Quanterix™
The Science of Precision Health

Simoa® HD-X Analyzer User Guide

USER-0033 06

simoa™

Customer Support

Customer support is available 8 AM to 5 PM, EDT.

email: techsupport@quanterix.com

Contact Us

Quanterix® Corporation

900 Middlesex Turnpike

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The Quanterix products referenced in this document are for research use only and are not for diagnostic or therapeutic procedures.

Contents

1 About This Document

Document Purpose

This document provides information about how to perform Simoa® assays with the Simoa HD-X Analyzer (termed "the instrument" subsequently). After reading this document, you will know how to operate the instrument, perform assays, maintain the instrument, and obtain help for instrument or assay problems.

Intended Audience

This guide is for anyone who operates the instrument, develops assays for the instrument, or maintains the instrument. Users should be familiar with standard laboratory practices and with computers running the Windows operating system.

Customer Support

Customer support is available 8 AM to 5 PM EST, Monday through Friday.

Email: techsupport@quanterix.com

When you contact Quanterix®, you may be asked to use the Customer Support Tool to generate a support package that will help troubleshoot the problem. See *Quanterix Customer Support Tool User Guide* for instructions.

Log in to the Quanterix portal to view training videos at http://portal.quanterix.com.

Purchasing Supplies

For information about purchasing reagent kits, consumables, and instruments, contact Sales at Quanterix:

Quanterix Corporation
900 Middlesex Turnpike
Billerica, MA 01821
Phone: 617-301-9400
Email: sales@quanterix.com

Abbreviations Used in This Document

CCD	charge-coupled device (camera imaging system)
ELISA	enzyme-linked immunosorbent assay
GUI	graphical user interface
SBG	Streptavidin-beta-galactosidase
RGP	resorufin-D-galactopyranoside

Notices Used in This Document

Note! This type of notice highlights important information about procedures or provides tips.

CAUTION! This type of notice provides information about minor potential hazards to the instrument or assay performance. Failure to comply with the notice may result in inaccurate results, instrument maintenance problems, or instrument failure.

WARNING! This type of notice provides information about situations that pose a danger to the instrument or to operators. Failure to observe the warnings in this notice may result in instrument damage or a safety hazard for operators.

2 Safety and Regulatory Information

Read the Safety and Regulatory Notices

Before you operate the Simoa® HD-X Analyzer, familiarize yourself with the safety and regulatory notices in this chapter. If improperly operated, the instrument can pose electrical or mechanical safety hazards to operators. You may also encounter chemical or biological hazards, depending on the assays that you perform with the instrument.

Do not perform any instrument procedures that are not described in this guide without the guidance of Quanterix® Customer Support.

The Simoa HD-X Analyzer must be installed by trained service professionals only. Do not interfere with system electrical grounding or move the instrument after installation without the assistance of Quanterix Customer Support.

General Safety Notices

CAUTION! As the Simoa HD-X Analyzer is designed for measuring biological samples, universal laboratory precautions should be used at all times.

WARNING! Do not install any software on the Simoa HD-X Analyzer computer unless instructed to do so by Quanterix Customer Support. Installing third-party software can result in system failure, job loss, and voiding of your warranty. Contact Technical Support for further details and assistance.

CAUTION! Do not move the instrument after the initial installation.

CAUTION! Do not remove the cover of the instrument.
Label located on pipette rail near cuvette loader.

CAUTION – Mechanical hazard! Do not insert your hands into the reagent bay, the sample bay, or the system bay if the instrument is turned on. Doing so may expose you to risk of personal injury. The instrument contains robotic pipettors that access these areas at times, and they can injure your hands.
Label is located on sample bay door, reagent bay door, drawers, waste container drawer (left and right side) and cuvette loader.

CAUTION! Hot surface!
Label is located on LSI unit.

Overheating!
Improper placement of the instrument may cause fire or serious instrument damage in case of overheating.

- Do not block or cover ventilation slots.
- The air shall be able to circulate.

Electrical Safety Notices

CAUTION! The appliance inlet is intended to be used as a disconnecting device in case of an emergency. Set up the instrument so that the appliance inlet is always easily accessible.

Improper connection of mains supply.

Improper connection of the instrument and the peripheral devices to the mains supply can cause serious personal injury with potentially deadly consequences and material damage (e.g. fire).

- Only use grounded connection and extension cables with sufficient capacity (voltage and current) to connect the instrument and any peripheral devices to the mains power supply.
- Never remove ground connections.
- Grounding of the instrument and its peripheral devices to the same protective earth potential shall be ensured.
- The use of a multi-outlet power strip is not allowed!
- Only use power cables that fulfill the minimum requirements for this instrument.

Chemical/Biological Safety Notices

CAUTION! Biohazard materials may be used on this instrument. Proper personal protective equipment (PPE) should be worn at all times to meet the level of biohazard materials used.

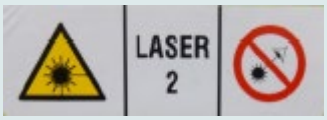
Risk of infection! Monthly maintenance and waste removal procedures put the user in close contact with biohazard materials. Proper personal protective equipment (PPE) should be worn at all times to meet the level of biohazard materials used. Label is located on drawers.

- Strictly follow the local and national provisions, legislation and laboratory regulations.
- Use appropriate gloves!
- Use an appropriate lab coat!
- Use an appropriate eye protection (e.g. protective glasses)!
- Avoid contact between skin/mucous membrane and samples/test reagents or parts of the instrument.
- Clean, disinfect and decontaminate the instrument immediately if potentially infectious material has been spilled.
- Do not use broken or chipped tubes or bottles.
- Observe the instructions in the package inserts for correct use of reagents.
- Observe the legal regulations for the handling of infectious material.
- Never use bio-hazardous liquids for testing the instrument!

- The instrument shall be cleaned, disinfected and decontaminated before servicing!

Laser and Electrical Safety Labels

The following regulatory labels are affixed to the Simoa HD-X Analyzer.

Label Image	Description of Label Information
	Laser safety label. The label is located on the side of the reagent bay (right) and the sample bay (left), adjacent to the laser inside of the bay.
	Laser information label. Provides the user with information on the class, wavelength, output power, and regulatory compliance status of the laser. The label is for the barcode readers next to the reagent bay and sample bay.
	Biohazard label. The label is located in five places: • The reagent bay door, in front of the reagent bay lanes. • The sample bay door, in front of the sample bay lanes. • The front of liquid waste bottles 1 and 2. • The solid waste intermediate trap door to the waste bin. • The system bay waste drawer, inside rear surface.
	Electrical hazard label. The label is located above and adjacent to the electrical power switch, on the side of the instrument.
	Heavy weight. The label is located on the inside of the reagent and sample bay doors. Do not place heavy objects on the doors. When the system bay drawers are open, do not step on them or apply additional weight.

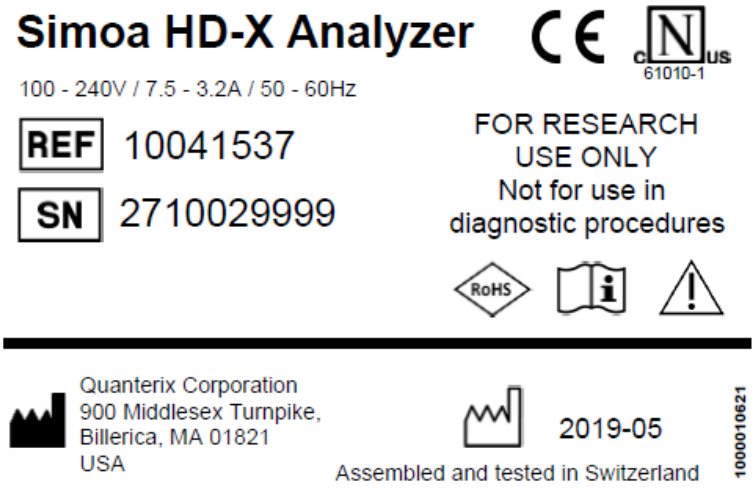
Regulatory Certifications

This product complies with the EMC standards listed below for class A devices and is suitable for use in commercial environments. In a domestic environment, this product may cause radio interferences, in which case the user may be required to take adequate measures.

