESCRIPTION OF CHANGE
CF-1089 B	August 2025	Added information about the SP200-T configuration
CF-1089 A	June 2025	Initial release

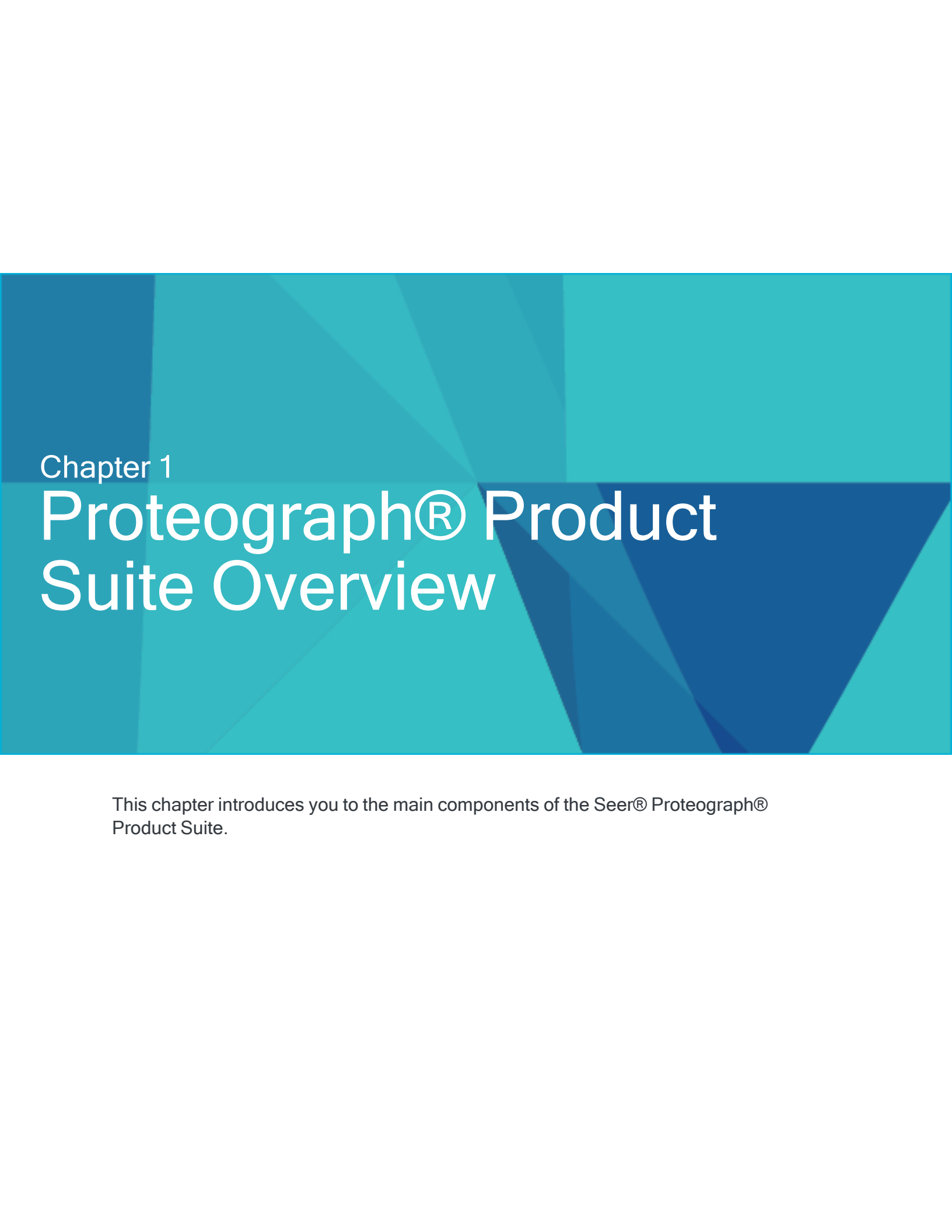
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Chapter 1

Proteograph® Product Suite Overview

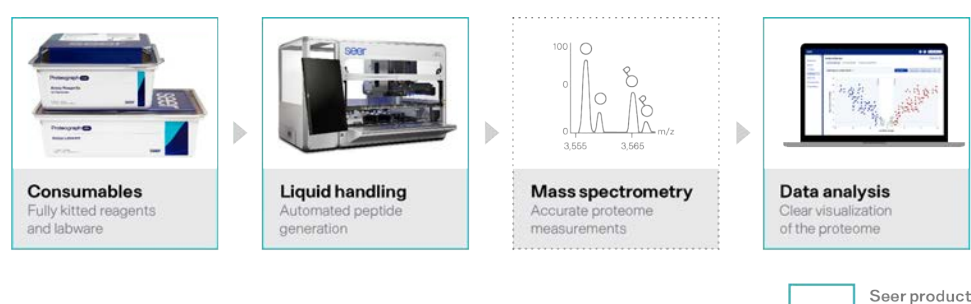
This chapter introduces you to the main components of the Seer® Proteograph® Product Suite.

Introduction

The Seer Proteograph® Product Suite provides integrated workflows for unbiased, deep, and large-scale access to the proteome. The Proteograph automated peptide preparation workflows leverage Seer's proprietary engineered nanoparticles in combination with the reagents, plasticware, and the SP200 Automation Instrument hardware needed to process a biosample into a single injection of mass spectrometry (MS) ready peptides. Following data acquisition, 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.

The following figure shows the high-level components that comprise the Proteograph Product Suite in the Proteograph ONE workflow with the products used at each step.

Figure 1. *Proteograph ONE workflow with products used*



The Proteograph Product Suite includes

- Proteograph ONE and DIRECT Assay Kits
 - Proprietary panel of nanoparticles used for protein corona formation in 40 and 80 sample Proteograph ONE assays
 - Proprietary nanoparticles used for peptide cleanup
 - Optimized reagents for corona formation, digestion, and purification steps
 - Plasticware for sample preparation and handling
 - Quality controls to compare results across assays or troubleshooting run issues
 - Additional support kits for quantifying and reconstituting peptides
- SP200 Automation Instrument
 - Automated workflow methods to go from proteins to peptides in a standard 8-hr work day
 - Intuitive Instrument Control Software (ICS) to set up and run the automated workflows
 - Methods for instrument maintenance and troubleshooting
- Proteograph Analysis Suite
 - Cloud based software for processing MS data, generating data visualizations and biological insights, documented separately in the Proteograph Analysis Suite user guide

The Proteograph Assay workflow

The Proteograph Product Suite has several automated assay methods that run on the SP200 Automation Instrument to output MS-ready peptides. The Proteograph ONE Assay methods process proteins using Seer's proprietary nanoparticles, and has method options for generating either 40 sample or 80 samples of peptides on a single 96 well plate. The Proteograph DIRECT Assay methods provide an automated option for direct digestion of proteins into peptides in an 80 sample format.

Overall, the workflow proceeds as follows:

Figure 2. Proteograph assay workflow



Details of the method steps are provided below, with the estimated duration and overall cumulative time provided for planning purposes. The average experienced user can expect to go from protein samples to quantified peptides in approximately 6.5 hours, with the resulting Collection Plate dried down and ready for -80 °C storage overnight.

Table 1. Timing of the Proteograph ONE Assay Steps

STEP	Approximate Time		Approx Cumulative Time (hours)
	80 sample	40 sample	
Proteograph Assay Setup	60 min ^a	45 min ^a	1.0
Sample and NP Dispense	35 min	25 min	1.5
Protein Corona and Wash	95 min		3.2
Denaturation and Digestion	105 min		4.9
Peptide Cleanup	55 min		5.9
Deck Cleanup	10 min ^a		6.0
Peptide Quantification	30 min ^b		6.5
Peptide Drying	15 min ^a to load then Dry Overnight		6.75
STOP POINT			
Peptide Reconstitution	15 min		7.0 ^c

^a Hands-on time.

^b Approximately 10 minutes are hands-on.

^c The DIRECT assay workflow excludes nanoparticle dispense and corona formation steps and completes in approximately 5.2 hours.

The Proteograph Product Suite

The Proteograph® Product Suite enables high-throughput, unbiased, deep, rapid proteomics analysis of sample types like plasma/serum, and cell and tissue lysate samples, at scale. The suite consists of the SP200 Automation Instrument, its Instrument Control Software, assay kits, and the Proteograph Analysis Suite software, which integrates with existing liquid chromatography-mass spectrometry (LC-MS) ecosystems.

SP200 Automation Instrument

The SP200 Automation Instrument (SP200) automates the sample processing workflows to prepare peptides for downstream analysis, enabling efficient experiment set-up, rapid protein processing, in a high throughput and reproducible manner. The Instrument Control Software provides the user interface point on a glove compatible touchscreen monitor attached to the instrument.

For a detailed overview of the SP200, including the instrument computer, work deck, and hardware components, see [Chapter 2 SP200 Automation Instrument Overview \(page 13\)](#).

NOTE

Additional specifications are described in the *Site Preparation Guide*.

Proteograph Instrument Control Software

The Proteograph Instrument Control Software (ICS) controls SP200 instrument operations, providing an interface for running assays and other methods and maintaining or troubleshooting the instrument. This software is preloaded onto the SP200 and its desktop icon appears on the home screen.

For more information about the ICS, workflows, and methods, see [Proteograph Instrument Control Software \(page 15\)](#) and [Chapter 4 Proteograph Assay \(page 39\)](#).

Proteograph Assay Kits

The Proteograph Product Suite uses the following assay and accessory kits for processing samples:

- **Proteograph® ONE Assay Kit (40 Samples):** a complete nanoparticle-based assay for processing 40 samples in a single 96-well plate.
- **Proteograph® ONE Assay Kit (80 Samples):** a complete nanoparticle-based assay for processing 80 samples in a single 96-well plate.
- **Proteograph® DIRECT Assay Kit (80 Samples):** a complete direct digestion assay for processing 80 samples in a single 96-well plate.
- **Proteograph® ONE PQR Kits:** an accessory kit for peptide quantification and reconstitution
- **Peptide Calibration Kit:** an accessory kit with spike in peptides and a preformulated elution buffer for peptide reconstitution. The peptide spike is utilized in the Proteograph Analysis Suite for normalization in MS related batch effects for large scale studies.

For more information on the assay kits, see [Kit contents and storage requirements \(page 28\)](#).

Proteograph Analysis Suite

The Proteograph Analysis Suite enables you to visualize mass spectrometry data, transforming raw data to biological insights, supporting efficient data processing and visualizations for large-scale studies.

This product is documented separately in the [Proteograph Analysis Suite Help system](#).



Chapter 2

SP200 Automation Instrument Overview

The SP200 Automation Instrument is a compact liquid handling robotic system that is used to automate the Proteograph ONE and DIRECT Assay workflows.

A number of critical pieces of hardware and their purposes are described below.

Instrument computer

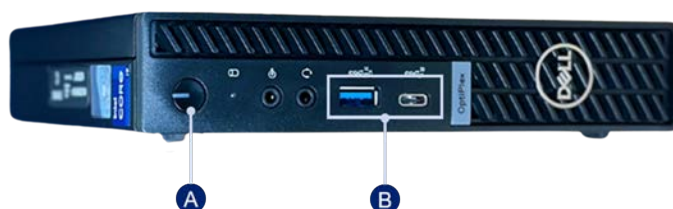
The SP200 includes an instrument computer that stores and runs the software needed to operate and troubleshoot the instrument.

The front panel of the instrument computer includes a power switch and USB ports, which are used to load or export data files.

NOTE

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

Figure 3. Instrument computer with power switch and USB ports



A Power switch

B One USB-A port and one USB-C port

NOTE

The computer shown above is an example of an installed computer. The actual computer used may vary.

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
 - LibreOffice

Proteograph Instrument Control Software

The SP200 includes dedicated Proteograph Instrument Control Software (ICS) that controls instrument operations, providing an interface for running methods, maintaining the instrument, and supporting training.

Figure 4. *Proteograph® ICS desktop icon*



This software is automatically loaded on the SP200 and displays the main menu when launched.

Figure 5. *Proteograph® ICS main menu*



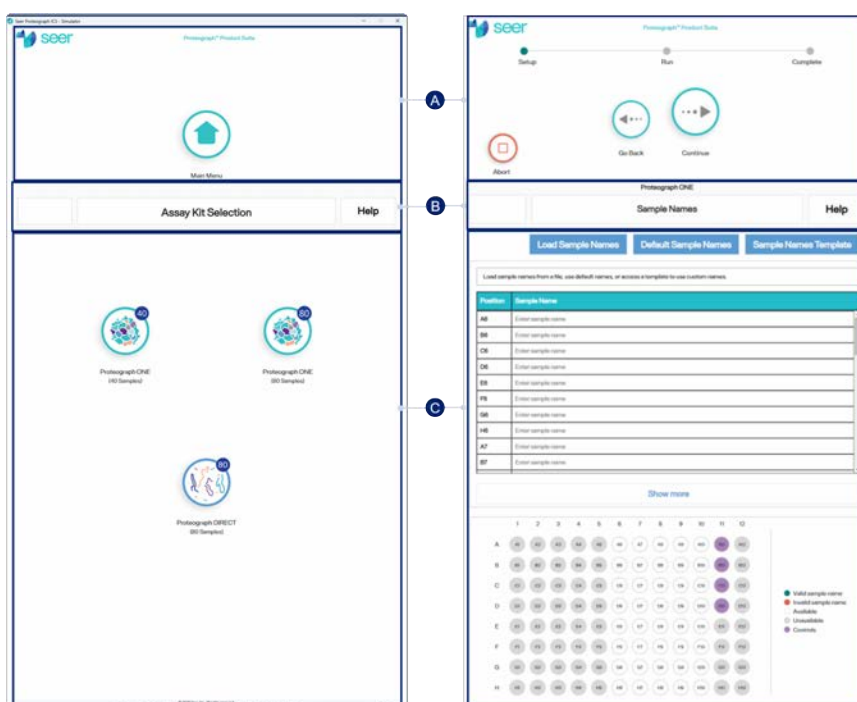
- A** Select to go to the *Assay Kit Selection* screen, where you can choose the assay that you want to run.
- B** Select to go to the *Peptide Quantification Registration* screen, to begin a peptide quantification run.
- C** Select to go to the *Peptide Reconstitution Registration* screen, to begin a peptide reconstitution run.
- D** Select to view the *Maintenance & Training* screen, where you can choose to perform daily or weekly maintenance tasks, begin water runs for training or troubleshooting purposes, perform a liquid waste check and waste flush to empty the waste container, or download the *Sample Names Template* file.
- E** Select to display and export information for Seer support.

The home screen also shows information about the last run, daily and weekly maintenance runs, and the last time the waste flush was performed.

ICS screens overview

Screens below the main menu share common areas.

Figure 6. *Proteograph® ICS secondary screens*



- A** Menu items appear here. Typically, you'll have a way to return to the main menu, either directly (like the Main Menu button above) or by aborting the current method run. Multi-step process screens also have a status bar to show your progression and buttons to return to the previous page or continue to the next page.
- B** The screen title appears in the center block, and a button to screen-level *Help* appears to the right.
- C** The lower pane is the active area of ICS, where you will enter information or select options to complete the actions or processes you've selected.

Work deck and autoloader tray

The SP200 has two work surfaces, the work deck and the autoloader tray. Enclosed by a front protective cover, the work deck is the area inside the instrument that holds the carriers during instrument operation. The autoloader 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 autoloader tray guide the carriers as they move along tracks between the work deck and the autoloader tray. Stop hooks attached to the back of the autoloader tray secure carriers and provide a stop point so that carriers are completely inserted.

Figure 7. Work surfaces

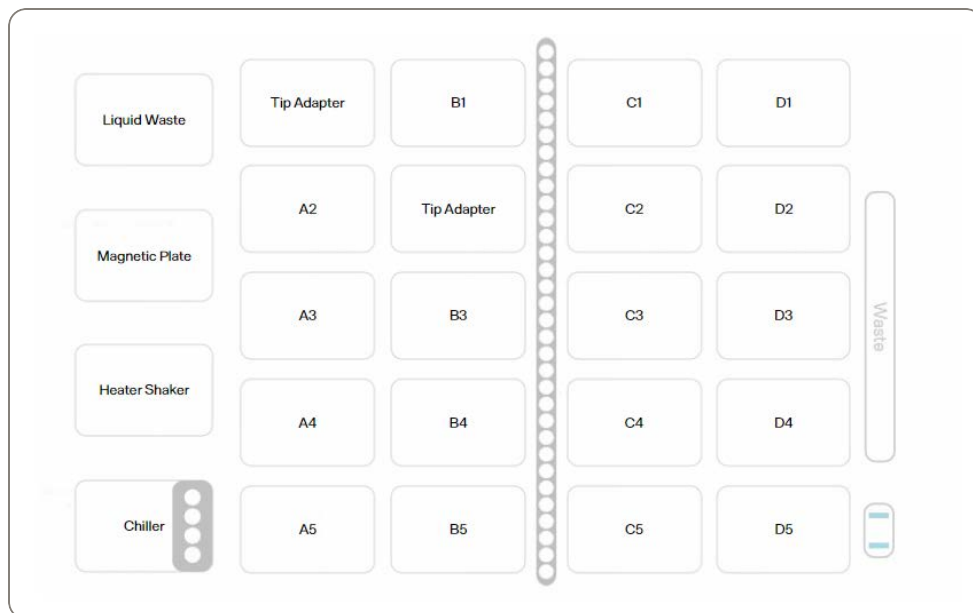


- A** Work deck
- B** Autoloader tray
- C** Stop hooks
- D** Side blocks

Work deck layout

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

Figure 8. Empty work deck



Serial number

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

Instrument hardware

The SP200 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 and to the sides of the instrument) 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 \(page 113\)](#).