- EN ISO 14971:2012 (medical devices – Application of risk management to med devices)
- EN 61010-1 and EN 61326-1 (International) Safety of operation in a laboratory environment

Labeling

The following label is affixed to the instrument:



Restriction of Hazardous Substances (RoHS2)

The instrument system complies with the RoHS2 Directive:

- Directive 2011/65/EU – Restriction of Hazardous Substances in Electrical and Electronic Equipment

3 Overview of the Simoa® HD-X Analyzer

Introduction

The Simoa HD-X Analyzer is a fully automated instrument that performs ELISA immunoassays at unprecedented levels of sensitivity. At the heart of the instrument is a precision-engineered, disposable disc with twenty-four ~238,000 femtoliter-sized reaction chambers, termed single molecule arrays (Simoa), that can isolate and detect single enzyme-labeled protein molecules. Because the array volumes are approximately 2 billion times smaller than those required for conventional ELISA, a rapid buildup of fluorescent product is generated if a labeled protein is present. With diffusion limited, this high local concentration of product can be readily observed.

Only a single molecule is needed to reach the detection limit.

Quanterix® provides a wide range of assay kits for the Simoa HD-X Analyzer, containing ready-to-load reagents. For those who wish to create their own assays, Quanterix also provides a Homebrew kit and assay development guide.

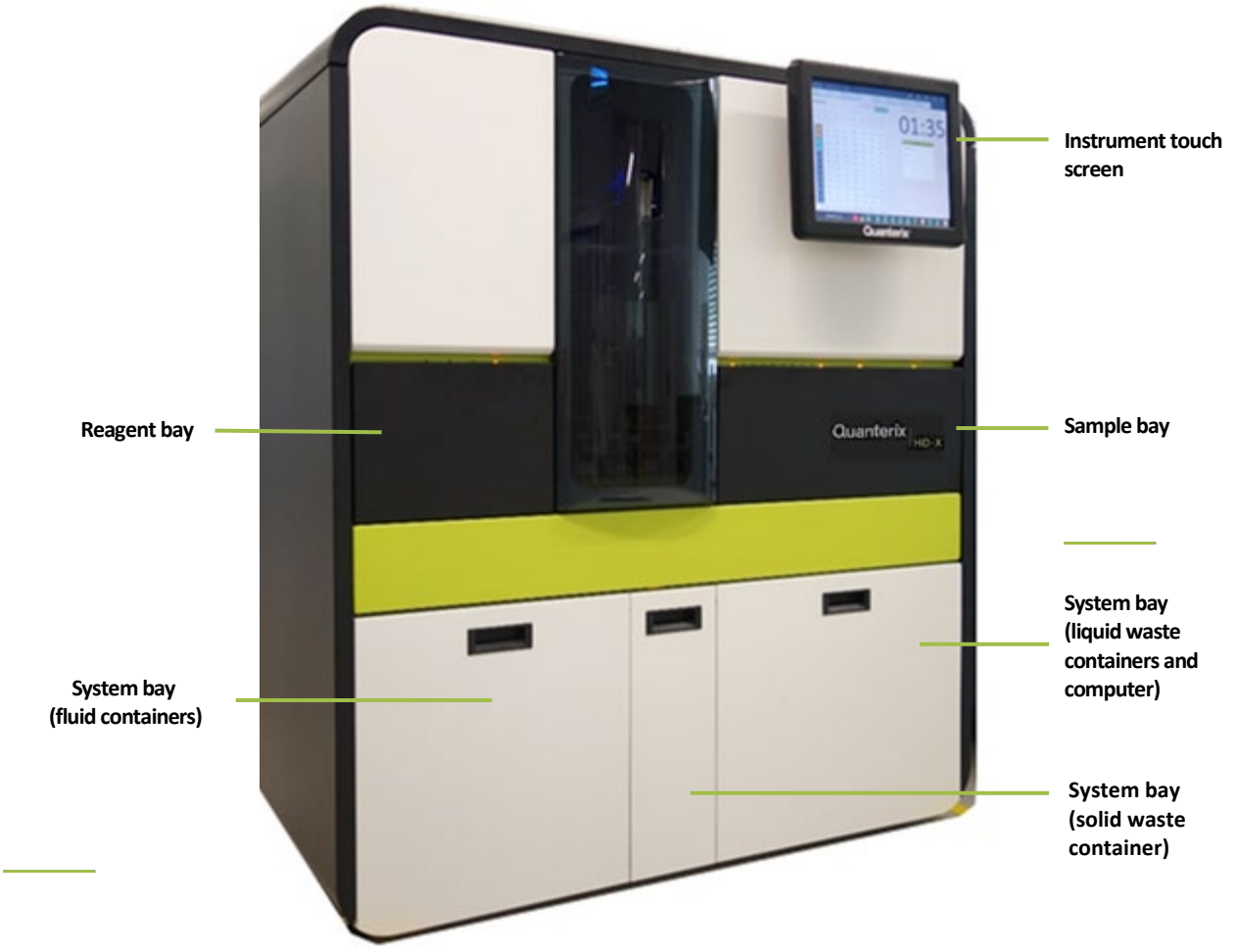
Performing a Simoa assay of any type is simple:

- Load assay reagents into racks in the reagent bay.
- Load plates or tubes of samples and a fluorescent substrate (typically RGP) into racks in the sample bay.

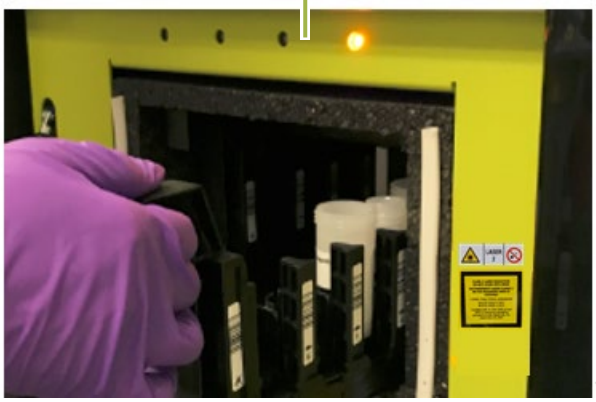
- Initiate the assay with the instrument's touch-screen software.
- View and analyze assay results in the instrument software or export them to analysis packages or a laboratory information management system (LIMS).