Barcode reader

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

Figure 9. Barcode reader. The model of barcode reader may vary.



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, and tips). For a schematic of the carriers, see [Work deck layout \(page 18\)](#).

Figure 10. Tube, plate, reservoir, and tip carriers



- A** Plate Carrier A
- B** Plate Carrier B
- C** Tube Carrier 1
- D** Plate Carrier C
- E** Tip Carrier D

Chiller unit

During the method, the chiller unit maintains the Trypsin/LysC Tube at the appropriate temperature.

Figure 11. Chiller unit



Compressed O-ring expansion (CO-RE II) head

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

WARNING

Do not touch the CO-RE head during operation.

Figure 12. Compressed O-ring expansion head



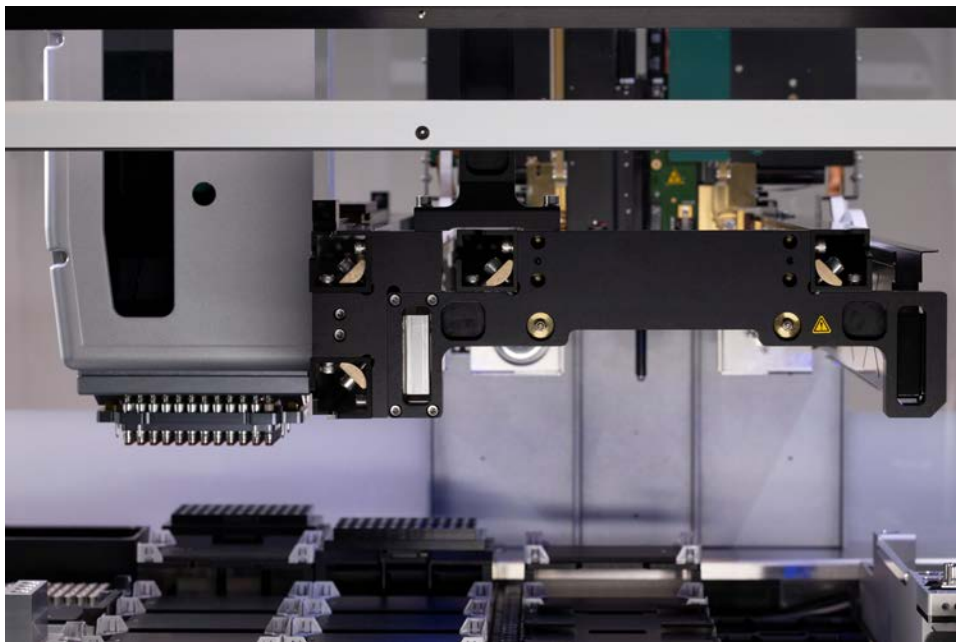
Gantry

During the method, the gantry moves the CO-RE head and independent 4-channel pipette heads.

WARNING

Do not touch the gantry during operation.

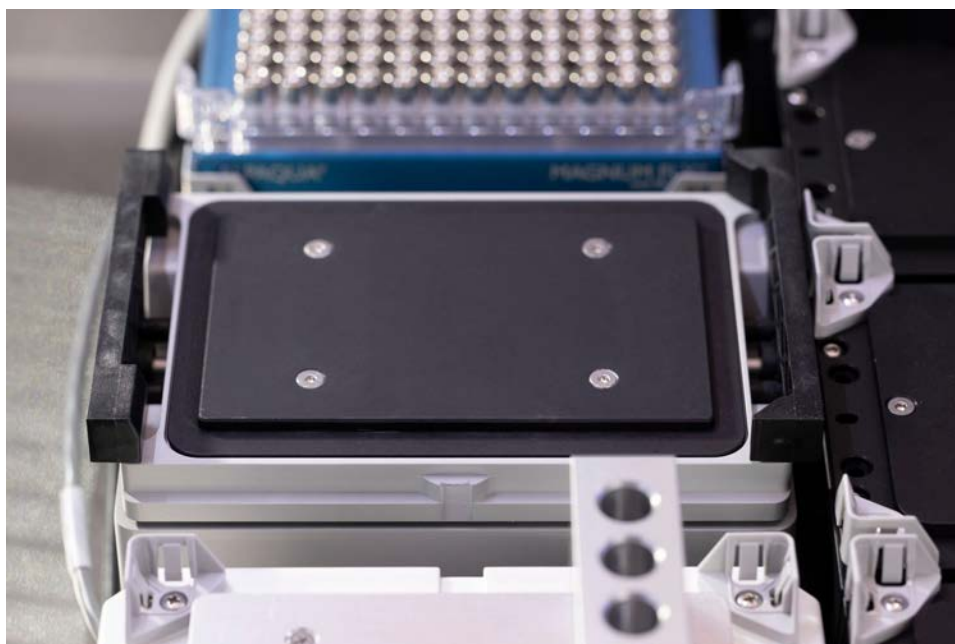
Figure 13. Gantry



Heater shaker

The heater shaker incubates and continuously mixes the Preparation Plate to facilitate sample denaturation. During Proteograph ONE Assay methods, the heater automatically heats to the appropriate temperature and reaches 95 °C.

Figure 14. Heater shaker



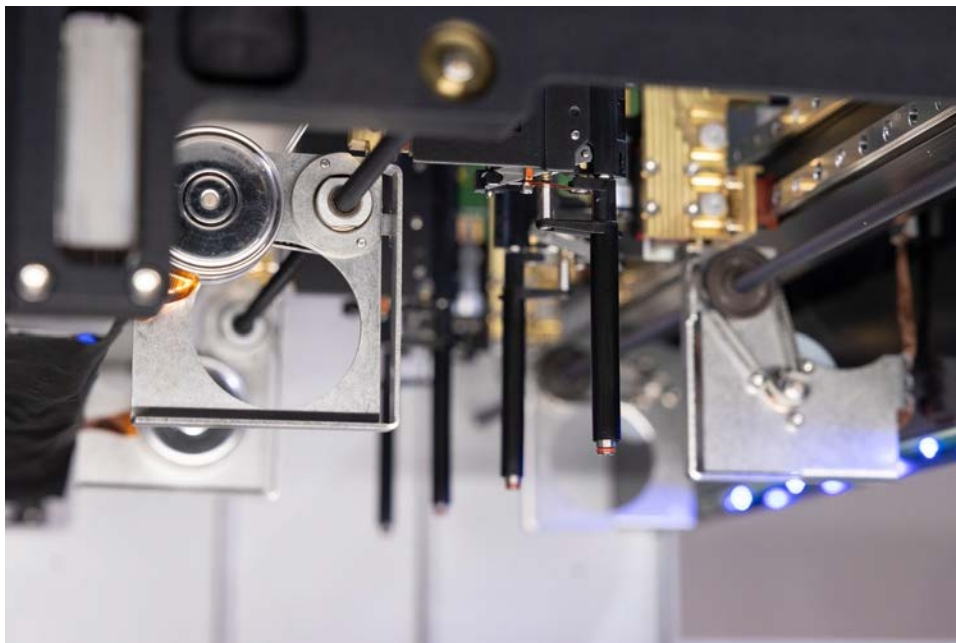
Independent 4-channel CO-RE pipette heads

Attached to the gantry, the independent 4-channel CO-RE pipette heads provide four separate channels for liquid handling.

WARNING

Do not touch the heads during instrument operation.

Figure 15. Independent 4-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 16. Magnetic plate

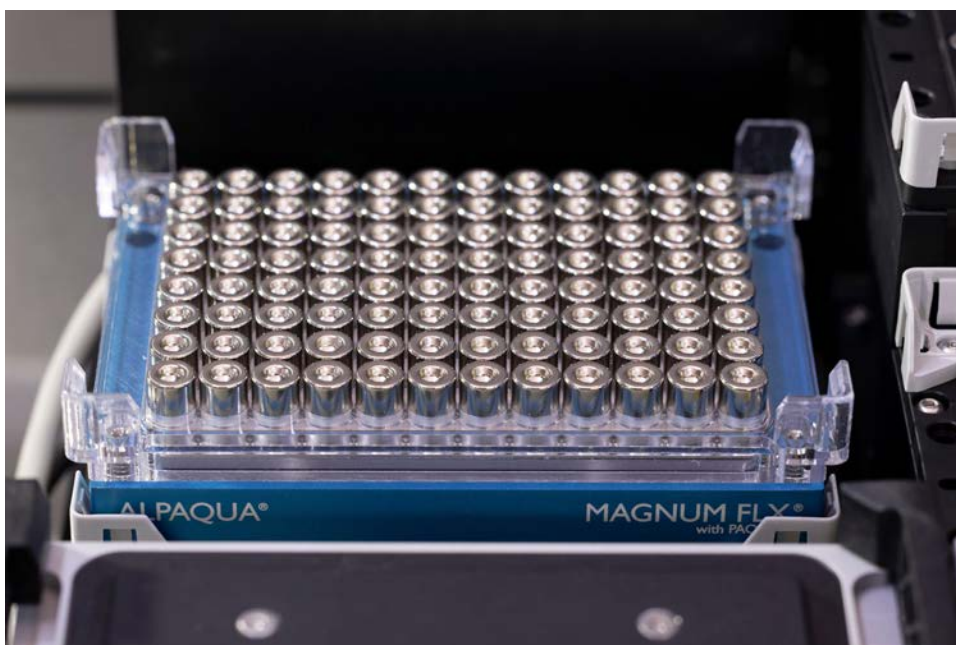


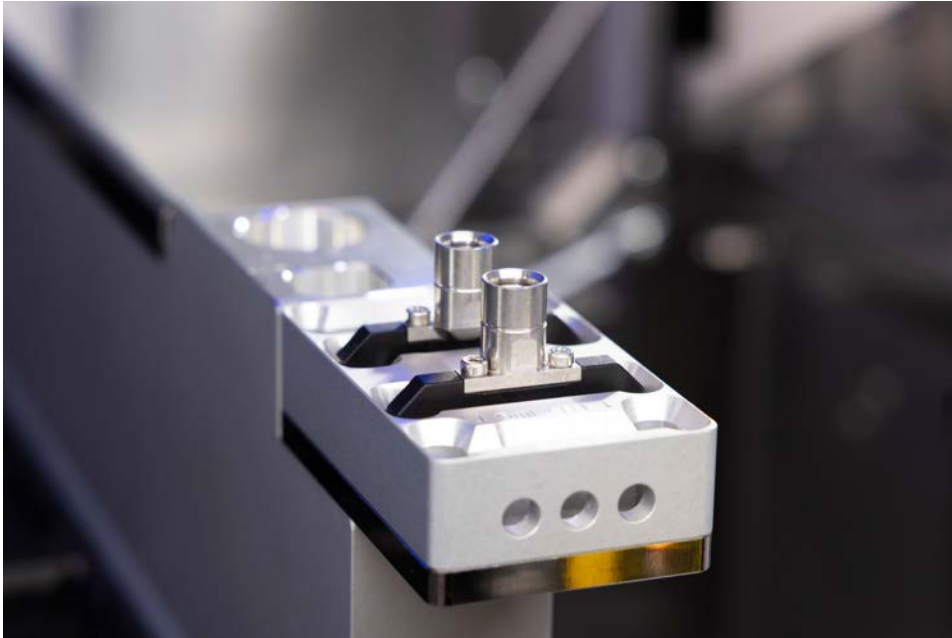
Plate grippers

Channels 3 and 4 of the independent 4-channel pipette heads use the plate grippers to transfer labware or NTRs to different locations on the deck.

CAUTION

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

Figure 17. Plate grippers



Teaching needles

A set of eight teaching needles are used during maintenance to check pressure tightness of the pipetting channels and ensure a good seal, as well as check whether capacitive detection is working. In addition, they are also used during instrument setup to teach labware positions.

CAUTION

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

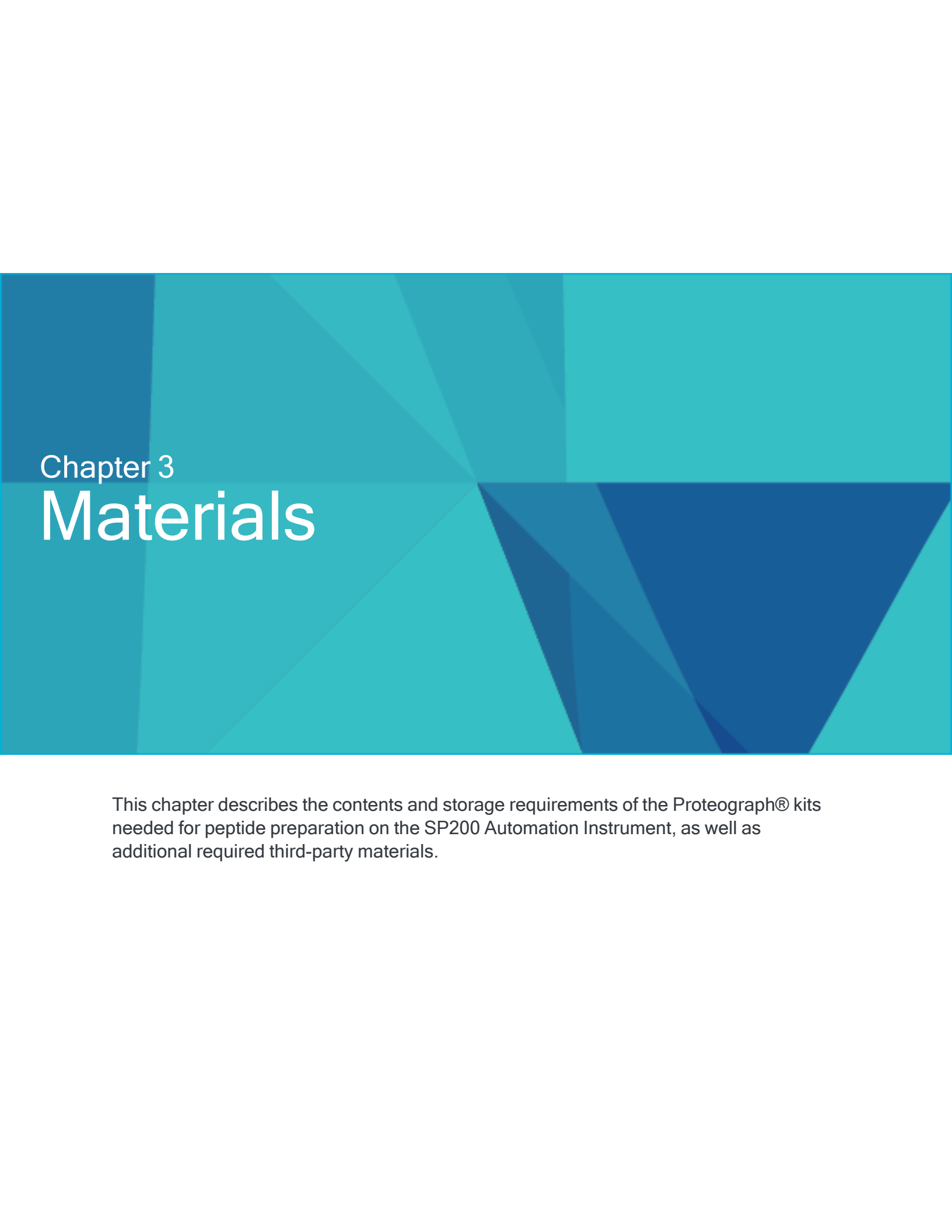
Figure 18. Teaching needles



Waste containers

The following containers collect waste from the SP200. 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.

- **CO-RE head waste** – Waste bin for 300 μ L NCTs from the CO-RE head.
- **Tip waste** – Waste bin for tip waste accessed below the unit or through a door on the right side of the SP200, depending on the instrument configuration.
- **Liquid waste** – Liquid waste is accessed in a container with waste sensor below the SP200.



Chapter 3

Materials

This chapter describes the contents and storage requirements of the Proteograph® kits needed for peptide preparation on the SP200 Automation Instrument, as well as additional required third-party materials.

Kit contents and storage requirements

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

Kits consist of two boxes: one of refrigerated reagents and one of labware.

CAUTION

After receiving an assay kit ***promptly store*** the contents of the refrigerated box at 2 - 8 °C. Labware can be stored at room temperature.

Figure 19. Assay kits ship in two boxes



Seer offers these proprietary kits:

- [Proteograph ONE Assay Kit 40 Samples \(P/N S55R5001\)](#), all consumables for a one-well proprietary nanoparticle-based assay run of up to 40 samples.
- [Proteograph ONE Assay Kit 80 Samples \(P/N S55R5002\)](#), all consumables for a one-well proprietary nanoparticle-based assay run of up to 80 samples.
- [Proteograph DIRECT Assay Kit 80 Samples \(P/N S55R5004\)](#), all consumables for one direct-digestion assay run of up to 80 samples.
- [Proteograph® ONE PQR Kit \(P/N S55R5006\)](#), consumables for four peptide quantification and reconstitution (PQR) runs.

In addition to the Proteograph ONE PQR Kit, peptide reconstitution runs requires a [Peptide Reconstitution Buffer](#), which can be prepared using the Seer provided Peptide Calibration Kit (below).

- [Peptide Calibration Kit \(P/N S55R5013\)](#), which contains the necessary reagents and consumables to prepare a Peptide Reconstitution Buffer for peptide calibration and data normalization during the Proteograph™ ONE and Proteograph™ DIRECT workflows.

NOTE

Additional third-party equipment and materials required for peptide preparation on the SP200 are described in [Required lab equipment \(page 36\)](#) and [Additional required materials \(page 37\)](#).

Proteograph® ONE Assay Kit (40 Samples)

The Proteograph ONE Assay Kit 40 Samples (P/N S55R5001) contains the labware and reagents needed to run the Proteograph ONE Assay method for up to 40 samples on the SP200. Each assay kit includes a refrigerated box of reagents and a box of labware.

CAUTION

After receiving an assay kit ***promptly store*** the contents of the refrigerated box at 2 - 8 °C. Labware can be stored at room temperature.

Refrigerated box of reagents

There are three pouches in the box, one containing the Nanoparticle tubes, one for the Controls (Plasma Control and Cleanup Particles Control), and one pouch with two tubes of Cleanup Particles.

Table 2. Contents of the Proteograph ONE Assay reagents box (40 samples)

DESCRIPTION	QUANTITY	CAP COLOR / LABEL	VOLUME (ML)	NOTES
Alkylation Solution	1	Green	7	
Binding Solution	1	White	115	
Cleanup Particles	2	Clear with black ring	n/a	
Cleanup Particles Control	1	Clear with black ring	n/a	
Denaturing Solution	1	Denature	25	
Enzyme Reconstitution Solution	1	White	7	
Nanoparticle (Tube 1)	1	Clear with black ring	n/a	
Nanoparticle (Tube 2)	1	Clear with black ring	n/a	
Nanoparticle (Tube 3)	1	Clear with black ring	n/a	
Nanoparticle Solution	1	Amber	14	
Plasma Control	1	Clear with black ring	n/a	
Reconstitution Solution	1	Clear	19	
Recovery Solution	1	Clear	24	
Reduction Solution	1	Blue	7	
Trypsin / LysC Protease MS Grade	2	Aluminum	n/a	
Trypsin / LysC Tube	1	Clear with black ring	n/a	a
Wash Solution	1	Amber	100	

a Not a reagent, but included in the Reagents box

*Labware box***Table 3.** Contents of the Proteograph ONE Assay labware box

DESCRIPTION	QUANTITY
Binding Reservoir	1
Black Lid	5
Cleanup Plate 1	1
Cleanup Plate 2	1
Clear Lid	1
Collection Plate	1
Nanoparticle Plate	1
Preparation Plate	1
Reagents Reservoir 1	1
Reagents Reservoir 2	1
Sample Transfer Plate	1
Wash Reservoir	1

Proteograph® ONE Assay Kit (80 Samples)

The Proteograph ONE Assay Kit 80 Samples (P/N S55R5002) contains the labware and reagents needed to run the Proteograph ONE Assay method for up to 80 samples on the SP200. Each assay kit includes a refrigerated box of reagents and a box of labware.

CAUTION

After receiving an assay kit ***promptly store*** the contents of the refrigerated box at 2 - 8 °C. Labware can be stored at room temperature.

Refrigerated box of reagents

There are four pouches in the box, one containing the Nanoparticle tubes, one for the Controls (Plasma Control and Cleanup Particles Control), and two pouches, each with two tubes of Cleanup Particles.

Table 4. Proteograph ONE Assay reagents (80 samples)

DESCRIPTION	QUANTITY	CAP COLOR / LABEL	VOLUME (ML)	NOTES
Alkylation Solution	1	Green	7	
Binding Solution	2	White	115	
Cleanup Particles	4	Clear with black ring	n/a	
Cleanup Particles Control	1	Clear with black ring	n/a	
Denaturing Solution	1	Denature	25	
Enzyme Reconstitution Solution	1	White	7	
Nanoparticle (Tube 1)	1	Clear with black ring	n/a	
Nanoparticle (Tube 2)	1	Clear with black ring	n/a	
Nanoparticle (Tube 3)	1	Clear with black ring	n/a	
Nanoparticle Solution	1	Amber	14	
Plasma Control	1	Clear with black ring	n/a	
Reconstitution Solution	1	Clear	19	
Recovery Solution	1	Clear	24	
Reduction Solution	1	Blue	7	
Trypsin / LysC Protease MS Grade	4	Aluminum	n/a	
Trypsin / LysC Tube	1	Clear with black ring	n/a	a
Wash Solution	2	Amber	100	

a Not a reagent, but included in the Reagents box

*Labware box***Table 5.** Contents of the Proteograph ONE Assay labware box

DESCRIPTION	QUANTITY
Binding Reservoir	1
Black Lid	5
Cleanup Plate 1	1
Cleanup Plate 2	1
Clear Lid	1
Collection Plate	1
Nanoparticle Plate	1
Preparation Plate	1
Reagents Reservoir 1	1
Reagents Reservoir 2	1
Sample Transfer Plate	1
Wash Reservoir	1

Proteograph® DIRECT Assay Kit (80 Samples)

The Proteograph DIRECT Assay Kit 80 Samples (P/N S55R5004) contains labware and all of the consumables you need to complete one direct-digestion assay of up to 80 samples. Each assay kit includes a refrigerated box of reagents and a box of labware.

CAUTION

After receiving an assay kit ***promptly store*** the contents of the refrigerated box at 2 - 8 °C. Labware can be stored at room temperature.

Refrigerated box of reagents

There are three pouches in the box, one for the Plasma Control tube, and two pouches of Cleanup Particles, each containing two tubes.

Table 6. Proteograph DIRECT Assay reagents (80 samples)

DESCRIPTION	QUANTITY	CAP COLOR / LABEL	VOLUME (ML)	NOTES
Alkylation Solution	1	Green	7	
Binding Solution	2	White	115	
Cleanup Particles	4	Clear with black ring	n/a	
Cleanup Particles Control	1	Clear with black ring	n/a	
Denaturing Solution	1	Denature	25	
Enzyme Reconstitution Solution	1	White	7	
Plasma Control	1	Clear with black ring	n/a	
Reconstitution Solution	1	Clear	19	
Recovery Solution	1	Clear	24	
Reduction Solution	1	Blue	7	
Trypsin / LysC Protease MS Grade	4	Gray	n/a	
Trypsin / LysC Tube	1	Clear with black ring	n/a	a

a Not a reagent, but included in the Reagents box

*Labware box***Table 7.** *Proteograph DIRECT Assay labware*

DESCRIPTION	QUANTITY
Binding Reservoir	1
Black Lid	4
Cleanup Plate 1	1
Cleanup Plate 2	1
Clear Lid	1
Collection Plate	1
Preparation Plate	1
Reagents Reservoir 1	1
Reagents Reservoir 2	1

Proteograph ONE PQR Kit

The Proteograph® ONE PQR Kit (P/N S55R5006) contains labware and all of the consumables you need to complete for four Peptide Quantification runs and four Peptide Reconstitution runs on the SP200.

CAUTION

When you receive this kit, **immediately** store the Recovery Solution at 2 - 8 °C. All other contents should be stored at room temperature.

Table 8. Contents of the Proteograph® ONE PQR Kit (P/N S55R5006)

DESCRIPTION	QUANTITY	NOTES
Black Lid	4	a
Quantification Plate	4	a
Quantification Reservoir	4	a
Reconstitution Reservoir	4	c
Recovery Solution	1	a, b
Standards Prep Plate	4	a

a For the Peptide Quantification method.

b Must be stored at 2 - 8 °C.

c For the Peptide Reconstitution method.

Peptide Calibration Kit

The Seer Peptide Calibration Kit (P/N S55R5013) contains the necessary reagents and consumables for peptide calibration and data normalization during the Proteograph ONE and Proteograph DIRECT workflows. Each kit provides enough prepared buffer solution for four plates.

NOTE

When you receive this kit, immediately store the PepCalMix at -20°C and Peptide Calibration Recon Buffer between 2 - 8 °C. All other components should be stored at room temperature.

Table 9. Contents of the Seer Peptide Calibration Kit (P/N S55R5013)

DESCRIPTION	QUANTITY	STORAGE
PepCalMix	2 tubes	-20°C
Peptide Calibration Recon Buffer	1 bottle (20 mL)	2 - 8 °C
Peptide Calibration Reconstitution Tube	4 tubes (5 mL each)	Ambient

NOTE

Seer recommends a single preparation of PepCalMix using the Peptide Calibration Recon Buffer and Peptide Calibration Reconstitution Tubes for a single study.

Required lab equipment

Peptide preparation, quantification, and reconstitution on the SP200 require the following third-party equipment. For additional information about these equipment, refer to the *Site Preparation Guide* (CF-1090).

Table 10. Required lab equipment

DESCRIPTION	REQUIREMENTS	SUPPLIER (OR EQUIVALENT)
Centrifuge with plate adapter	Can achieve a relative centrifugal force of 500 x g and accommodate a standard 96 well microplate.	Centrifuge - Thermo Fisher Scientific, catalog # 75009521 Centrifuge Plate Adapter - Thermo Fisher Scientific, catalog # 75003624
Fluorometric microplate reader	Can measure peptide concentration via fluorescence (Ex. 390, Em. 475 nm) in a 96-well plate format.	Molecular Devices, SpectraMax, M2
Refrigerated microcentrifuge	Can achieve a relative centrifugal force of 5000 x g, maintain a temperature of 4C, and accommodate sample tubes.	Thermo Fisher Scientific, catalog # 75002441
Vacuum concentrator	Capable of maintaining refrigerated temperature at vacuum and accommodate a standard 96 well microplate.	Thermo Fisher Scientific, catalog # SRF110P1-115

Additional required materials

Peptide preparation, quantification, and reconstitution on the SP200, 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 2 - 8 °C.

NOTE

Suggested suppliers for these materials are listed below. Where indicated, you may use alternate suppliers whose materials meet the stated requirements.

Table 11. Additional required materials

DESCRIPTION	RECOMMENDED SUPPLIER
1-10 mL pipette with tips or 10 mL serological pipettes	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
2-20 µL pipette with tips.	Rainin, part # 17014392
100-1000 µL pipette with tips	Rainin, material # 17014382 ^a
300 µL Nested Conductive Tips (NCTs)	Hamilton, part # 235950
50 µL Nested Conductive Tips (NCTs)	Hamilton, part #: 235947
70% isopropyl alcohol or 70% ethanol	General lab supplier
Sealing foil (5 × 3 inch)	VWR, catalog # 60941-126 ^{a, c}
Axygen AxyMats 96 round well sealing mat for PCR microplates	VWR, product # AM-96-PCR-RD
Deionized water	General lab supplier
Disposable latex gloves	General lab supplier
Kimwipes or similar lint-free tissues	General lab supplier
Lab coats	General lab supplier
Pierce quantitative fluorometric peptide assay	Thermo Fisher Scientific, catalog # 23290 ^b
Protective goggles	General lab supplier
Waste bags with biohazard labeling	Hamilton, part # 199203 ^a
Waste container biohazard box Approximately 11 in (28 cm) l x 20 in (51cm) w X 15 in (38 cm) h	General lab supplier

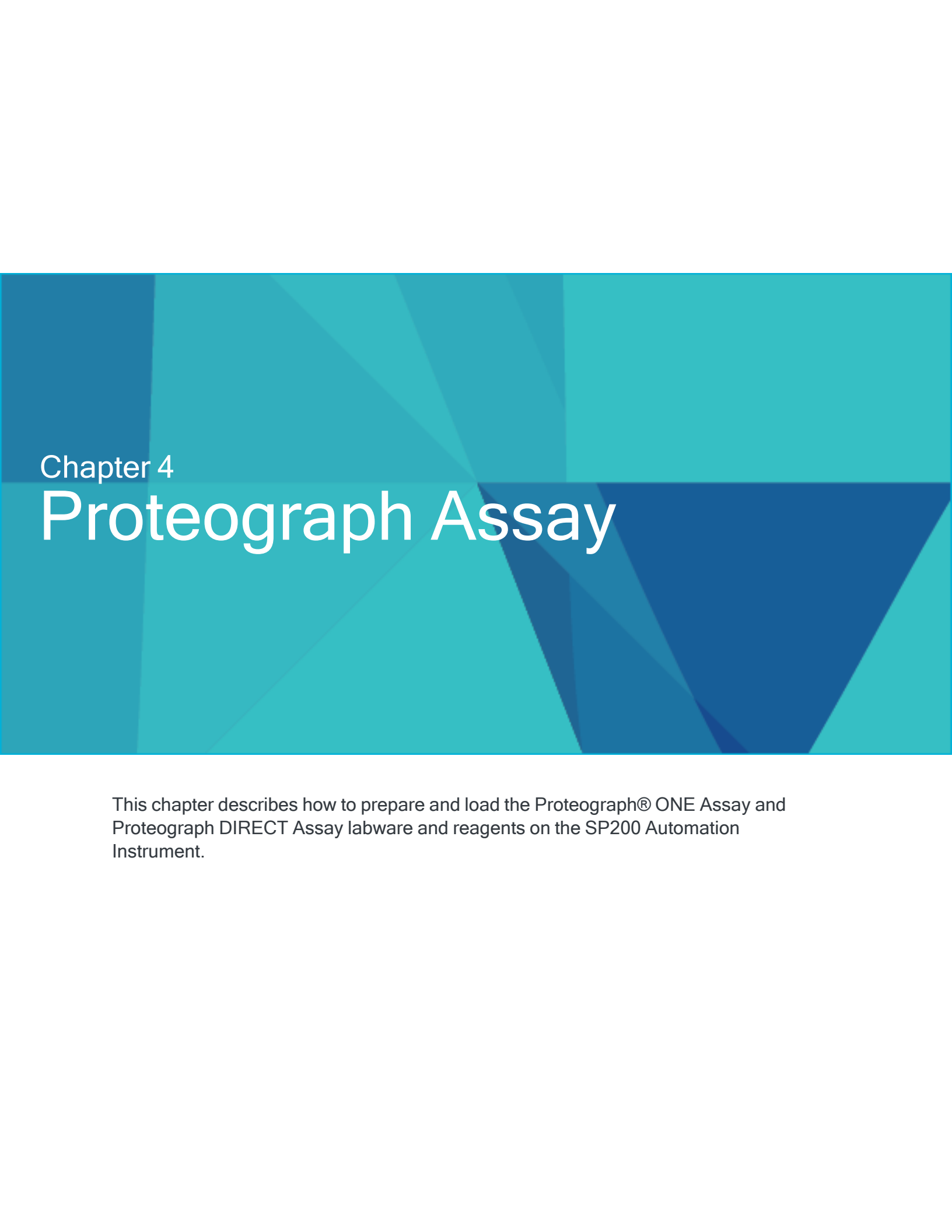
^a Or equivalent.

^b Must be stored at 2 - 8 °C.

^c Must be able to withstand freezing at -80°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® ONE Assay and Proteograph DIRECT Assay labware and reagents on the SP200 Automation Instrument.

Proteograph Assays

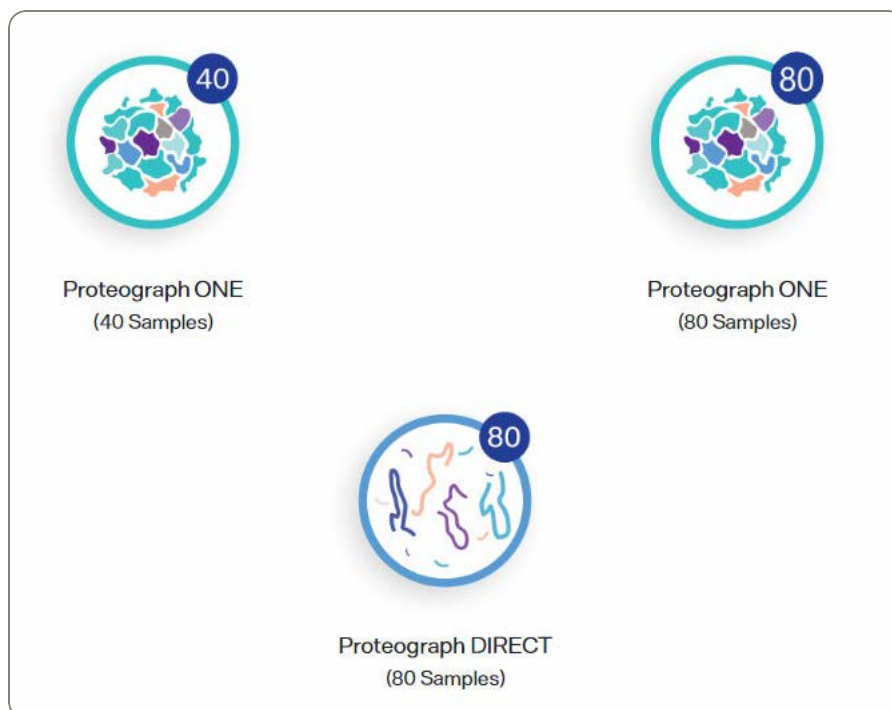


Figure 20. Proteograph Assay Types

The Proteograph® ONE Assay method on the SP200 Automation Instrument prepares peptides for analysis. To run the method, prepare the instrument and materials and follow the on-screen 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 \(page 110\)](#).
- Confirm you have all [Proteograph ONE Assay Kit](#) items and other materials. See [Required lab equipment \(page 36\)](#) and [Additional required materials \(page 37\)](#).
- Review best practices to help load the work deck correctly and efficiently. See [Best practices \(page 51\)](#).