Key Instrument Parts

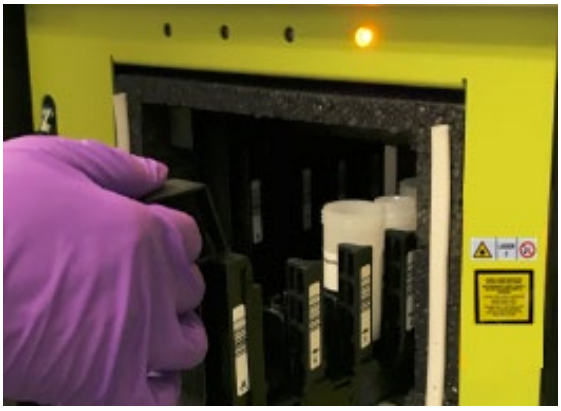
External View



Reagent bay showing four reagent rack lanes



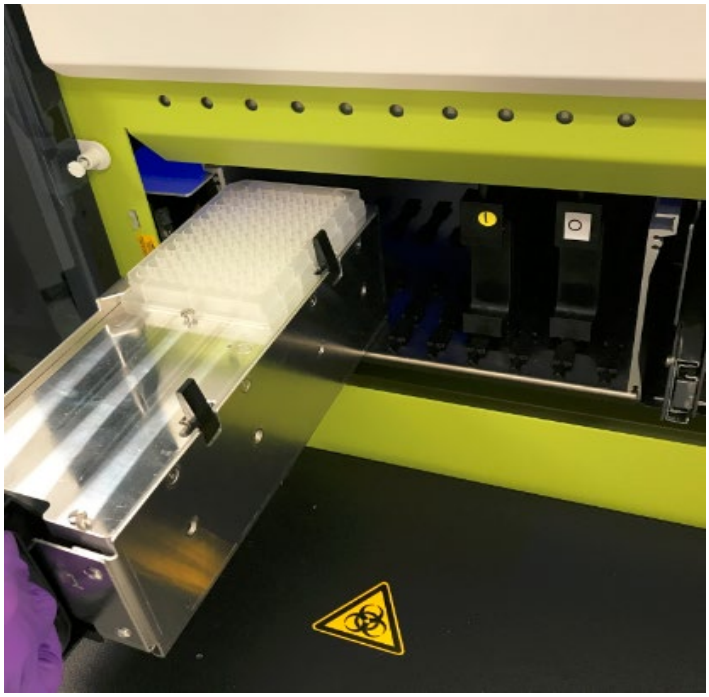
Lane 1 2 3 4

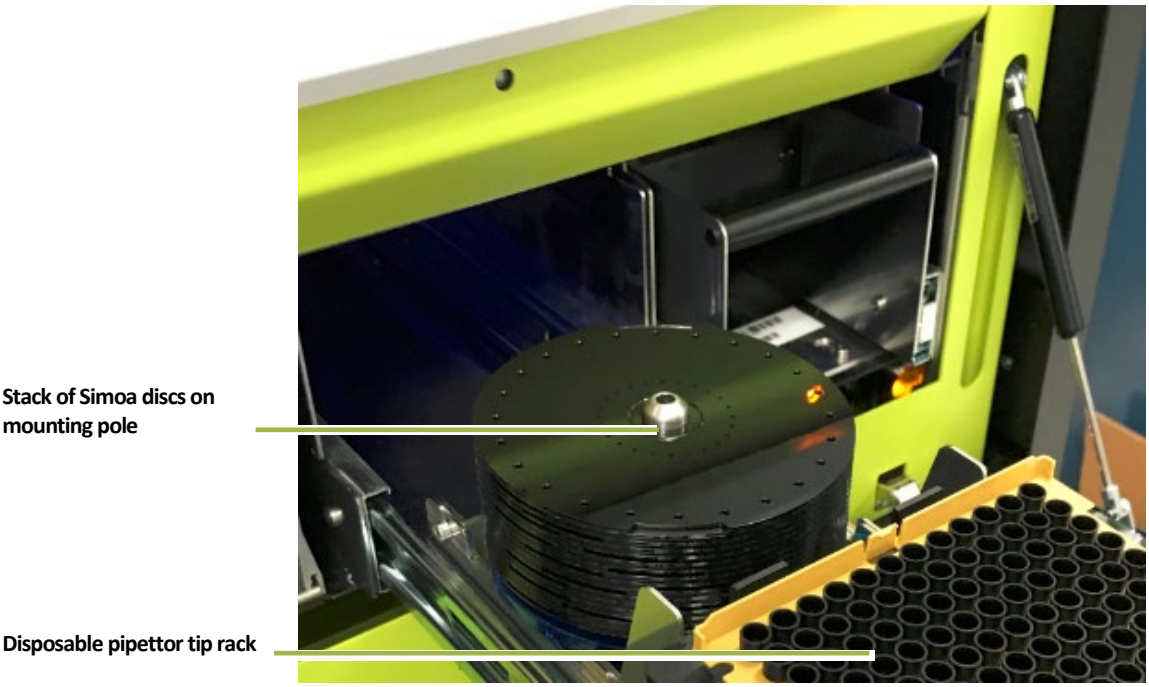


Loading a reagent rack Into reagent bay lane 4

Sample Bay

Loaded plate rack loaded into sample bay lanes 1-4 and RGP rack loaded into RGP lane 1





System Bay

System fluid containers, page 14 **Solid waste container, page 15** **Liquid waste containers, page 15**

Labeling for System fluid (left) and Liquid waste containers (right)

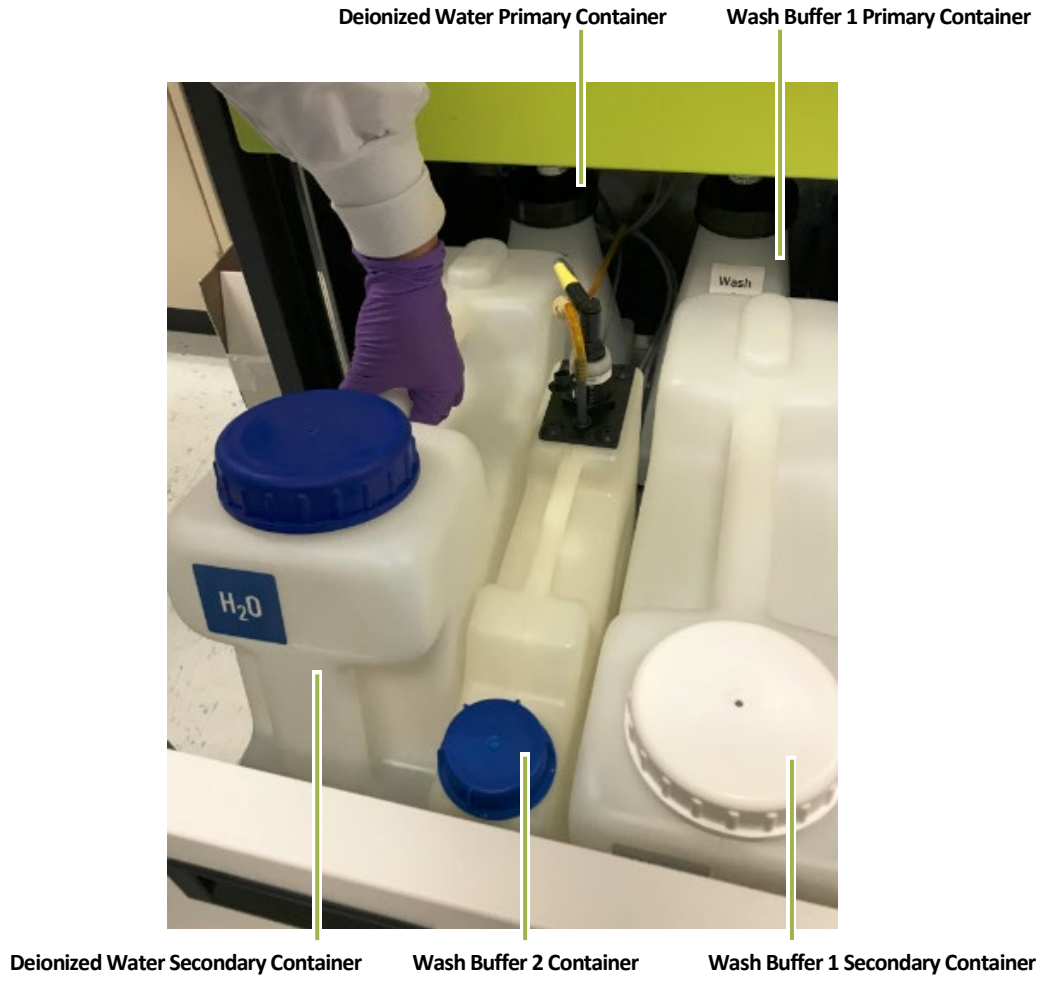
System Fluid Containers

The system uses three system fluids:

- Deionized water (system fluid and needle wash)
- Wash buffer 1 (main assay wash buffer)
- Wash buffer 2 (bead wash pre-loading)

The deionized water and wash buffer 1 each have a secondary container that feeds into a primary container. The instrument draws fluid from the primary containers, which in turn replenish themselves passively from their secondary containers. The container caps connect to tubing that delivers system fluids where needed in the instrument. The secondary containers connect to the primary containers via a plug.

Each secondary system fluid container feeds into a primary container via a short connector. The instrument monitors fluid levels in the containers. The software notifies operators when fluids need replenishing via color-coded icons.



System Waste Containers

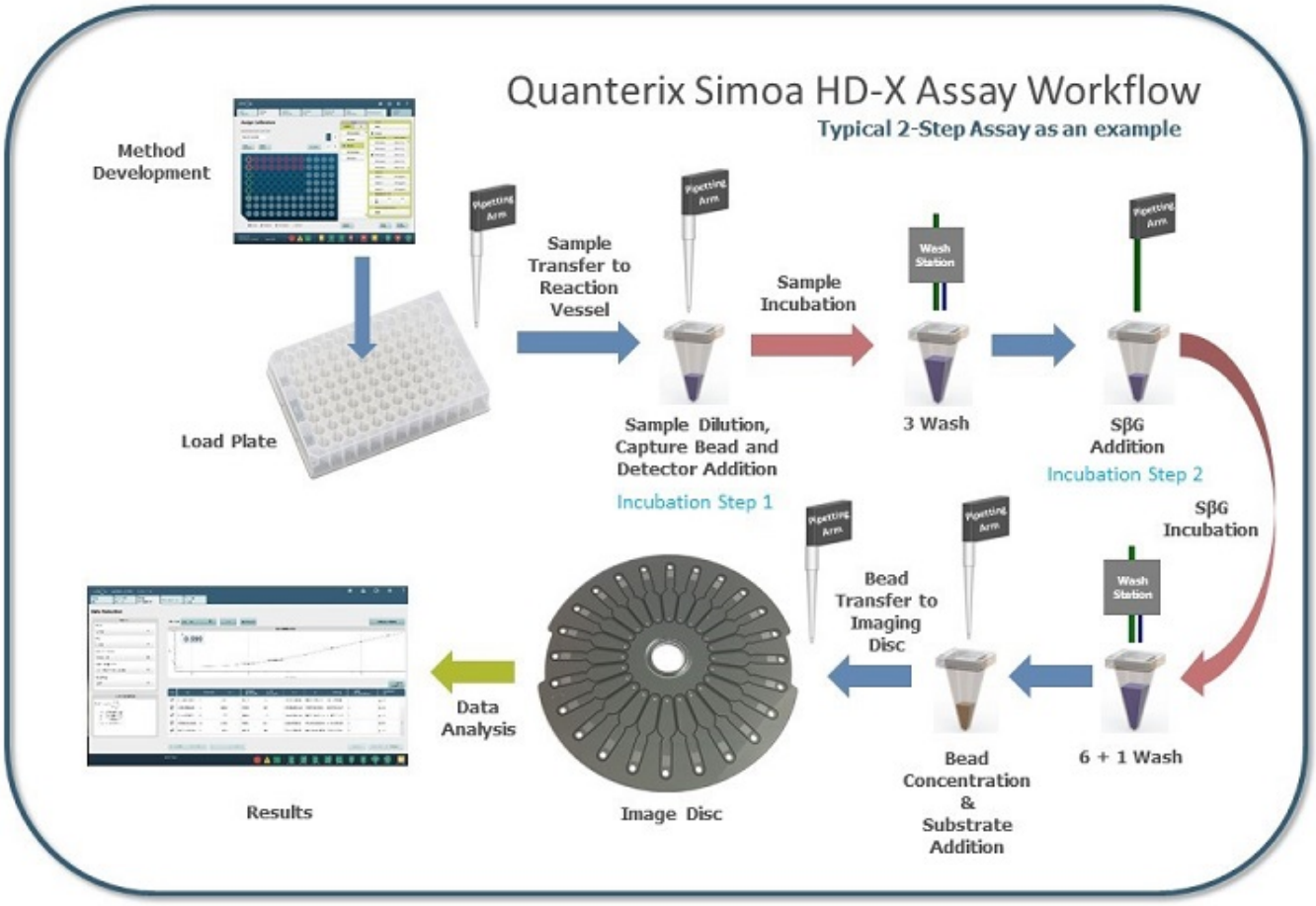
The instrument discards used cuvettes, tips, and Simoa Discs into the solid waste container and the effluent from sample washing and incubation into the liquid waste container.

The caps on the liquid waste containers hold liquid-level sensors that allow the instrument to determine when the containers are full and need to be emptied.

Solid waste container

Liquid waste containers

Overview of Key Instrument Operations



- **Method Development** – Set up your assay using the HD-X touch screen.
- **Load Plate** – Load reagents, samples, and consumables.
- **Sample Transfer to Reaction Vessel** – Robotic pipettors deliver samples into the cuvettes.
- **Sample Dilution, Capture Bead and Detector Addition** – Samples are diluted; capture beads and detector reagents are added to the cuvette.
- **Sample Incubation** – The system implements a one-, two-, or three-step incubation series by moving the cuvette within the washer/incubator ring per the details of the user-specified assay protocol.
- **3 Washes/6+1 Washes** – The washer/incubator ring moves the cuvettes through a series of pelleting, mixing, and washing, according to the protocol. All washes, except the final wash, are performed using Wash Buffer 1. Wash Buffer 2 is used for the final wash preceding substrate addition.

- **SBG Addition** / **SBG Incubation** – SBG is added to the cuvette and the washer/incubator ring implements the protocol.
- **Bead Concentration & Substrate Addition** – Beads are pelleted, wash buffer is removed and beads are re-suspended via mixing with enzyme substrate, RGP.
- **Bead Transfer to Imaging Disc** – The disposable tip pipettor transfers the mixture of beads and RGP into the sample inlet of a microarray on the Simoa Disc. The instrument loads and seals individual beads into microwells within the array. Loose beads that have not settled into microwells are flushed away.
- **Image Disc** – The instrument moves the disc to the imaging station, where a camera images the sealed wells, capturing the growth of fluorescent signal generated by enzyme labeled beads.
- **Data Analysis/Results** – The Simoa software analyzes the image, determines the average enzymes per bead (AEB), and generates a curve.

4 Quick Start Guide

This quick start guide walks you through performing an assay run on the Simoa® HD-X Analyzer. Be sure to read the entire user guide before you perform your first run.

After that, you can use this guide as a ready reference and to locate explanations of instrument procedures.

Log in to the Quanterix® portal to view training videos for additional detail at http://portal.quanterix.com/.

Note! The Instrument and the computer must be power-cycled once every 24 hours to permit internal systems to reinitialize.