Types of Assays

The Seer® SP200 Automation Instrument supports the following assays:

Proteograph® ONE Assay

The Proteograph ONE assay involves Seer's proprietary engineered nanoparticles to enrich and prepare peptides for mass spectrometry analysis. Depending on the kit you have, you can run an assay of up to either 40 or 80 samples.

The Proteograph ONE Assay requires either:

- [Proteograph® ONE Assay Kit \(40 Samples\)](#)
- [Proteograph® ONE Assay Kit \(80 Samples\)](#)

Proteograph DIRECT Assay

The Proteograph DIRECT assay involves a Seer peptide preparation workflow that does not utilize Seer's proprietary nanoparticle technology.

The Proteograph DIRECT assay requires:

- [Proteograph® DIRECT Assay Kit \(80 Samples\)](#)

Because this protocol does not use Seer's nanoparticle technology, some of the steps to load the instrument are different from the Proteograph ONE Assay workflow. These differences are noted in the on-screen instructions.

NOTE

Additional lab equipment and materials are required. For more information, see [Required lab equipment \(page 36\)](#) and [Additional required materials \(page 37\)](#).

Number of samples and controls

The Proteograph ONE Assay method requires either 40 or 80 plasma samples. During the assay, each sample incubates with a Proteograph ONE nanoparticle, producing a single well output of peptides in a 96-well plate for up to 40 or 80 samples.

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

- **Process Control** – A pooled plasma sample that is processed throughout the entirety of the workflow, including corona formation, trypsin digestion, and peptide cleanup.
- **Digestion Control** – A pooled plasma sample that is processed throughout workflow steps after the corona formation, including trypsin digestion and peptide cleanup. This control is used for diagnostic and troubleshooting efforts.
- **User Control** – An available well for the end user to supply their own on plate control sample of interest.
- **Cleanup Particles Control** – A digested peptide sample generated from pooled plasma that is only processed through the peptide cleanup steps of the assay. This control is used for diagnostic and troubleshooting efforts.

Plate Maps

The output of the Proteograph ONE Assay method is the Collection Plate containing the purified peptides. Samples are incubated with Seer's proprietary engineered nanoparticles in columns 6-10 (for the 40-sample assay) or columns 1-10 (for the 80-sample assay).

Controls occupy wells A11, B11, C11, and D11.

The Proteograph DIRECT assay does not involve nanoparticle incubation like the Proteograph ONE assay. Instead, this protocol utilizes denaturation, reduction, alkylation, digestion incubation, and cleanup steps to prepare the peptides for mass spectrometry.

Proteograph DIRECT assay controls occupy wells A11, C11, and D11.

NOTE

You can add your User Control to well A11.

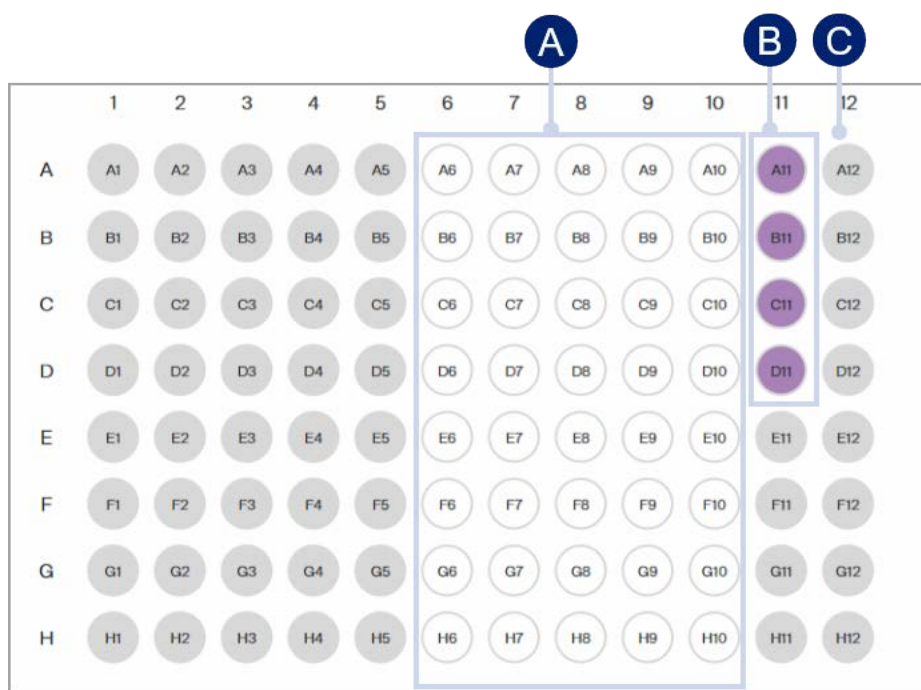
The remaining controls are placed in the tube carrier during setup, and are added to the Collection Plate by the instrument during the assay method.

Proteograph ONE assays

For enriched assays, the control well contents are:

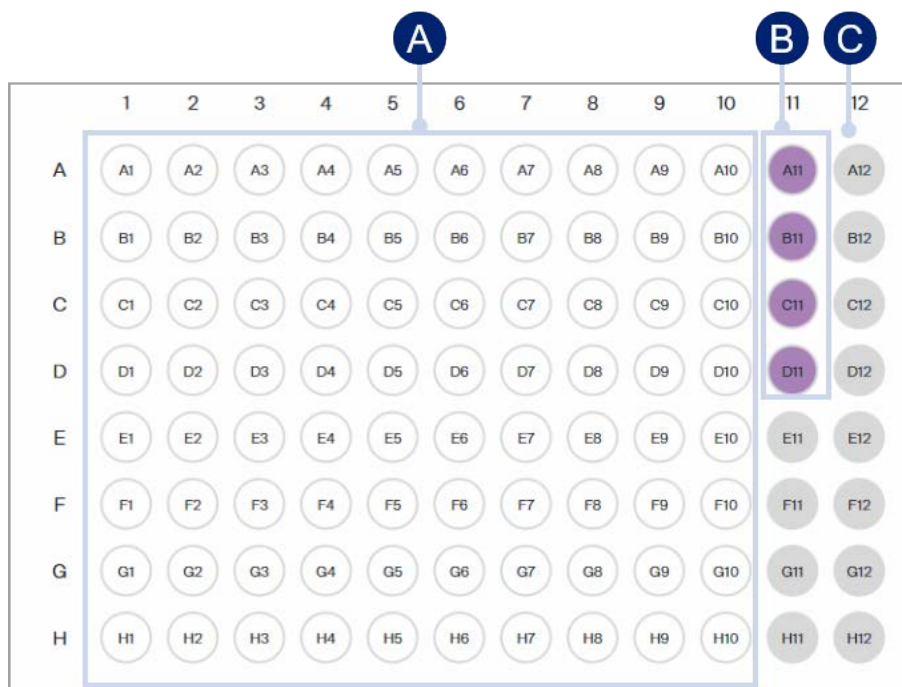
- A11: User Control (You can add your own control)
- B11: Process Control (Added by the instrument during the assay)
- C11: Digestion Control (Added by the instrument during the assay)
- D11: Cleanup Particles Control (Added by the instrument during the assay)

Figure 21. Layout of the post-assay 40-sample Proteograph ONE assay Collection Plate



- A** Sample wells (white).
B Control well (purple).
C Unavailable wells (shaded).

Figure 22. Layout of the post-assay 80-sample Proteograph ONE assay Collection Plate



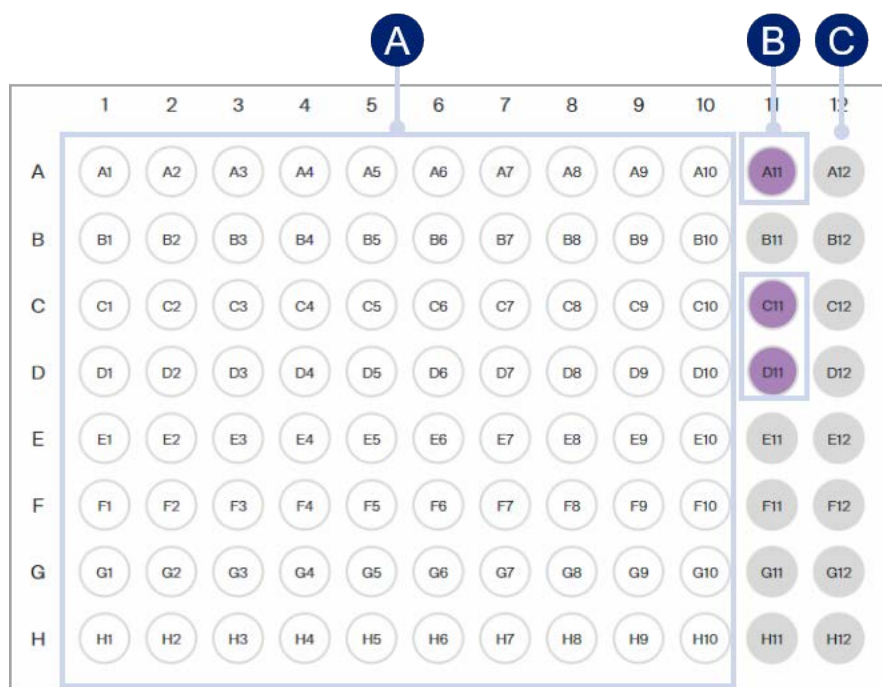
- A** Sample wells (white).
- B** Control well (purple).
- C** Unavailable wells (shaded).

Proteograph DIRECT assays

For Direct Digest assays, the control well contents are:

- A11: User Control (You can add your own control)
- C11: Direct Digest Control (Added by the instrument during the assay)
- D11: Cleanup Particles Control (Added by the instrument during the assay)

Figure 23. Layout of the post-assay 80-sample Proteograph DIRECT assay Collection Plate



- A** Sample wells (white).
B Control well (purple).
C Unavailable wells (shaded).

Work deck quick reference

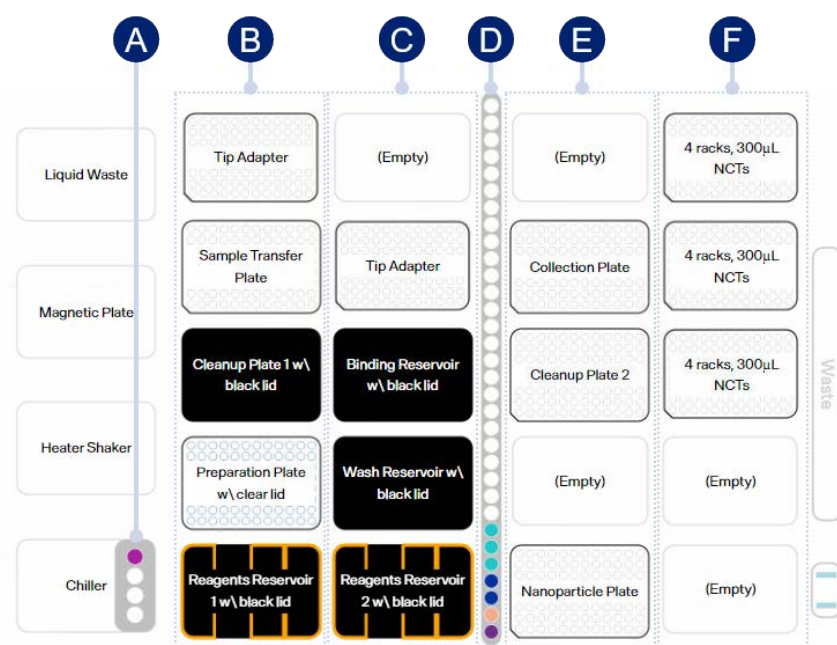
The following figures show the layout of the work deck after it is loaded with labware and reagents for the Proteograph™ ONE Assay method.

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.

Work deck layout for the Proteograph ONE Assay (40 Samples)

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



- A** Chiller
- B** Carrier A
- C** Carrier B
- D** Tube Rack
- E** Carrier C
- F** Carrier D

Table 12. Work deck layout for reference, Proteograph ONE Assay (40 Samples)

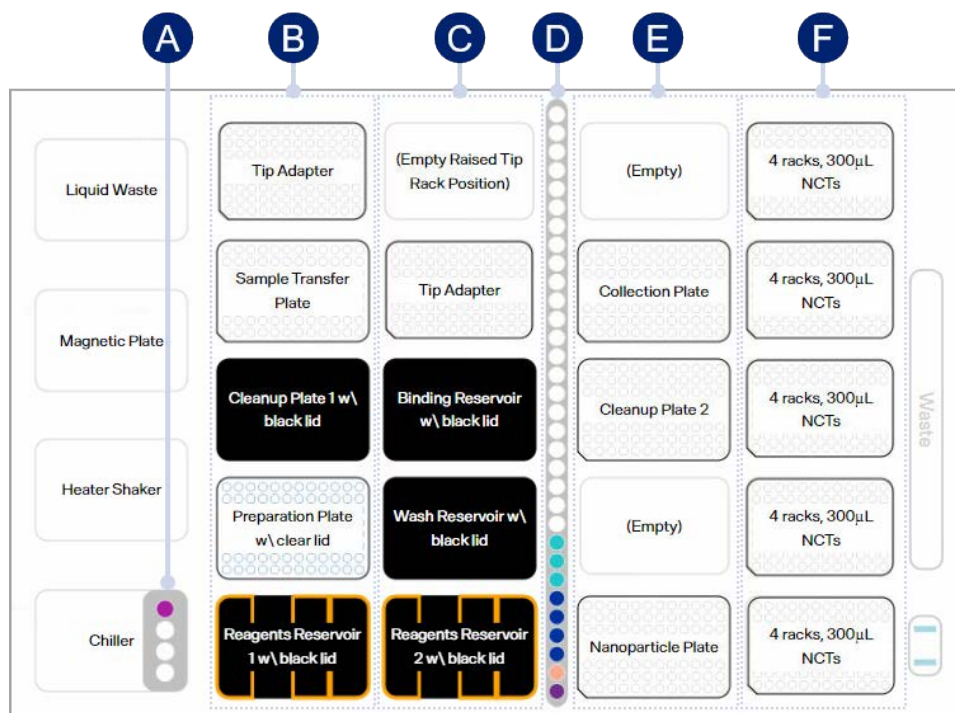
CARRIER	POSITION	ITEM	NOTES
Chiller with Tube Carrier	1	Tube of reconstituted Trypsin/LysC	
Carrier A	A1	Tip adapter	a
	A2	Sample Transfer Plate with prepared samples	
	A3	Cleanup Plate 1 w/ black lid	a
	A4	Preparation Plate w/ clear lid	a
	A5 - well 1	Denaturation Solution	
	A5 - well 3	Reduction Solution	
	A5 - well 4	Alkylation Solution	
Carrier B	B2	Tip adapter	
	B3	Full Binding Reservoir	
	B4	Full Wash Reservoir	
	B5 - well 1	Nanoparticle Solution	
	B5 - well 3	Reconstitution Solution	
	B5 - well 4	Recovery Solution	
Carrier C	C2	Collection Plate	a
	C3	Cleanup Plate 2	a
	C5	Nanoparticle Plate	a
Carrier D	D1	Four racks of 300 µL Nested Conductive Tips	b
	D2	Four racks of 300 µL Nested Conductive Tips	b
	D3	Four racks of 300 µL Nested Conductive Tips	b
Tube Rack	T26	Nanoparticle (Tube 1)	
	T27	Nanoparticle (Tube 2)	
	T28	Nanoparticle (Tube 3)	
	T29	Cleanup Particles	
	T30	Cleanup Particles	
	T31	Plasma Control	
	T32	Cleanup Particles Control	

a This labware is loaded empty.

b One stack of four NCT racks.