1. Turn on the Simoa HD-X Analyzer computer.	Chapter 5, page 23
2. If the instrument is not running, turn it on. If the instrument has been running continuously overnight, turn it off, wait 15 seconds, and turn it on again.	Chapter 5, page 24
3. Log in to the Simoa software.	Chapter 5, page 25
4. If performing the first run of the day:	
• Perform the End of Day task if it was not performed the previous night.	Chapter 11, page 157
• Perform the Start of Day task.	Chapter 11, page 156
5. Prepare reagents, samples, and calibrators.	Chapter 8, page 92
6. If needed:	
• Empty the solid and liquid waste containers.	Chapter 5, page 51
• Refill system fluid containers (deionized water, system wash buffer 1 and 2).	Chapter 5, page 46
• Load cuvettes, disposable pipettor tips, and Simoa discs.	Chapter 5, page 36

7. Mix the bead reagent according to the kit instructions. Re-mix the bead reagent if it sits for >5 minutes before being loaded.	Chapter 8, page 97
8. Set up the software for sample plates or sample tubes.	Chapter 12, page 170
9. Load bead, detector, and SBG reagents into the instrument.	Chapter 8, page 97
• Place bead, detector, and SBG in a reagent rack.	
• **If using the handheld barcode scanner:** Identify the rack position of each reagent bottle and the lane into which you plan to load them. Scan the bottle barcodes and insert the reagent rack.	
• **If using the on-board scanner:** Enable the on-board scanner in the Load Reagents screen and select the lane into which you will load the reagent rack. Insert the rack.	
10. Load RGP reagent into the instrument.	Chapter 8, page 102
• Place RGP reagent in the RGP rack.	
• **If using the handheld barcode scanner:** Identify the rack position of the RGP reagent and the lane into which you plan to load it. Scan the RGP bottle barcode and insert the RGP rack.	
• **If using the on-board scanner:** Enable the on-board scanner and select the lane into which you will load the RGP rack. Insert the rack.	
11. Load calibrators and samples:	
• Assign calibrators in the software.	Chapter 8, page 113
• Assign samples in the software.	Chapter 8, page 121
• Insert calibrator and sample plates or tubes.	Chapter 8, page 127

12. Start the run.	Chapter 8, page 128
13. Monitor the assay in the Current Run tab.	Chapter 8, page 132

5 Operating the Simoa® HD-X Analyzer

Connecting the Simoa HD-X Analyzer to Electrical Power

The Simoa HD-X Analyzer must be connected to a dedicated electrical outlet that conforms to the instrument specifications for electrical power (see *Simoa HD-X Analyzer Site Requirements*). Your instrument will be equipped with the correct type of electrical plug for your country.

CAUTION! If you must use an extension cord to connect the instrument to its dedicated electrical outlet, use only a cord with a three-prong connector on both ends. Using a two-prong extension cord defeats the grounding circuit of the outlet, creating a hazard of shock.

CAUTION! Quanterix® recommends that the dedicated outlet that you use for the instrument be equipped with a ground fault circuit interrupter.

Note! Do not disconnect the instrument from its electrical power source when the instrument is running an assay. Doing this will cause the instrument to discard the Simoa Disc and the cuvettes for the assay when you reconnect it to power.

Turning the Instrument Computer On and Off

The instrument computer is inside of the system bay. Complete all assay operations and log out of the Simoa software before you turn off the computer.

To turn the computer on

Press the on/off power switch at the top of the computer, wait three minutes, power up the instrument (see “Turning the Instrument On and Off” on page 24), and open the HD-X software (see “Logging In to and Out of the Simoa Software” on page 25).

To turn the computer off

Close the software and shut down the computer through Windows Shut down.

Turning the Instrument On and Off

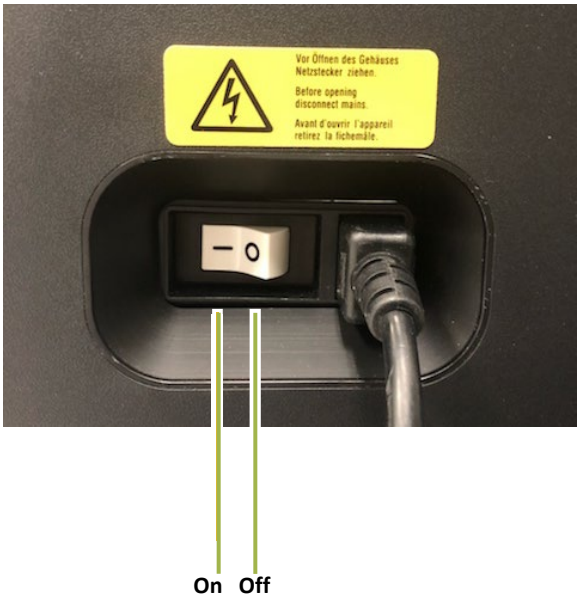
❖ To turn the instrument on

Move the power switch to the on position: Push down the end of the switch with the dash (—). The instrument initializes itself over the next three minutes.

❖ To turn the instrument off

Move the power switch to the off position: Push down the end of the switch with the circle (O).

Note! Do not turn off the instrument during an assay run. Doing this will cause the instrument to discard the Simoa Disc and the cuvettes for the assay when you reconnect it to power.



Note! You must turn the instrument on and off once a day to power cycle instrument systems. During power cycling, the instrument performs a series of hardware and software initialization processes. Wait 15 seconds after you power down before powering up again.

If the instrument has not been power cycled in over 24 hours, the software will prompt you to turn it off with the following message:

Select “Yes” to close the software and shut down the instrument computer. You must then manually turn off the instrument by moving the power switch to the off position.

Logging In to and Out of the Simoa Software

To log in to the software, you need a user name and a password. Only a user with administrative privileges can add users to the system. For information on managing users, see “Managing Users” on page 177.

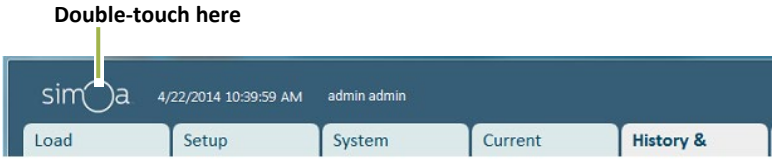
To log in to the software

1. Double-touch the Simoa software icon on the instrument desktop.

2. Enter your user name and password > touch **Enter**.
3. Touch **Accept** in the Terms of Use dialog. Depending on how your system is configured, the first time you open the software, you may see the startup screen.

If you check the “Do not show this screen next time” checkbox, the next time you open the software, the tabbed user interface appears.

To minimize the software GUI, double-touch the letter **O** in simoa.

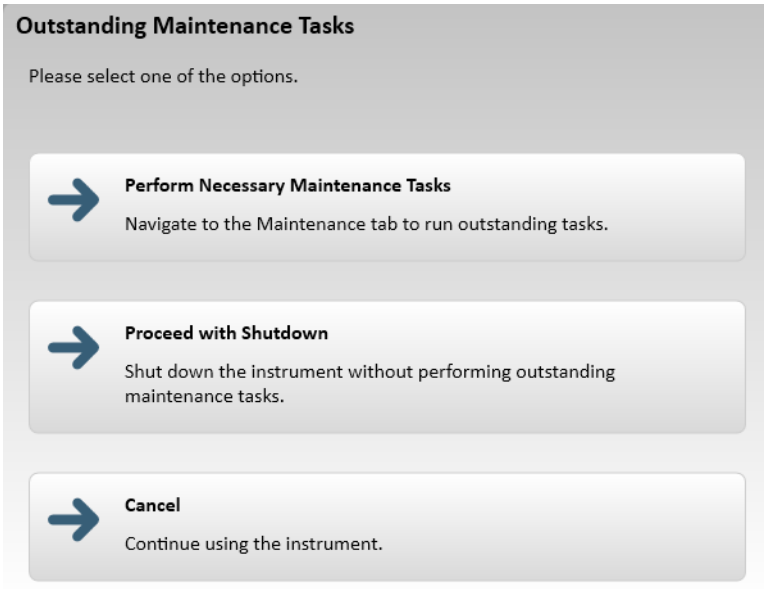


To Log Out of the software

1 Touch the Logout icon at the top right of the software screen.

2 Click Logout to log out the current user (without closing the software) or Shutdown to log out and exit the software.

3 Perform maintenance tasks, exit, or cancel the log out.



Note! Do not shut down the software during an assay run or while data analysis is in progress, as this will result in run failure or the cancellation of data analysis.

Preparing the Instrument for the First Run of the Day

Before the first run of the day:

- Power cycle the instrument if it was not turned off the preceding night by turning the instrument off, waiting for 15 seconds, and turning it on again (see page 24).
- If the End of Day task was not performed the preceding night, perform it now (see page 157).
- Run the Start of Day task (see page 156).
- Refill the system fluid containers if necessary (see page 46).
- Empty the waste containers if necessary (see page 51).

Shutting Down the Instrument After the Last Run of the Day

After the last run of the day:

- Remove any leftover reagents and samples from the instrument. Recap the reagents and store at 2–8° C or as instructed in the assay kit instructions.
- Empty the solid and liquid system waste containers (see page 51).
- Perform the End of Day task (see page 157).

Operating the Barcode Scanners

You have the option to use the handheld or on-board barcode scanners to scan reagent label barcodes.

Using the Handheld Scanner

The handheld scanner is used to scan reagent, RGP, and Simoa disc barcodes.

The barcode scanner is connected to the instrument computer by default. If you need to connect it, plug it into one of the USB ports on the front of the instrument computer, which is in the system bay. The scanner beeps once when it becomes active.

When not in use, store the scanner in the holster that is mounted on the right side of the instrument.

To operate the scanner, point it at the barcode and press the scan button. The scanner transmits the barcode data to the computer, which displays it in the software.

Using the On-Board Scanner

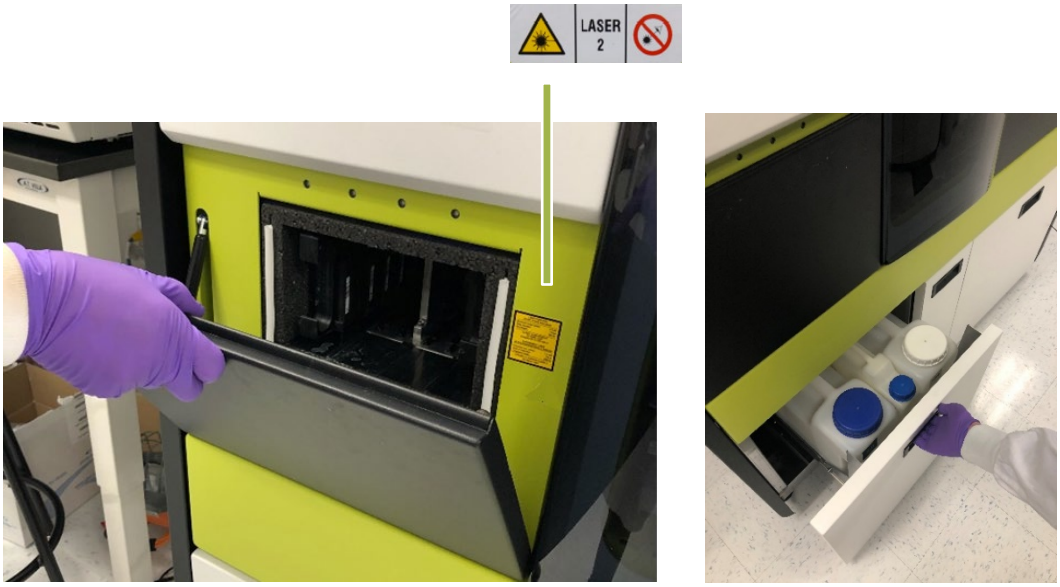
You can use the on-board scanner as an alternative to the handheld device for scanning reagent, RGP, and sample barcodes. It cannot be used to scan Simoa discs.

The on-board scanner is located on the inside of the reagent and sample bays and reads the barcoded reagents, RGP and samples as you insert the racks into the instrument. To use the on-board scanner, you must enable it by turning on the “Enable On-Board Scanner” toggle in the Load Reagents or Setup Run tab. For more information about using the on-board scanner, see Loading Reagents and RGP into the Instrument on page 95.

Note! The on-board barcode scanner will only work if the red scanner light is visible on the inside of the reagent or sample bay. Make sure that the red light is visible before you load barcoded items into the instrument.

Opening and Closing the Instrument Bay Drawers

Pull the door/drawers to open the reagent, sample, and system bays. Keep all instrument drawers closed except when you need to open them for access into the instrument or the system bay.



Inserting and Removing a Reagent Rack

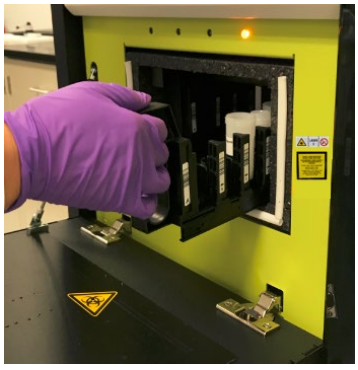
Each of the four lanes in the reagent bay can hold one reagent rack. Each reagent rack can hold six 15-mL bottles of assay reagents, for a maximum of 24 reagent bottles.

The first three positions in the reagent racks oscillate during runs and are intended to hold reagents that must be held in suspension (for example, capture bead reagent).

To insert and remove a reagent rack

To insert a rack into a reagent bay lane, line it up between the lane guides and push the rack into the instrument until the catch engages.

To remove a rack, grasp the handle, push the rack in slightly to release the catch, and pull the rack straight out.



Inserting and Removing a Plate Rack

Lanes 1 to 8 in the sample bay can hold two plate racks. Each plate rack can hold two 96-well plates, for a maximum of four loaded plates. Plate rack 1 goes in lanes 1–4, and plate rack 2 in lanes 5–8.

If you are loading only one plate, place it in position 1 in plate rack 1.

CAUTION! Use only Quanterix-supplied sample plates. These plates have been verified to be nonreactive with Simoa assay reagents and to be correctly sized to work with the instrument pipettors. Quanterix cannot guarantee system performance if you use any other type of plate. For information on ordering plates, see “Purchasing Supplies” on page 2.

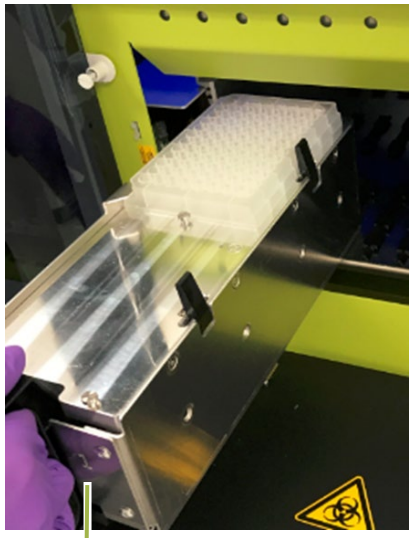
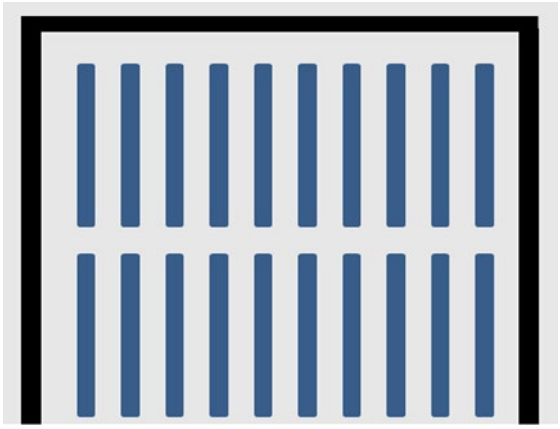
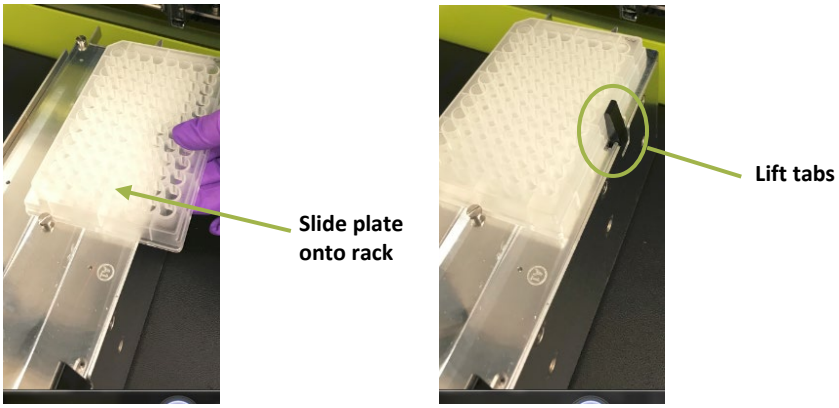