Work deck layout for the Proteograph ONE Assay (80 Samples)

Figure 25. Work deck loaded for the Proteograph ONE assay method (80 samples)



- A** Chiller
- B** Carrier A
- C** Carrier B
- D** Tube Rack
- E** Carrier C
- F** Carrier D

Table 13. Work deck layout for reference, Proteograph ONE Assay (80 Samples)

CARRIER	POSITION	ITEM	NOTES
Chiller with Tube Carrier	1	Tube of reconstituted Trypsin/LysC	
Carrier A	A1	Tip adapter	a
	A2	Sample Transfer Plate with prepared samples	
	A3	Cleanup Plate 1 w/ black lid	a
	A4	Preparation Plate w/ clear lid	a
	A5 - well 1	Denaturation Solution	
	A5 - well 3	Reduction Solution	
	A5 - well 4	Alkylation Solution	

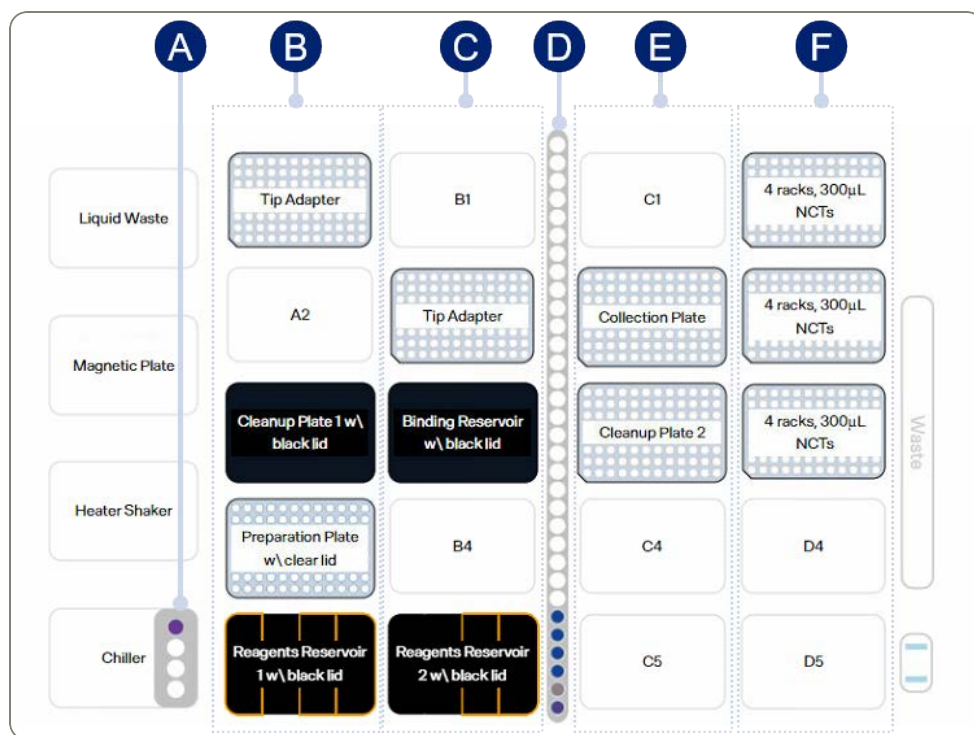
CARRIER	POSITION	ITEM	NOTES
Carrier B	B2	Tip adapter	
	B3	Full Binding Reservoir	
	B4	Full Wash Reservoir	
	B5 - well 1	Nanoparticle Solution	
	B5 - well 3	Reconstitution Solution	
	B5 - well 4	Recovery Solution	
Carrier C	C2	Collection Plate	a
	C3	Cleanup Plate 2	a
	C5	Nanoparticle Plate	a
Carrier D	D1	Four racks of 300 µL Nested Conductive Tips	b
	D2	Four racks of 300 µL Nested Conductive Tips	b
	D3	Four racks of 300 µL Nested Conductive Tips	b
	D4	Four racks of 300 µL Nested Conductive Tips	b
	D5	Four racks of 300 µL Nested Conductive Tips	b
Tube Rack	T24	Nanoparticle (Tube 1)	
	T25	Nanoparticle (Tube 2)	
	T26	Nanoparticle (Tube 3)	
	T27	Cleanup Particles	
	T28	Cleanup Particles	
	T29	Cleanup Particles	
	T30	Cleanup Particles	
	T31	Plasma Control	
	T32	Cleanup Particles Control	

a This labware is loaded empty.

b One stack of four NCT racks.

Work deck layout for the Proteograph DIRECT Assay (80 samples)

Figure 26. Work deck loaded for the Proteograph DIRECT Assay (80 samples)



- A** Chiller
- B** Carrier A
- C** Carrier B
- D** Tube Rack
- E** Carrier C
- F** Carrier D

Table 14. Work deck layout for reference, Proteograph DIRECT Assay Kit (80 Samples)

CARRIER	POSITION	ITEM	NOTES
Chiller with Tube Carrier	1	Tube of reconstituted Trypsin/LysC	
Carrier A	A1	Tip adapter	a
	A3	Cleanup Plate 1 w/ black lid	a
	A4	Preparation Plate w/ clear lid	a
	A5 - well 1	Denaturation Solution	
	A5 - well 3	Reduction Solution	
	A5 - well 4	Alkylation Solution	

CARRIER	POSITION	ITEM	NOTES
Carrier B	B2	Tip adapter	
	B3	Full Binding Reservoir	
	B5 - well 3	Reconstitution Solution	
	B5 - well 4	Recovery Solution	
Carrier C	C2	Collection Plate	a
	C3	Cleanup Plate 2	a
Carrier D	D1	Four racks of 300 µL Nested Conductive Tips	b
	D2	Four racks of 300 µL Nested Conductive Tips	b
	D3	Four racks of 300 µL Nested Conductive Tips	b
Tube Rack	T27	Cleanup Particles	
	T28	Cleanup Particles	
	T29	Cleanup Particles	
	T30	Cleanup Particles	
	T31	Plasma Control	
	T32	Cleanup Particles Control	

a This labware is loaded empty.

b One stack of four NCT racks.

Best practices

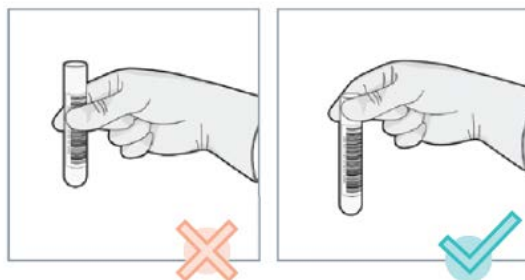
When setting up the work deck, observe the following best practices to ensure the proper techniques for pipetting liquids, placing labware and reagents, and placing lids. Improper work deck setup can cause errors.

For information on resolving setup errors, see [Chapter 9. Troubleshooting \(page 106\)](#).

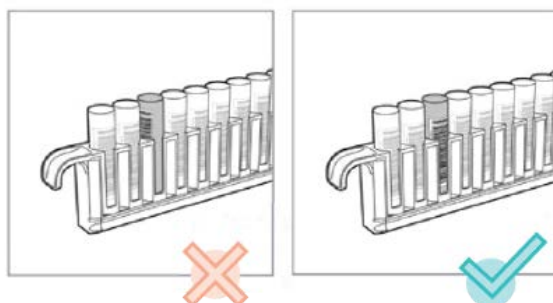
Loading labware

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.

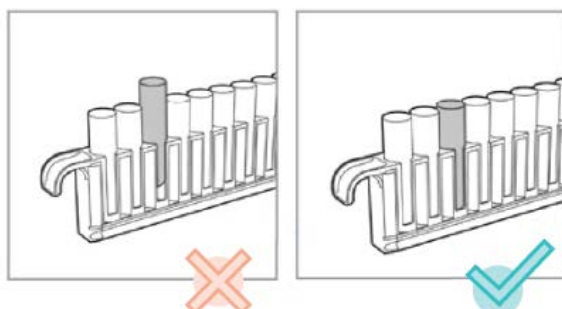
- **Tubes** – Avoid placing your fingers over barcodes.



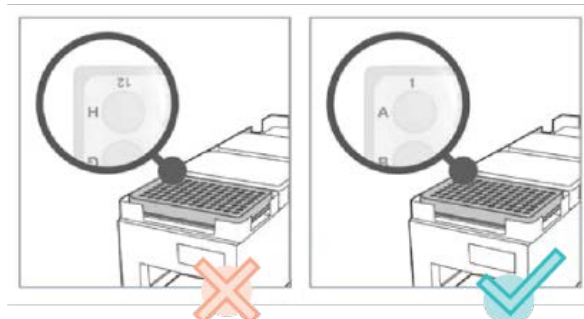
Face all tube barcodes to the right so the barcode reader can scan them. Incorrect orientation makes the barcodes unreadable.



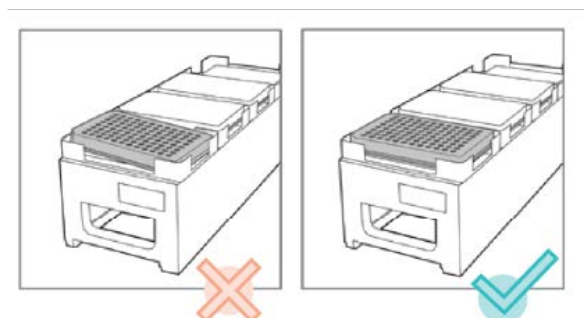
Fully insert tubes into the tube carrier. Improper seating can cause malfunction.



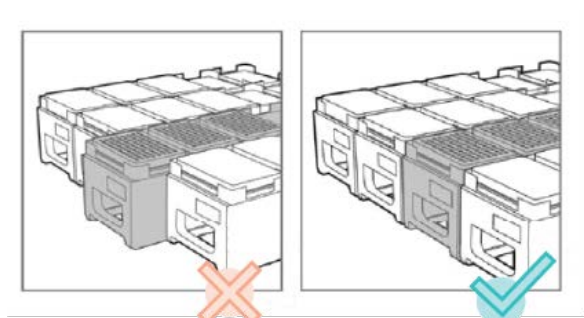
- **Wells** – Position wells on plate carriers so that well A1 is in the upper-left corner.



- **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.

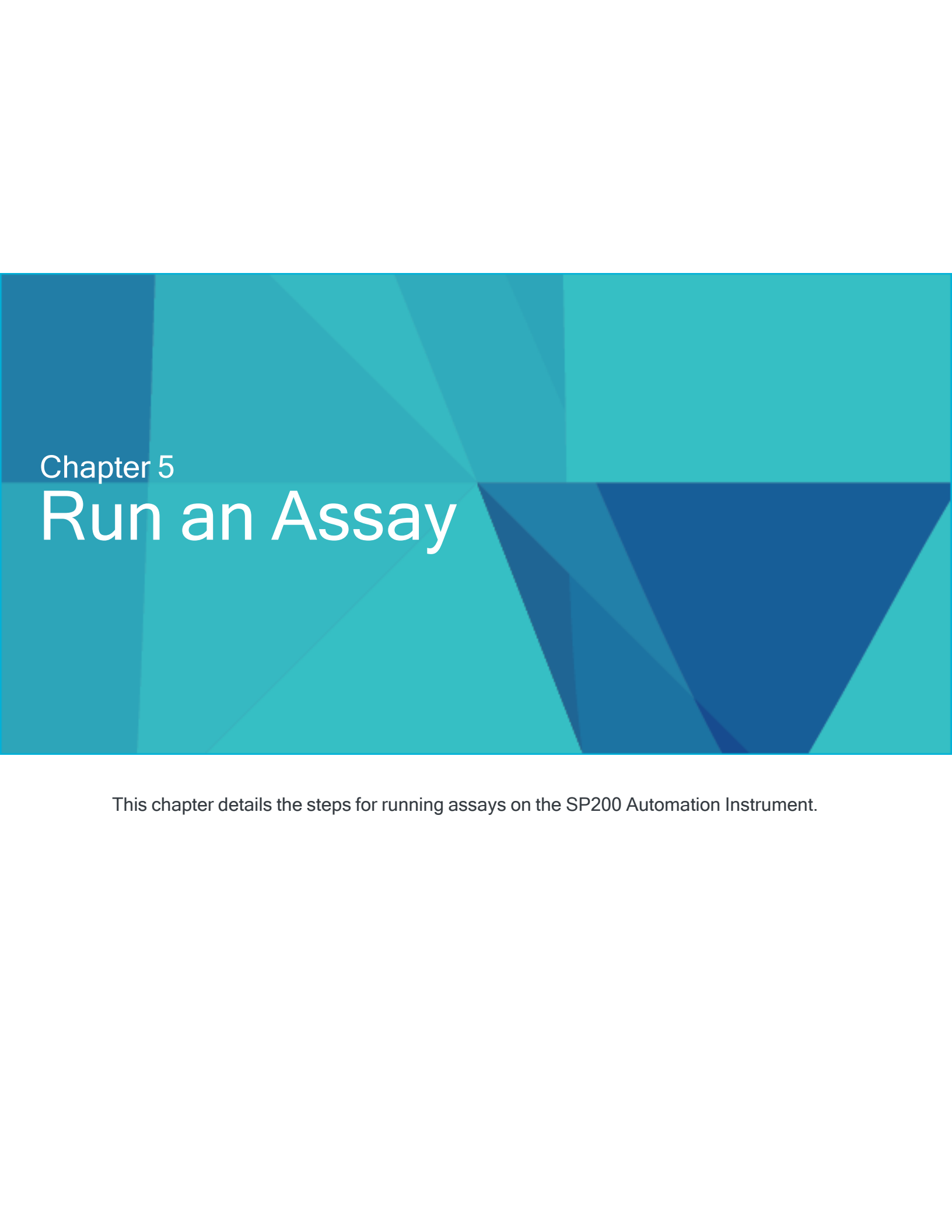


Placing lids

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

Figure 27. Misaligned lid





Chapter 5

Run an Assay

This chapter details the steps for running assays on the SP200 Automation Instrument.

Steps to run an assay

Performing daily or weekly maintenance and then running the assay are the first steps of the assay workflow.

Figure 28. *The Maintenance and Run Assay segments of the Proteograph Workflow*



NOTE

Before you begin an assay, ICS will prompt you to perform maintenance. Daily and weekly maintenance steps are described in [Instrument Maintenance & Training \(page 91\)](#).

The Proteograph Assay protocol consists of the following steps:

1. [Prepare the instrument \(next page\)](#)
2. [Prepare the assay materials \(page 57\)](#)
3. [Set up the Proteograph Assay method \(page 57\)](#)
4. [Deck Setup 1 of 4: Load Plate Carrier A \(page 60\)](#)
5. [Deck Setup 2 of 4: Load Plate Carrier B \(page 61\)](#)
6. [Deck Setup 3 of 4: Load Carriers C and D \(page 62\)](#)
7. [Deck Setup 4 of 4: Load tube carriers \(page 63\)](#)
8. [Prepare and place samples \(page 65\)](#)
9. [Complete setup and start the method \(page 69\)](#)
10. [Unload the work deck \(page 71\)](#)

NOTE

You must begin peptide quantification within **1 hour** after completing the assay.

Prepare the instrument

1. Turn on the instrument:



- A** Location of the power switch on the instrument.

NOTE

Turning on the SP200 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:
 - **SP200** – Press the green switch on the front of the instrument.
 - **Instrument computer** – Press the power button on the front of the instrument computer.
- b. After the computer has initialized, select the **Proteograph® ICS** desktop shortcut to launch ICS.



Prepare the assay materials

1. Set the centrifuge to 4 °C.
2. 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 2 - 8 °C until they are loaded into the sample prep plate and onto the work deck.

3. Remove labware from room-temperature storage and arrange as follows:
 - Place the Trypsin/LysC Tube on ice.
 - Check all labware for appropriate barcode labels.
4. Remove the refrigerated box from 2 - 8 °C storage and arrange reagents as follows:
 - Place the Enzyme Reconstitution Solution on ice.
 - Place the vials (the number of vials depends on your Proteograph ONE Assay Kit version) of Trypsin/LysC Protease MS Grade on ice.
 - Set aside the remaining reagents at room temperature.

Set up the Proteograph Assay method

1. On the ICS main menu, select **Proteograph Assays**.
The *Safety Confirmation* dialog appears.
2. Close the instrument's front protective cover if open, and then select **Confirm**.
The *Run Maintenance* dialog appears.

The daily maintenance reminder appears if more than 24 hours elapsed since the last daily maintenance was performed, and the weekly maintenance reminder if more than seven days elapsed since the last weekly maintenance process was performed. For more information, see [Instrument Maintenance & Training \(page 91\)](#).
3. Select **Run** to run maintenance tasks.

If you select **Run**, the maintenance screens guide you through the maintenance tasks. When complete, you are returned to the *Assay Kit Selection* screen.
4. At **Assay Kit Selection**, select the assay you want to run. You can choose a 40- or 80-sample Proteograph ONE assay, or an 80-sample Direct Digest assay.

You should have the appropriate assay kit ready.

5. At **Registration**, do the following:

- a. In the **Your Name** field, enter your username.

NOTE

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

- b. In the **Project Name** field, enter a name for your experiment.

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

TIP

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

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

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

- d. Select **Continue** to advance.

6. At **Sample Names**, enter names to correlate sample names with their well position on the sample collection plate. You can:

- Manually enter the sample names in the *Sample Name* field for each position.
- Select **Sample Names Template** to download a template file in compressed format (seer-sample-names-templates.zip). You can open this file and replace the sample names and their designated positions and save as a .csv file.
- Select **Load Sample Names** to load a list of sample names from a .csv file saved locally.
- Select **Default Sample Names** to have ICS load sample names for all positions.

7. Select **Continue** to advance.

8. At **Liquid Waste Check**, do the following:

- 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 gray button to disconnect the nozzle from the attachment.
 - ii. Unplug the cable from the sensor attached to the waste container.
 - iii. Unscrew the cap from the container.
 - iv. Dispose of the contents per your laboratory policy.

WARNING

Never use disinfectants containing oxidizing agents such as bleach to decontaminate liquid waste from the SP200. Doing so can cause toxic fumes and other adverse chemical reactions.

- v. Screw the cap onto the container.
 - vi. Reconnect the sensor to the container.
 - vii. Ensure that the tubing is connected to the waste bottle.
- c. Select the **I have checked the liquid waste container** checkbox.
 - d. Select **Continue** to advance.

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

- a. If necessary, empty tip waste.
- b. Pull all carriers out onto the autoloader tray and ensure that the work deck is clear of any labware or spills.
- c. Push the carriers to the stop hooks at the back of the autoloader tray.

CAUTION

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

- d. Close the front protective cover.
- e. Select **Continue** to advance.

CAUTION

The gantry will move after you select Continue.

10. Proceed to [Deck Setup 1 of 4: Load Plate Carrier A \(next page\)](#).

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.

NOTE

Pipette reagents into their plates or reservoirs once they are pulled out onto the autoloader tray.