Plate rack number engraved here



To load a plate onto a rack

1. Slide the plate onto the rack.
2. Ensure the lip of the plate sits below the head of both positioning screws.
3. Lift the tabs to secure the plate.

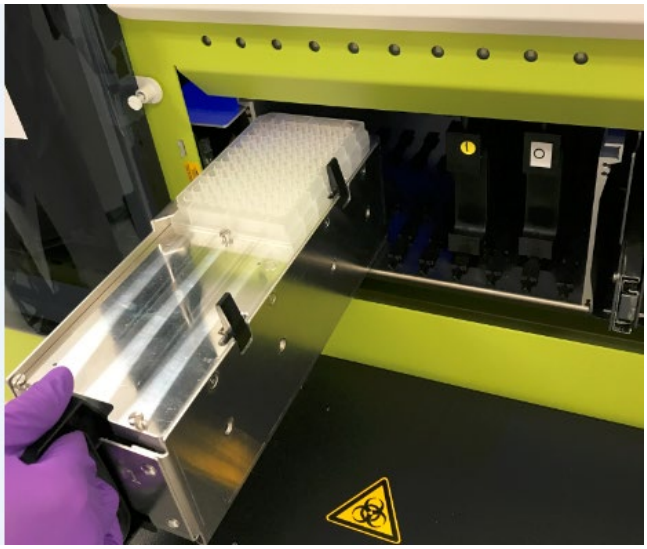


To insert and remove a plate rack

The plate racks are numbered and must be inserted into the instrument in a specific position. Plate rack 1 is inserted in lanes 1 to 4, and plate rack 2 in lanes 5 to 8.

To insert a plate rack, line it up over four lane guide rails in the sample bay and push the rack into the instrument until the catch engages.

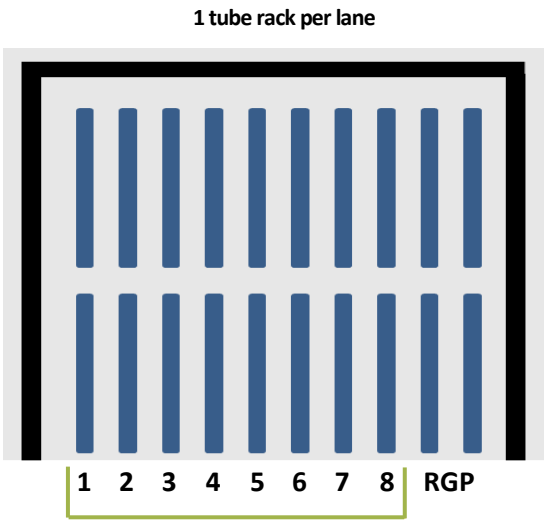
To remove a plate rack, grasp the handle, push the rack in slightly to release the catch, and pull the rack straight out.



Inserting and Removing a Tube Rack

Lanes 1 to 8 in the sample bay can hold tube racks.

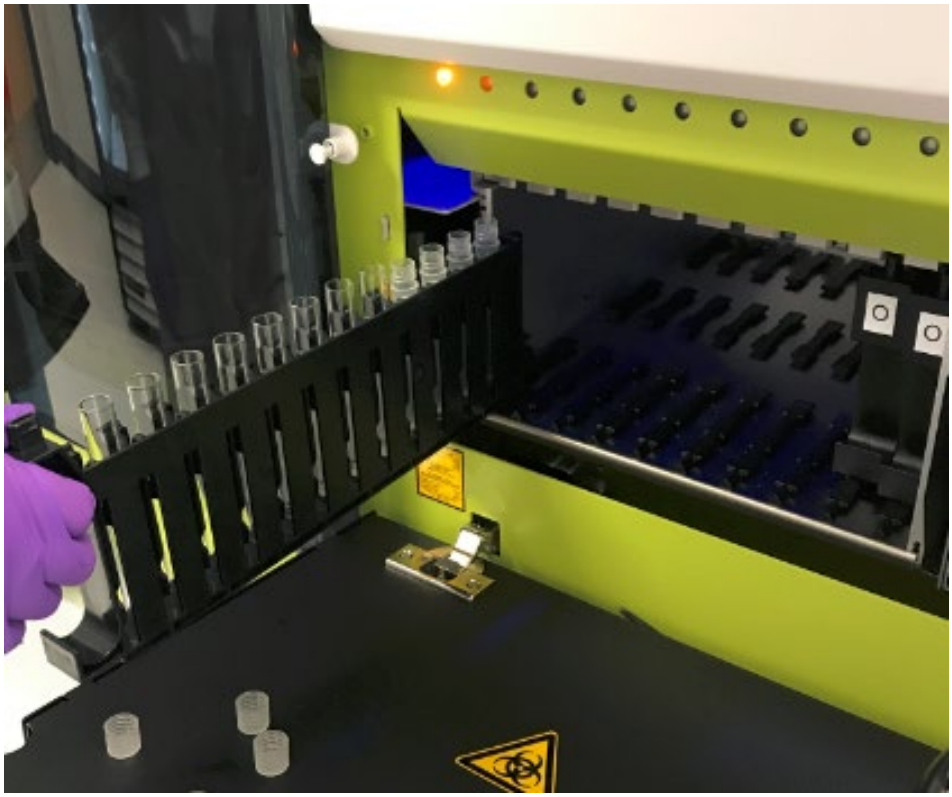
One tube rack can hold 12 tubes, for a maximum of 96 tubes in the sample bay. The tube racks accommodate tubes up to 100 mm tall and 10 to 16 mm in diameter.



To insert and remove a tube rack

To insert a tube rack, center the rack over a lane guide rail in the sample bay and push the rack into the instrument until the catch engages.

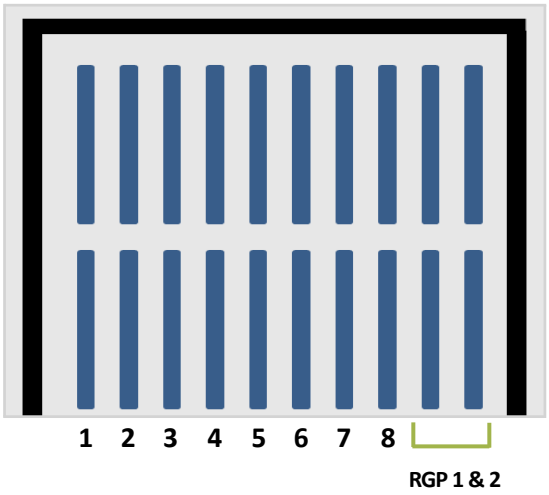
To remove a tube rack, grasp the handle, push the rack in slightly to release the catch, and pull the rack straight out.