CAUTION

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

Table 15. Deck Setup 1 of 4 (Load Plate Carrier A)

POSITION AND CONTENTS		PROTEOGRAPH ONE 40 SAMPLES	PROTEOGRAPH ONE 80 SAMPLES	PROTEOGRAPH DIRECT ASSAY 80 SAMPLES
A1	Tip adapter (verify it is present)	✓	✓	✓
A2	(Leave this position empty for now)	✗	✗	✗
A3	Cleanup Plate 1, covered with a black lid	✓	✓	✓
A4	Preparation Plate, covered with a clear lid	✓	✓	✗
A5	Reagents Reservoir 1, covered with a black lid			
	Well 1: Denaturation Solution	✓	✓	✓
	Well 2: (Leave this empty for now)	✗	✗	✗
	Well 3: Reduction Solution	✓	✓	✓
	Well 4: Alkylation Solution	✓	✓	✓

2. Select each on-screen checkbox as you verify that the proper reservoirs and contents are in place.
3. Select **Continue** to advance.

Deck Setup 2 of 4: Load Plate Carrier B

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

1. Place reservoirs and reagents into Plate Carrier B as shown in the work deck layout on-screen. Load reservoirs in the order listed.

The assay size you chose determines the number of Binding Solution and Wash Solution bottles you add in their respective reservoirs. 40-sample assays require one bottle; 80-sample assays require two bottles.

Table 16. Deck Setup 2 of 4 (Load Plate Carrier B)

POSITION AND CONTENTS		PROTEOGRAPH ONE 40 SAMPLES	PROTEOGRAPH ONE 80 SAMPLES	PROTEOGRAPH DIRECT ASSAY 80 SAMPLES
B1	(Empty)	⊘	⊘	⊘
B2	Tip Rack Adapter (verify one is present)	✓	✓	✓
B3	Place Binding Reservoir, add binding solution, and cover with a black lid	✓	✓	✓
B4	Place Wash Reservoir, fill with wash solution, and cover with a black lid	✓	✓	⊘
B5	Place Reagent Reservoir 2, fill applicable wells with solution, and cover with a black lid			
	Well 1: Nanoparticle Solution	✓	✓	⊘
	Well 2: (Empty)	⊘	⊘	⊘
	Well 3: Reconstitution Solution	✓	✓	✓
	Well 4: Recovery Solution	✓	✓	✓

2. Select each on-screen checkbox as you verify that the proper reservoirs and contents are in place.
3. Select **Continue** to advance.

Deck Setup 3 of 4: Load Carriers C and D

At **Deck Setup 3 of 4**, do the following.

1. Place labware and NCTs into Plate Carriers C and D as shown in the work deck layout on-screen.

POSITION AND CONTENTS		PROTEOGRAPH ONE 40 SAMPLES	PROTEOGRAPH ONE 80 SAMPLES	PROTEOGRAPH DIRECT ASSAY 80 SAMPLES
Carrier C				
C1	(Empty)	⊘	⊘	⊘
C2	Collection Plate	✓	✓	✓
C3	Cleanup Plate 2	✓	✓	✓
C4	(Empty)	⊘	⊘	⊘
C5	Nanoparticle Plate	✓	✓	⊘
Carrier D				
D1	Four racks of 300 µL Nested Conductive Tips	✓	✓	✓
D2	Four racks of 300 µL Nested Conductive Tips	✓	✓	✓
D3	Four racks of 300 µL Nested Conductive Tips	✓	✓	✓
D4	Four racks of 300 µL Nested Conductive Tips	⊘	✓	⊘
D5	Four racks of 300 µL Nested Conductive Tips	⊘	✓	⊘

2. Select each on-screen checkbox as you verify that the proper reservoirs and contents are in place.
3. Select **Continue** to advance.

Deck Setup 4 of 4: Load tube carriers

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

1. On ice, prepare the Trypsin/LysC Tube:
 - a. Add 500 µL Enzyme Reconstitution Solution to one vial of Trypsin/LysC Protease MS Grade for a final concentration of 0.2 µg/µL.
 - b. Pipette 2-3 times to mix.
 - c. Repeat the two steps above for the remaining Trypsin/LysC vials.

NOTE

A 40-sample assay requires two Trypsin/LysC vials. An 80-sample assay requires four Trypsin/LysC vials.

- d. Transfer 500 µL from each Trypsin/LysC vial into the Trypsin/LysC Tube.
2. Verify that the chiller unit is cold (4 °C) and then transfer the Trypsin/LysC Tube to the chiller.

If the chiller has not yet reached the proper temperature, wait until that temperature is achieved before removing the Trypsin/LysC Tube from the ice.

TIP

If the chiller unit is on but is not achieving the correct temperature, contact your field service representative or Seer support. See [Technical Support \(page 127\)](#) for contact information.

- Load tubes into the chiller and tube rack as shown in the on-screen instructions.

NOTE

Remove caps from tubes prior to placing them into the tube rack.

Table 17. Deck Setup 4 of 4 (Load chiller and tube carriers)

POSITION AND CONTENTS	PROTEOGRAPH ONE 40 SAMPLES	PROTEOGRAPH ONE 80 SAMPLES	PROTEOGRAPH DIRECT ASSAY 80 SAMPLES
Chiller			
Place Trypsin/LysC tube in the top hole of thermal block	✓	✓	✓
Tube Rack (Remove cap from tubes before placing.)			
Nanoparticle (tube 1)	T26	T24	⊘
Nanoparticle (tube 2)	T27	T25	⊘
Nanoparticle (tube 3)	T28	T26	⊘
Cleanup Particles tube	⊘	T27	T27
Cleanup Particles tube	⊘	T28	T28
Cleanup Particles tube	T29	T29	T29
Cleanup Particles tube	T30	T30	T30
Plasma Control tube	T31	T31	T31
Cleanup Particles Control tube	T32	T32	T32

- Select each on-screen checkbox as you verify that the proper tubes and contents are in place.
- Select **Continue** to advance.

Prepare and place samples

After you've loaded the work deck, you must prepare and add your samples and controls.

BEST PRACTICE

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

- For Proteograph ONE assays, aspirate 120 μL from the bottom of the tube, avoiding lipid layers and precipitates. Slowly dispense plasma at the bottom of the Sample Transfer Plate.
- For Proteograph DIRECT assays, aspirate 10 μL from the bottom of the tube, avoiding lipid layers and precipitates. Slowly dispense into the bottom of the Preparation Plate.
- While dispensing, avoid using the second stop of the pipette so as not to introduce bubbles.

1. Prepare and place the samples.

For Proteograph® ONE assays:

- a. Confirm that the centrifuge has reached 4 °C, and then centrifuge the samples at 5,000 $\times$ g for 2 minutes.
- b. Pipetting from the bottom of the tubes, transfer 120 μL of sample plasma into wells 1-80 (A1 - H10) for 80-sample assays or 41-80 (A6-H10) for 40-sample assays of the Sample Transfer Plate.

BEST PRACTICE

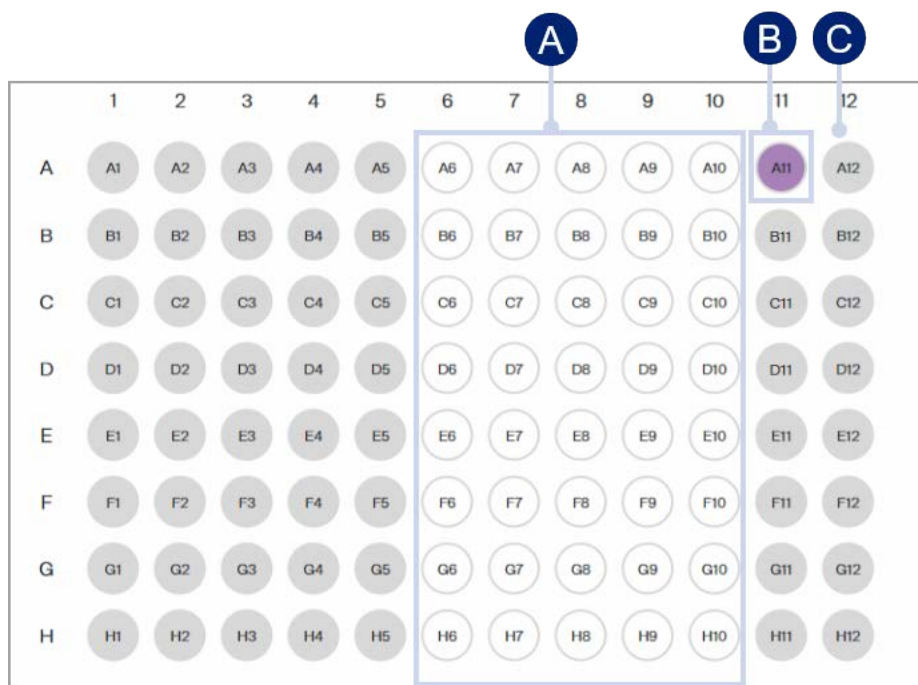
When running multiple replicates of a sample, transfer the sample to a new container after centrifugation, then pipette mix 5-10 times to ensure the sample is uniform before aliquoting to the Sample Transfer plate.

CAUTION

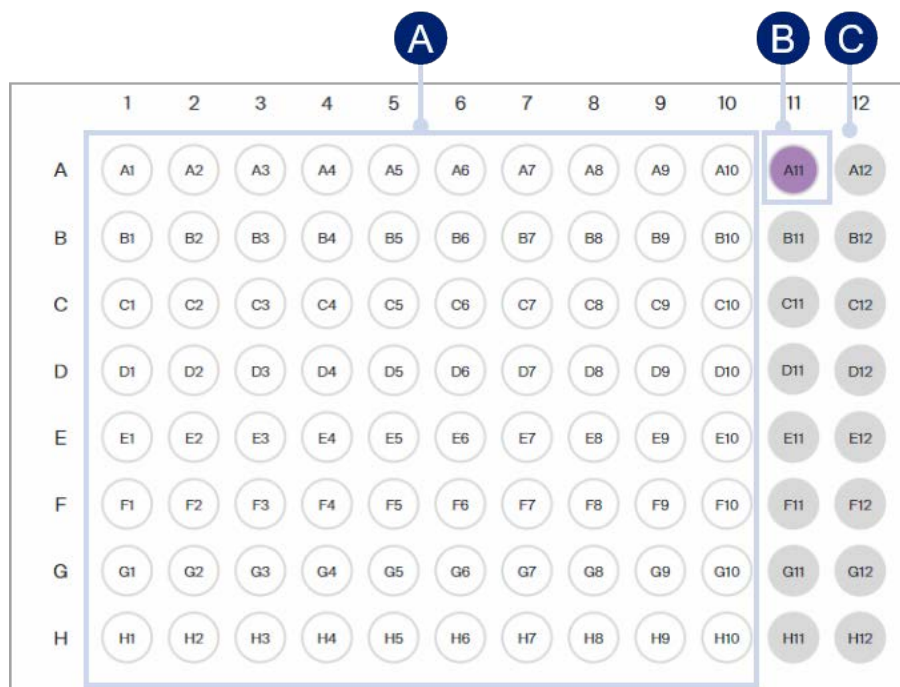
If using fewer samples than the assay kit you've chosen, fill the remaining wells with 120 μL of water. Additionally, if not using a control sample, add 120 μL of water to well A11.
For example, for an 80-sample assay, if you have 78 samples, fill wells 79 and 80 with water.

- c. Add 120 μL of your control to A11.
- d. Seal the Sample Transfer Plate.
- e. Centrifuge at 500 $\times$ g for 15 seconds.
- f. Unseal and place the Sample Transfer Plate in Carrier A in position A2.

Figure 29. Layout of the Sample Transfer Plate for a 40-sample Proteograph ONE assay



- A** Sample wells (white).
- B** Control well (purple).
- C** Unavailable wells (shaded).

Figure 30. Layout of the Sample Transfer Plate for a 80-sample Proteograph ONE assay

- A** Sample wells (white).
- B** Control well (purple).
- C** Unavailable wells (shaded).

For Proteograph® DIRECT assays:

- a. Confirm that the centrifuge has reached 4 °C, and then centrifuge the samples at 5,000 × g for 2 minutes.
- b. Determine sample protein concentration before running the Proteograph DIRECT assay.
- c. Transfer 10 µL of each sample – Cell Lysate or Tissue Lysate – from your assay sample list into wells 1–80 of the Preparation Plate. The recommended input protein concentration is 0.5 µg/µL, with a total protein mass of no less than 0.25 µg and no more than 10 µg per sample.

If the protein concentration is higher than recommended, dilute the sample with water, TE buffer, or PBS.

NOTE

The recommended lysis buffer for use with the Proteograph DIRECT workflow is 25 mM HEPES (pH 7.6) containing 0.5% Triton X-100.

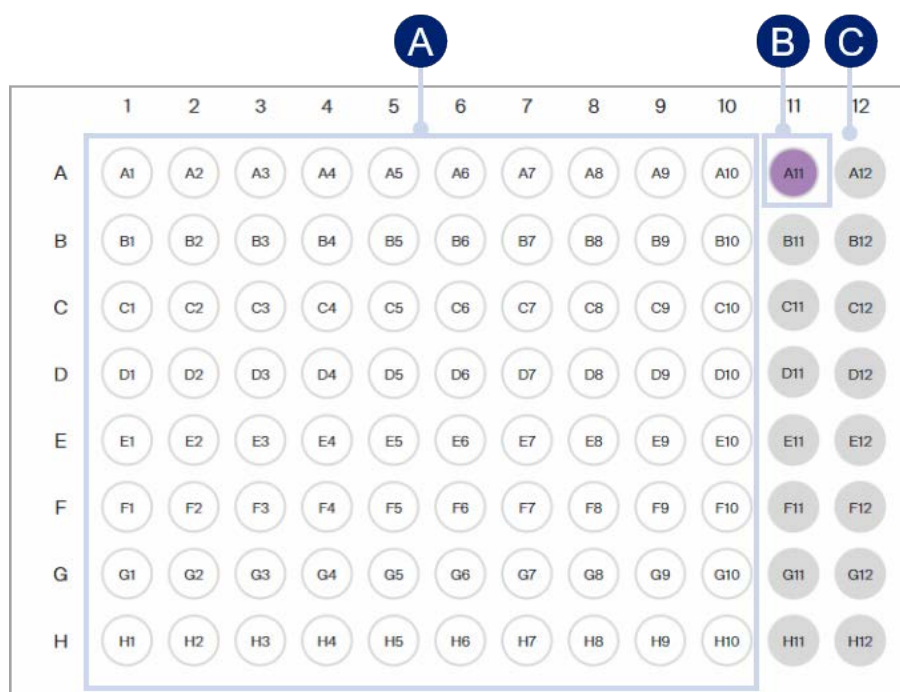
Other sample types may be compatible with the DIRECT workflow; however, they have not been validated.

BEST PRACTICE

When running multiple replicates of a sample, transfer the sample to a new container after centrifugation, then pipette mix 5-10 times to ensure the sample is uniform before aliquoting to the Preparation Plate.

- d. Add 10 μ L of your Control to A11 in the Preparation Plate. (Optional.)
- e. Seal the Preparation Plate.
- f. Centrifuge at 500 x g for 15 seconds.
- g. Unseal and place the Preparation Plate in Carrier A in position A4.
- h. Place the clear lid on top of the Preparation Plate in A4.

Figure 31. Layout of the Preparation Plate for a 80-sample Proteograph DIRECT assay



- A** Sample wells (white).
- B** Control well (purple).
- C** Unavailable wells (shaded).

2. Confirm that all barcodes in the tube carriers are visible and facing to the right, then return the carriers to the autoloader tray.
3. Select each on-screen checkbox as you verify that the proper reservoirs and contents are in place.
4. 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 \(page 46\)](#).

- b. For each of the covered reservoirs, lift 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 well (A1, B1, C1...), while the second column lists user-defined sample names. For example: A1, <SampleA1> ... H10, <SampleH10>
To download a sample names template file, select **Sample Names Template** to download *seer-sample-names-templates.zip*. Unzip and open this file, replace the sample names in their designated positions, and save as a .csv file that you can import using the **Load Sample Names** button.

- 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 well of the Sample Transfer Plate 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 autoloader tray.
- d. Select **Continue** to advance.

ICS moves the carriers from the autoloader 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.

The method takes approximately 5 hours to complete. During the method, ICS displays each stage with time estimates. For information about time estimates, see [The Proteograph Assay workflow \(page 10\)](#).

When the method has finished, proceed to [Unload the work deck \(next page\)](#).

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.

NOTE

If you encounter an unrecoverable error during the Proteograph® ONE Assay method, contact your field service representative or Seer support. See [Technical Support \(page 127\)](#) for contact information.

Unload the work deck

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

1. Ensure that the autoloader 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\Proteograph\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 **Unload Carriers Only** – Unload the carriers .
The carriers immediately move from the work deck to the autoloader tray. ICS returns to the main menu.
4. Immediately retrieve the Collection Plate, which contains the peptides:
 - a. Remove the Collection Plate from the work deck.
 - b. Seal the plate with sealing foil (5 × 3 Inch).
 - c. Centrifuge the sealed plate at 500xg for 15 seconds.
 - d. Set the plate aside at room temperature.

WARNING

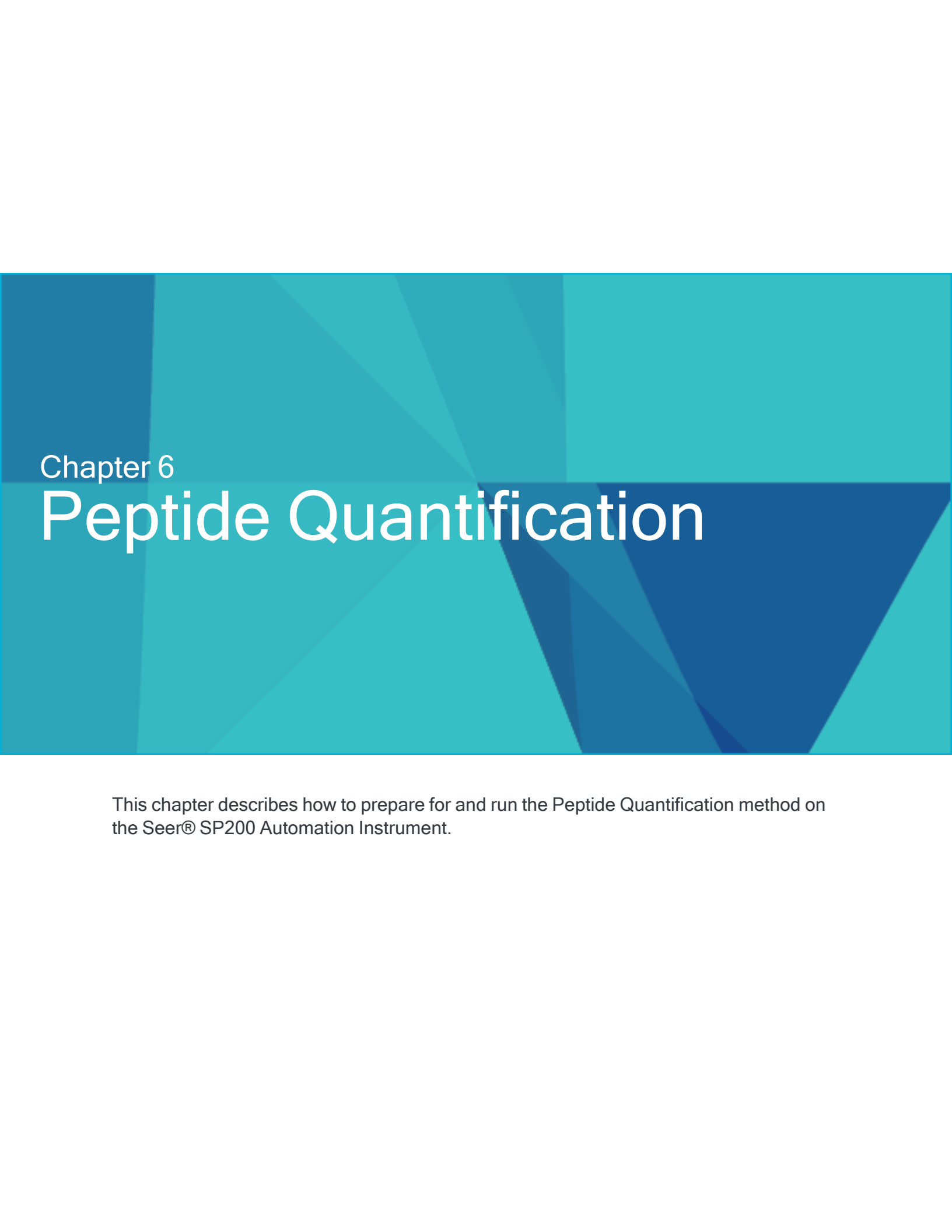
You must begin peptide quantification within 1 hour after completing the assay.

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. Dispose of other labware and leftover reagents in the appropriate waste containers per laboratory policy.
6. Proceed to [Chapter 6. Peptide Quantification \(page 72\)](#).



Chapter 6

Peptide Quantification

This chapter describes how to prepare for and run the Peptide Quantification method on the Seer® SP200 Automation Instrument.

Peptide Quantification method

After you run the Proteograph ONE 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.

Figure 32. The Peptide Quantification segment of the Proteograph workflow



NOTE

Start quantification within **one hour** of removing the Collection Plate from the SP200. 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 ONE PQR Kit \(page 35\)](#).

Additional required equipment and materials are listed below. (For supplier information, see [Required lab equipment \(page 36\)](#) and [Additional required materials \(page 37\)](#).)

Required equipment

- Acid-resistant CentriVap centrifugal vacuum concentrator
- Fluorescence intensity microplate reader

Additional required materials

- 50 µL Nested Conductive Tips (NCTs)
- 300 µL Nested Conductive Tips (NCTs)
- 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
 - 100-1000 µL pipette with tips
- Sealing foil (5 × 3 Inch)

Protocol steps

The Peptide Quantification protocol consists of the following steps:

1. [Prepare the instrument \(below\)](#)
2. [Prepare the quantification consumables \(next page\)](#)
3. [Set up and run the Peptide Quantification method \(page 76\)](#)
4. [Unload the work deck and dry peptides \(page 78\)](#)

Prepare the instrument

1. If the SP200 is not already on, turn on the instrument:

NOTE

Turning on the SP200 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:
 - **SP200** – Press the green switch on the front of the instrument.
 - **Instrument computer** – Press the power button on the front of the instrument computer.
- b. 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 \(page 94\)](#).)

Prepare the quantification consumables

1. Remove the following reagents from 2 - 8 °C storage:
 - Recovery Solution (1 bottle)
 - Pierce quantitative fluorometric peptide assay (sold separately)

NOTE

These materials must be removed from 2 - 8 °C storage an hour 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 ONE PQR Labware Kit:
 - Quantification Reservoir
 - Black Plate Lid
 - Black Quantification Plate
 - Standards Prep Plate

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 **Registration**, do the following:

- a. In the **Your Name** field, enter your username.

NOTE

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

- b. Select **Continue** to advance.

3. At **Deck Setup** (2 screens), 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.

NOTE

Pipette reagents into their plates or reservoirs once they are pulled out onto the autoload tray.

CAUTION

Ensure correct orientation of the Standards Prep Plate and Quantification Reservoir on the work deck **before adding reagents** to the reservoir. The barcode should be facing to the right (toward the barcode reader).

POSITION(S)	ITEM(S)	INSTRUCTION
A2	Collection Plate	Place on deck
A3	Standards Prep Plate	Place on deck
Well A1	150uL of Digest Standard	
A4	Quantification Reservoir	
Well 1	2.4mL Peptide Assay Reagent	
Well 10	3mL Recovery Solution	
Well 11	5 mL Peptide Assay Buffer	
Well 12	5 mL Peptide Assay Buffer	
B1	Single 300uL Tip Rack	
B3	300mL Reservoir filled with 150mL of 70% Isopropyl alcohol	
B4	Quantification Plate	Place on deck
C4	Black Plate Lid	
D1	Place a single rack of 50µL conductive tips	

- b. Leave all other positions 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 **Peptide Quantification - Setup Complete**, do the following:
 - a. Close the front protective cover.

ICS locks the front protective cover.

The method takes approximately 30 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 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 Quantification Plate:
 - i. Remove the Black Quantification 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 \(page 116\)](#).

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 **Total Recon Volume (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. Unseal the plate.
- ii. Transfer the plate to a vacuum concentrator.
- iii. Confirm that the vacuum concentrator is balanced appropriately.
- iv. Set the temperature to $\leq 20^{\circ}\text{C}$.
- v. Run the vacuum concentrator until the peptides are fully dried, which can take several hours.

TIP

Seer recommends running the vacuum concentrator overnight.

- vi. 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 **Main Menu** to return to the ICS main menu.

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

3. If you intend to reconstitute peptides immediately, proceed to [Peptide Reconstitution \(page 81\)](#).

Otherwise, if you intend to reconstitute peptides later:

- a. Seal the Collection Plate with sealing foil (5 × 3 Inch) and store at -80 °C.

TIP

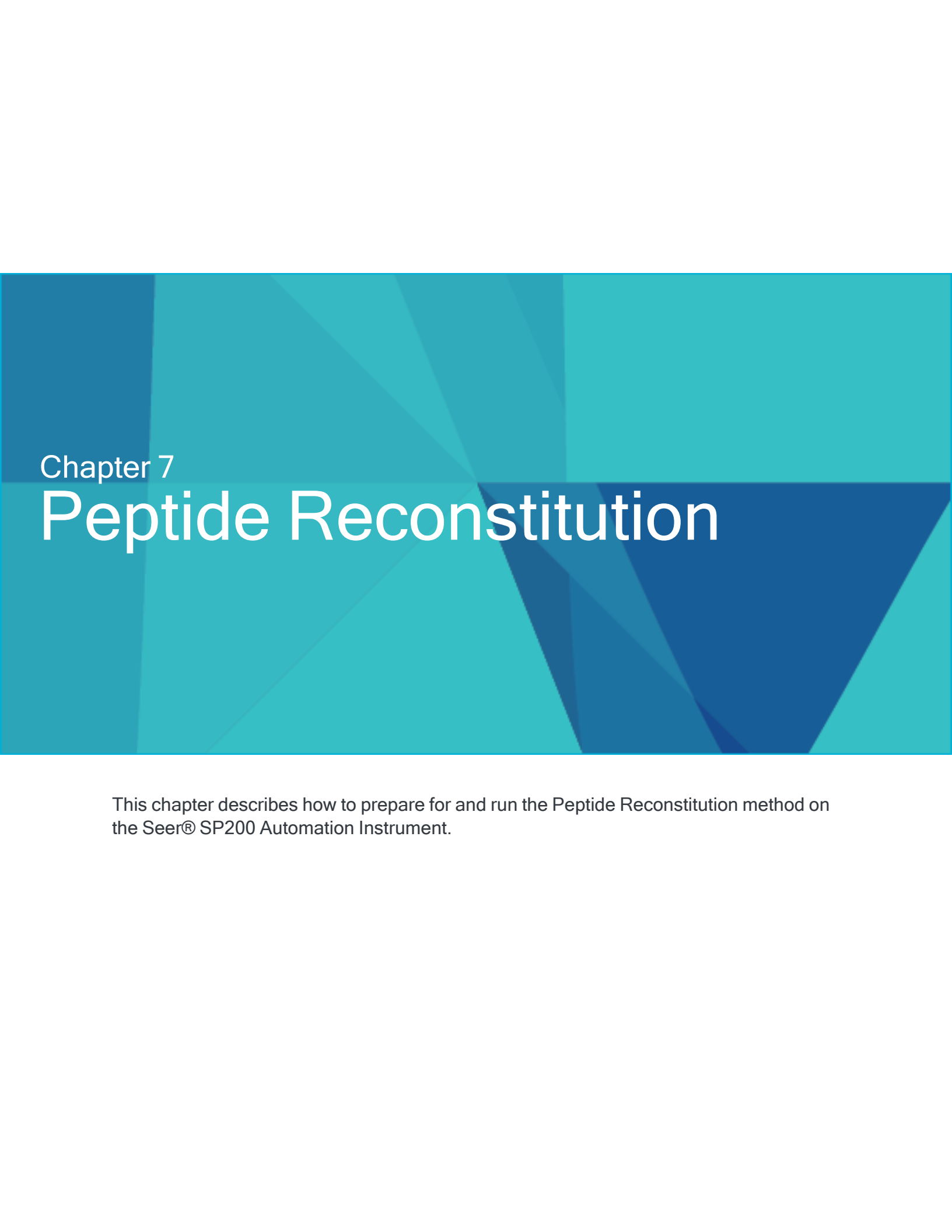
You can store peptides this way for up to one year before reconstitution.

- b. Turn off the instrument and peripherals.

NOTE

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

- **SP200** – Press the green switch on the front of the instrument.
- **Instrument computer** – Press the power button on the front of the instrument computer.



Chapter 7

Peptide Reconstitution

This chapter describes how to prepare for and run the Peptide Reconstitution method on the Seer® SP200 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.

Figure 33. *The Peptide Reconstitution segment of the Proteograph Workflow*



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® ONE PQR Kit (P/N S55R5006).

Additional required equipment and materials are listed below. (For supplier information, see [Required lab equipment \(page 36\)](#) and [Additional required materials \(page 37\)](#).)

Additional required materials

- 300 µL Nested Conductive Tips (NCTs)
- Axygen AxyMats 96 round well sealing mat for PCR microplates
- Peptide reconstitution buffer (see [Peptide reconstitution buffer preparation \(next page\)](#))
 - [Peptide Calibration Kit \(page 36\)](#)
- 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
- Sealing foil (5 × 3 inch)

Peptide reconstitution buffer preparation

Preparing the peptide reconstitution buffer requires the [Peptide Calibration Kit \(page 36\)](#). You'll also need the following labware and equipment:

Table 18. Equipment

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

Preparation steps

CAUTION

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

To create the Peptide Reconstitution Buffer

1. Obtain an appropriate sized bottle large enough to hold sufficient volume to make a single batch for your anticipated study size.

Assume 5 mL of reconstitution buffer will be required for each plate.

For example, an 800 sample study uses 10 plates / kits, requiring 50 mL of Peptide Calibration Reconstitution Buffer.

2. Prepare the Peptide Reconstitution Buffer:

NOTE

You may need to use multiple Peptide Calibration Kits to produce a sufficient volume of Peptide Reconstitution Buffer to run the entire planned study. Each kit provides enough prepared buffer to complete 4 plates.

- a. Thaw tubes of frozen PepCalMix at room temperature.
- b. Add 1 mL of Peptide Calibration Recon Buffer to each PepCalMix tube to reconstitute it.
- c. Vortex the PepCalMix tubes for 10 to 15 seconds.
- d. Place the PepCalMix tubes in the microcentrifuge and spin down at 16,000 x g for 1 minute.
- e. In the large bottle, combine the contents of the PepCalMix tubes with the remaining Peptide Calibration Reconstitution Buffer.
- f. Invert the bottle 8-10 times to mix.

3. Aliquot the prepared Peptide Reconstitution Buffer into the provided Peptide Calibration Reconstitution Tubes at 5 mL per tube.

Label each tube with:

Peptide Reconstitution Buffer

Preparation date

Expiration date (1 year after prep)

Operator name / initials

4. Freeze 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 \(page 87\)](#)
4. [Unload the work deck and export data \(page 90\)](#)

NOTE

Before beginning the reconstitution protocol, ensure that you have already prepared and stored the Peptide reconstitution buffer. See [Peptide reconstitution buffer preparation \(page 83\)](#).

Prepare the instrument

1. If the SP200 is not already on, turn on the instrument:

NOTE

Turning on the SP200 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:
 - **SP200** – Press the green switch on the front of the instrument.
 - **Instrument computer** – Press the power button on the front of the instrument computer.
- b. 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 \(page 94\)](#).)

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. Sonicate for approximately 10 seconds in a room-temperature water bath.
 - b. Vortex briefly.

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 **Registration**, do the following:
 - a. In the **Your Name** field, enter your username.

NOTE

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

- b. Select **Continue** to advance.
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:**
 - A minimum volume of at least 2.5 mL (even if the calculated volume is less than 2.5 mL).
OR
 - 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).

CAUTION

If you add a total buffer volume of less than 2.5 mL, the instrument's pipettes may fail to pick up the liquid.

The maximum recon volume is 150 μL per well.

NOTE

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

- b. Place labware and reagents on the work deck according to the layout shown on-screen. Load items in the order listed.

NOTE

Pipette reagents into their plates or reservoirs once they are pulled out onto the autoloader tray.

POSITION(S)	ITEM	INSTRUCTION
A2	Collection Plate	
A3	Reconstitution Reservoir	
Well 4	Reconstitution Buffer	
B1	300 μ L Tip Rack	
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 autoloader tray.
 - d. Select **Continue** to advance.

ICS moves the carriers from the autoloader 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 \(page 116\)](#):

- [Input Fluorescence Data \(page 117\)](#)
- [Reconstitution \(page 117\)](#)
- [Standard Curve \(page 118\)](#)
- [Peptide Concentration \(page 118\)](#)
- [Peptide Recovered \(page 118\)](#)
- [Outliers \(page 119\)](#)

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.

NOTE

Plate map files are saved to the following directory on the instrument computer: C:\Users\Public\Seer\Proteograph\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*.

- b. Unload labware from the work deck:
 - i. Open the front protective cover.
 - ii. Remove the Collection Plate from the work deck.
 - iii. Seal the Collection Plate with an MS-compatible seal and store at 2 - 8 °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.

- iv. 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.

- **SP200** – Press the green switch on the front of the instrument.
- **Instrument computer** – Press the power button on the front of the instrument computer.



Chapter 8

Instrument Maintenance & Training

This chapter describes procedures and best practices for maintenance of the Seer® SP200 Automation Instrument. It also includes methods that use deionized water instead of plasma samples for training with Seer staff.

Maintenance and training methods

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

Table 19. Maintenance methods

ICON	METHOD	APPROXIMATE DURATION	FREQUENCY
	Daily Maintenance	10 minutes	At the start of each day (typically, before running the Proteograph™ ONE Assay or Peptide Reconstitution methods)
	Weekly Maintenance	30 minutes	At the end of each week
	Water Run Proteograph ONE (40 Samples)	100 minutes	During training or troubleshooting with Seer support
	Water Run Proteograph ONE (80 Samples)	100 minutes	During training or troubleshooting with Seer support
	Water Run Proteograph DIRECT (80 Samples)	100 minutes	During training or troubleshooting with Seer support
	Waste Flush	3 minutes	Typically performed on Quantification runs during incubation; can be run separately

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 \(page 127\)](#) 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.

NOTE

Preventative maintenance visits are included in the Proteograph Plus Extended Warranty service plan.

Required materials for maintenance

All maintenance methods require the following materials. (For supplier information, see [Additional required materials \(page 37\)](#).)

- 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 \(page 96\)](#).

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 ONE Assay or Peptide Reconstitution method.)

NOTE

Waste is typically placed on the table under the instrument.

Run the Daily Maintenance method

1. On the ICS main menu, select **Maintenance**, and then select **Daily Maintenance**.
2. At **Liquid Waste Check**, do the following:
 - 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 gray button to disconnect the nozzle from the attachment.
 - ii. Unplug the cable from the sensor attached to the waste container.
 - iii. Unscrew the cap from the container.
 - iv. Dispose of the contents per your laboratory policy.

WARNING

Never use disinfectants containing oxidizing agents such as bleach to decontaminate liquid waste from the SP200. Doing so can cause toxic fumes and other adverse chemical reactions.

- v. Screw the cap onto the container.
 - vi. Reconnect the sensor to the container.
 - vii. Ensure that the tubing is connected to the waste bottle.
- c. Select the **I have checked the liquid waste container** checkbox.
- d. Select **Continue** to advance.
3. At **Registration**, do the following:
 - a. In the **Your Name** field, enter your username.

NOTE

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

- b. Select **Continue** to advance.

4. At **Prepare the SP200**, follow the onscreen instructions and select **Continue**.
5. At **Clean the Work Deck**, do the following:
 - a. Open the front protective cover and inspect the work deck.
 - b. If the deck needs cleaning, dampen a lint-free tissue with 70% isopropyl alcohol and wipe down gently to clean it.
 - c. After the deck is clean, select **Continue**.
6. At **Empty Tip Waste**, do the following:
 - a. Empty both tip waste containers located on the sides of the SP200.
 - b. Ensure the tip waste container door is properly closed
 - c. Select **Continue**.

The instrument performs maintenance checks.
7. At **Pipette Tightness Check**, follow the onscreen instructions and select **Continue**.
8. At **cLLD Check**, follow the onscreen instructions and select **Continue**.

The instrument displays a Check Results Summary to show the success or failure of individual checks.
9. Select **Main Menu** to return to the menu, or select **Save Report** to save the report as a CSV file.

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 SP200. If you need assistance with maintenance, contact your field service representative or Seer support. See [Technical Support \(page 127\)](#) for contact information.

Required materials for weekly maintenance

In addition to the materials required for all maintenance methods (see [Required materials for maintenance \(page 93\)](#)), the Weekly Maintenance method requires the following materials. (For supplier information, see [Additional required materials \(page 37\)](#).)

- 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 \(previous page\)](#).

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 placed on the table under the instrument.

1. On the ICS main menu, select **Maintenance & Training**, and then select **Weekly Maintenance**.
2. At **Registration**, do the following:
 - a. In the **Your Name** field, enter your username.

NOTE

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

- b. Select **Continue** to advance.
3. At **Prepare the SP200**, follow the onscreen instructions and select **Continue**.
4. At **Power off the SP200**, follow the onscreen instructions to turn off power to the instrument, and then select **Continue**.
5. At **Remove/Clean Carriers**, follow the onscreen instructions and select **Continue**.
6. At **Check Autoload Tray**, follow the onscreen instructions and select **Continue**.
7. At **Clean 4 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.

8. 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.

9. 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. Re-install 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.

10. At **Clean Barcode Scanner**, do the following:

- a. Prepare a lint-free tissue dampened with deionized water.
- b. Wipe the inside and outside of the cover.
- c. Select **Continue**.

11. At **Power on the SP200**, close the front protective cover, power on the instrument, and then select **Continue**.

12. At **Clean the Work Deck**, do the following:
 - a. Open the front protective cover and inspect the work deck.
 - b. If the deck needs cleaning, dampen a lint-free tissue with 70% isopropyl alcohol and wipe down gently to clean it.
 - c. When the deck is clean, select **Continue**.
13. At **Empty Tip Waste**, do the following:
 - a. Empty both tip waste containers located on the sides of the SP200.
 - b. Ensure the tip waste container door is properly closed
 - c. Select **Continue**.

The instrument performs maintenance checks.
14. At **Pipette Tightness Check**, follow the onscreen instructions and select **Continue**.
15. At **cLLD Check**, follow the onscreen instructions and select **Continue**.

The instrument displays a Check Results Summary to show the success or failure of individual checks.
16. Select **Main Menu** to return to the menu, or select **Save Report** to save the report as a CSV file.
17. The **Complete** screen indicates the successful completion of the method. Do the following:
 - a. Return all carriers to the autoloader 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.

Water Runs

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™ ONE 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 a Water Run method

The Water Run method uses the same work deck layout as the Proteograph™ ONE Assay method, but replaces the reagents with water. The Seer® SP200 Automation Instrument Proteograph Instrument Control Software (ICS) includes a water run methodology for each assay type:

- Water Run Proteograph® ONE (40 Samples)
- Water Run Proteograph® ONE (80 Samples)
- Water Run Proteograph® DIRECT (80 Samples)

To run a Water Run method:

1. On the ICS main menu, select **Maintenance & Training**, and then select the type of **Water Run** you want to perform.
2. At the **Registration** screen, select **Continue**.
The **Registration** screen is already populated, with *Water Run* in the **Your Name** field.
3. On the **Sample Names** screen, enter sample names manually, select **Default Sample Names** to autopopulate the sample names, or select **Load Sample Names** to import a csv file of sample names.
4. Select **Continue**.
5. On the **Liquid Waste Check** screen, follow the onscreen instructions for checking and emptying the waste container if needed, and then select the **I have checked the liquid waste container** box.
6. Select **Continue**.
7. On the **Device Initialization** screen, follow the onscreen instructions to prepare the instrument, and then select **Continue**.

8. When ICS prompts you to load the work deck (beginning at **Deck Setup 1 of 4**), follow the onscreen instructions for placing labware and replace reagents with the minimum amounts of deionized water shown below:

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

Table 20. Water Run Deck Setup 1 of 4 (Load Plate Carrier A)

		DEIONIZED WATER VOLUME (MIN)		
POSITION AND CONTENTS		40-SAMPLE	80-SAMPLE	DIRECT DIGEST
A5	<i>Reagents Reservoir 1, covered with a black lid</i>			
	Well 1: Denaturation Solution	25 mL	25 mL	25 mL
	Well 3: Reduction Solution	7 mL	7 mL	7 mL
	Well 4: Alkylation Solution	7 mL	7 mL	7 mL

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

Table 21. Water Run Deck Setup 2 of 4 (Load Plate Carrier B)

		DEIONIZED WATER VOLUME (MIN)		
POSITION AND CONTENTS		40-SAMPLE	80-SAMPLE	DIRECT DIGEST
B3	Place Binding Reservoir, add binding solution, and cover with a black lid	116 mL	232 mL	232 mL
B4	Place Wash Reservoir, fill with wash solution, and cover with lid	100 mL	200 mL	N/A
B5	<i>Reagent Reservoir 2, fill applicable wells with solution, and cover with lid</i>			
	Well 1: Nanoparticle Solution	14 mL	14 mL	N/A
	Well 3: Reconstitution Solution	19 mL	19 mL	19 mL
	Well 4: Recovery Solution	24 mL	24 mL	24 mL

- c. At **Deck Setup 3 of 4**, place the labware as instructed (no liquids are added this step).

POSITION AND CONTENTS		40 SAMPLES	80 SAMPLES	DIRECT DIGEST
Carrier C				
C1	(Empty)	⊘	⊘	⊘
C2	Collection Plate	✓	✓	✓
C3	Cleanup Plate 2	✓	✓	✓
C4	(Empty)	⊘	⊘	⊘
C5	Nanoparticle Plate	✓	✓	⊘
Carrier D				
D1	Four racks of 300 μ L Nested Conductive Tips	✓	✓	✓
D2	Four racks of 300 μ L Nested Conductive Tips	✓	✓	✓
D3	Four racks of 300 μ L Nested Conductive Tips	✓	✓	✓
D4	Four racks of 300 μ L Nested Conductive Tips	⊘	✓	⊘
D5	Four racks of 300 μ L Nested Conductive Tips	⊘	✓	⊘

d. At **Deck Setup 4 of 4**:**Table 22.** Water Run Deck Setup 4 of 4 (Load chiller and tube carriers)

POSITION AND CONTENTS	40-SAMPLE	80-SAMPLE	DIRECT DIGEST
Chiller			
Place Trypsin/LysC tube in the top hole of thermal block			
Add water to the Trypsin/LysC tube	1 mL	2 mL	2 mL
Tube Rack (Remove cap from tubes before placing.)			
Nanoparticle (tube 1)	T26	T24	n/a
Nanoparticle (tube 2)	T27	T25	n/a
Nanoparticle (tube 3)	T28	T26	n/a
Cleanup Particles tube	n/a	T27	T27
Cleanup Particles tube	n/a	T28	T28
Cleanup Particles tube	T29	T29	T29
Cleanup Particles tube	T30	T30	T30
Plasma Control tube	T31	T31	T31
Cleanup Particles Control tube	T32	T32	T32
Sample Transfer Plate in position A2			
Transfer water to each well in the Sample Transfer Plate for the number of samples in the assay.	120 µL in wells 41-80 (A6 - H10)	120 µL in wells 1-80 (A1 - H10)	n/a
Transfer water to the control location (well A11)	120 µL	120 µL	n/a
Preparation Plate (in position A4)	n/a	n/a	15 mL in wells 1-80 (A1 - H10)

9. Select **Continue** on this and the remaining screens to begin the method.

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

Waste Flush

The Waste Flush method is typically run automatically during the incubation time of a Peptide Quantification run. However, you can also run it manually.

Run the Waste Flush method

1. On the ICS main menu, select **Maintenance**, and then select **Waste Flush**.
2. At **Liquid Waste Check**, do the following:
 - 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 gray button to disconnect the nozzle from the attachment.
 - ii. Unplug the cable from the sensor attached to the waste container.
 - iii. Unscrew the cap from the container.
 - iv. Dispose of the contents per your laboratory policy.

WARNING

Never use disinfectants containing oxidizing agents such as bleach to decontaminate liquid waste from the SP200. Doing so can cause toxic fumes and other adverse chemical reactions.

- v. Screw the cap onto the container.
 - vi. Reconnect the sensor to the container.
 - vii. Ensure that the tubing is connected to the waste bottle.
 - c. Select the **I have checked the liquid waste container** checkbox.
 - d. Select **Continue** to advance.
3. At **Registration**, do the following:
 - a. In the **Your Name** field, enter your username.

NOTE

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

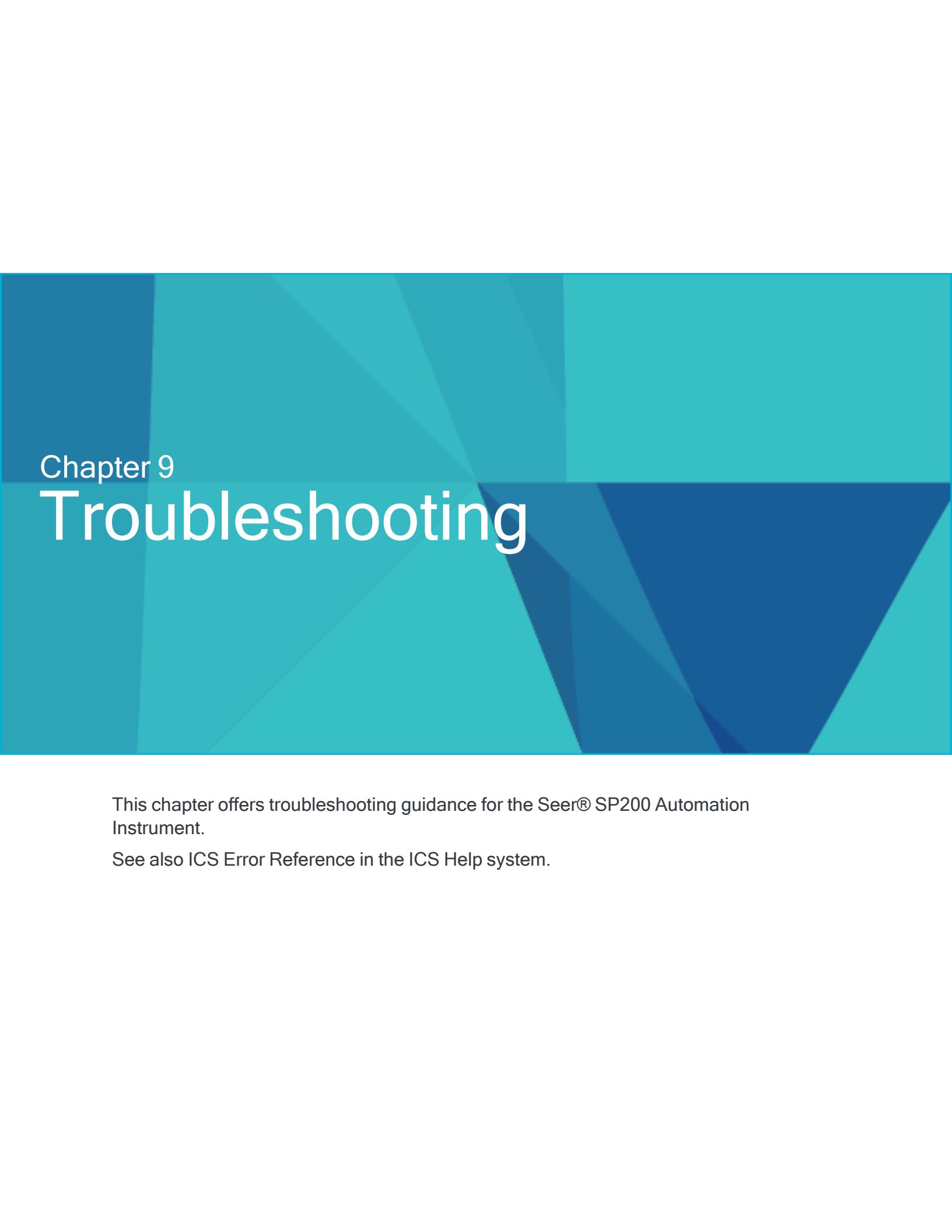
- b. Select **Continue** to advance.

4. At **Device Initialization**, do the following:
 - a. Verify that the Seer SP200 Automation Instrument is powered on.
 - b. Pull all carriers onto the autoloader tray.
 - c. Ensure that the work deck is free of obstructions.
 - d. Close the front protective cover.
 - e. Select **Continue** to begin the automated equipment initialization process.

CAUTION

The gantry will move after you select Continue.

5. At **Deck Setup**, do the following:
 - a. Place a rack of 300 μ L tips, nested into position B1 of carrier B.
 - b. Place a monowell reservoir filled with 70% isopropyl alcohol with no lid into position B3 of carrier B.
 - c. Select the checkboxes as you place items.
 - d. Select **Continue**.
6. At **Starting Waste Flush**, do the following:
 - a. Verify the carriers are on the autoloader tray.
 - b. Close the front cover.
 - c. Select **Continue** to begin the waste flush.
7. At **Waste Flush Complete**, unload all labware from the carriers and select **Continue**.



Chapter 9

Troubleshooting

This chapter offers troubleshooting guidance for the Seer® SP200 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, 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, 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 \(page 127\)](#) 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 \(page 51\)](#).

If an error persists or the instrument experiences intermediate or critical failures, contact your field service representative or Seer support. See [Technical Support \(page 127\)](#) 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 \(page 127\)](#) 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.

The background of the top half of the page is a teal-colored geometric pattern. It consists of several overlapping triangles and polygons in various shades of teal, creating a modern, abstract design.

Appendix A

Safety and Compliance

This appendix provides safety and regulatory compliance information for the proper use of the Seer® SP200 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 SP200 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-1090).

Intended use

The Seer® SP200 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 SP200 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 SP200. If at risk of electric shock, also unplug the instrument.

Noise level

The instrument operates at a noise level of < 65 dBA. On standby, the noise level is < 46 dBA.

Hazardous waste disposal

The Proteograph® assays produce 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 SP200. 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 23. Hazardous reagents

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

Safety hazards

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

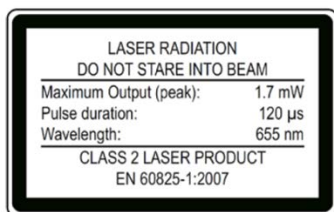
Table 24. Safety hazard symbols

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
	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 SP200 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 SP200 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 25. 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 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.

The background of the top half of the page is a teal-colored geometric pattern. It consists of several overlapping triangles and polygons in various shades of teal and blue, creating a modern, abstract design.

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 147, as this is the volume of peptides collected at the end of the Proteograph™ ONE 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 147 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 147 μL. (For a typical assay with a single peptide quantification run performed, this should be 137 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 ($\mu\text{g}/\mu\text{L}$)** – 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 $\mu\text{g}/\mu\text{L}$), 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.

MS

Mass spectrometry.

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™ ONE Assay icsMethod.

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™ ONE 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 SP200.

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 SP200 used to operate the instrument.

Proteograph ONE Assay Kit

A Seer® kit containing the reagents and labware for preparing samples on the SP200.

Proteograph ONE PQR Labware Kit

A Seer® kit containing labware and reagents needed for four Peptide Quantification runs and four Peptide Reconstitution runs on the SP200.

Proteograph® Product Suite

The bundle of Seer® kits, instrument, and analysis software.

Q**QC**

Quality control.

R**rack**

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

S

sleeve

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

SP200 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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