Inserting and Removing an RGP Rack

The RGP racks are similar to tube racks. They have been customized to hold the short RGP reagent vials.

The RGP racks must be inserted into lanes RGP 1 and RGP 2 only (also termed the “substrate lanes”).



RGP racks are inserted and removed in the same manner as the tube racks (see “Inserting and Removing a Tube Rack” on page 33).

Loading Instrument Consumables

CAUTION! Use only Simoa cuvettes and Simoa disposable pipettor tips. Using any other type of cuvette or disposable pipettor tip may cause unreliable assay results or damage to the instrument. Quanterix cannot guarantee system performance if you use any other type of cuvette or disposable tip. For information on ordering consumables, see page 2.

Loading Cuvettes

The instrument uses 1-mL plastic cuvettes as reaction vessels. Cuvettes are snapped together into stacks of 50 to make them easy to load.

The cuvette holder has 10 chutes into which you insert stacked cuvettes. The instrument monitors the number of cuvettes in each chute. When a chute contains only five cuvettes, that chute will be considered empty. This chute will be refilled the next time by rotating to the front of the instrument when the cuvettes are loaded.

Note! Always load full stacks of 50 cuvettes. The instrument counts partial stacks as a single cuvette.

Single Cuvette

Cuvette Stack

To determine whether you need to load cuvettes

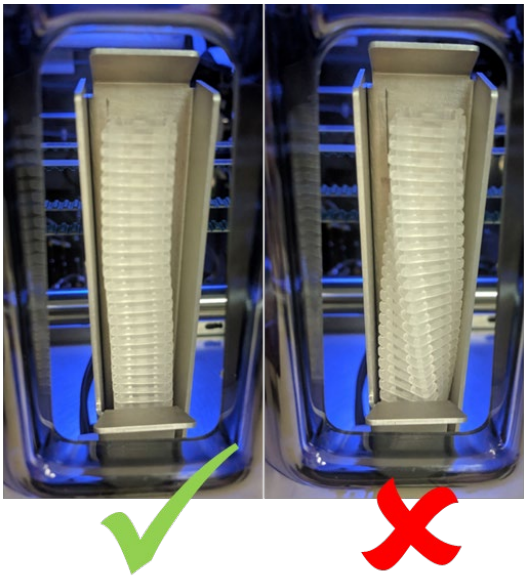
Touch the System Resources tab. If there are insufficient cuvettes, the cuvette diagram is red.

To load cuvettes

1. Touch the System Resources tab > touch the cuvette diagram. The Solid Resources screen appears. For information on reading this screen, see page 131.
2. Ensure the instrument status in the lower left corner shows the system is in a “Ready” state.
3. Unwrap a stack of cuvettes and insert it into the cuvette holder, as illustrated below, with the point down. The cuvette number in the Solid Resources screen will update.

Cuvette number updates

CAUTION! When loading the chute, ensure the stack does not become twisted (see below). Twisting can occur if the stack is dropped too quickly into the chute and may cause a jam in the cuvette loader.



4 The cuvette loader rotates and the cuvette stack drops into the loader. "Maintenance" appears in the lower left corner of the screen. **Wait until the rotation is finished and the system status changes from "Maintenance" to "Ready" before loading a new stack.**

Loading Disposable Pipettor Tips

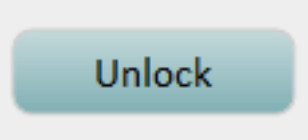
Each of the two System Resources drawers holds up to three disposable pipette tip racks. A full rack holds 96 tips, so the maximum number of tips that may be loaded at one time is 576, or 6 full racks.

Use only Quanterix-supplied tips to ensure proper instrument functioning and transfer of liquids. The instrument uses two tips per sample – one when the sample is picked up for processing and another when the sample is picked up for imaging.

When you load tips, you indicate in the software the position of each loaded tip rack. Doing so specifies the number of loaded tips and allows the software to count down from this number and to notify you if you need to load more tips.

❖ To determine whether you need to load disposable pipettor tips

1. Touch the System Resources tab > touch one of the Tip diagrams to view the Solid Resources screen (for information on reading the Solid Resources screen, see page 131.)
2. If the System Resources drawer is locked, touch the **Unlock** button located under the System Resources Drawer diagram (see page 131).



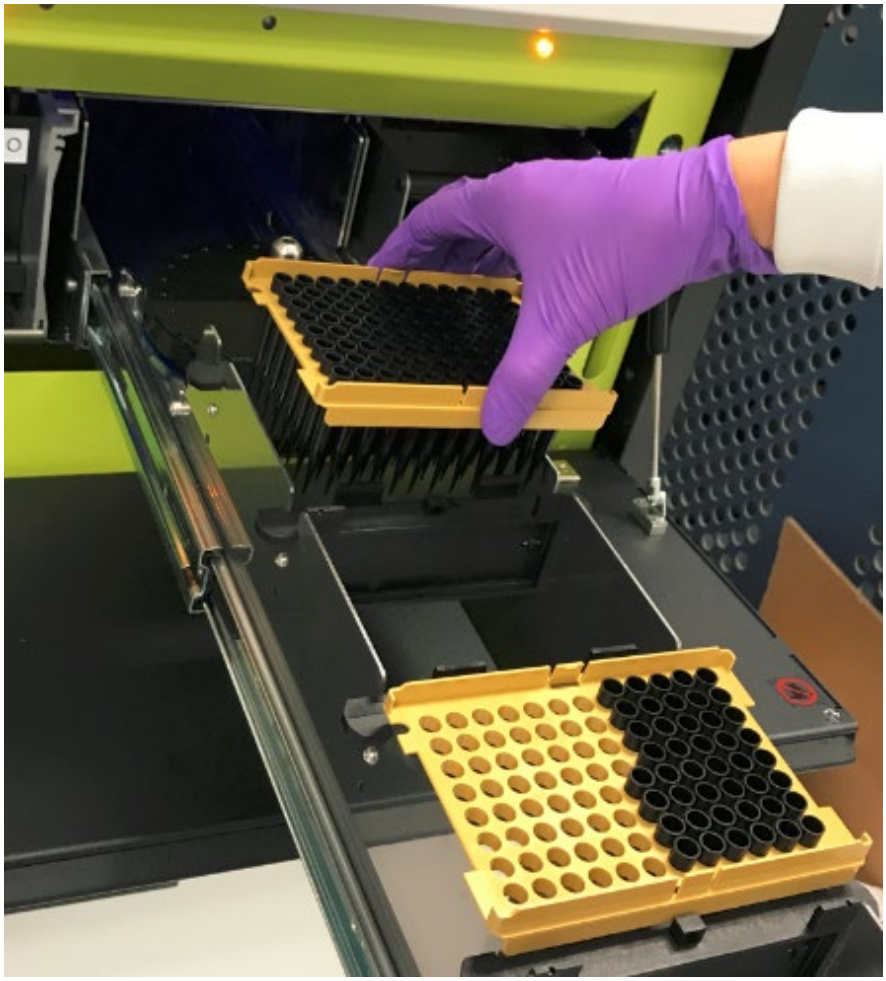
3. Pull out both System Resources drawers and verify that enough tips are loaded to complete the next planned assay run.
4. Double the total number of tests to be run in the assay to determine the number of tips required.

❖ To load disposable pipettor tips

CAUTION! The software only allows loading of full tip racks to prevent contamination that could result from handling tips**.**

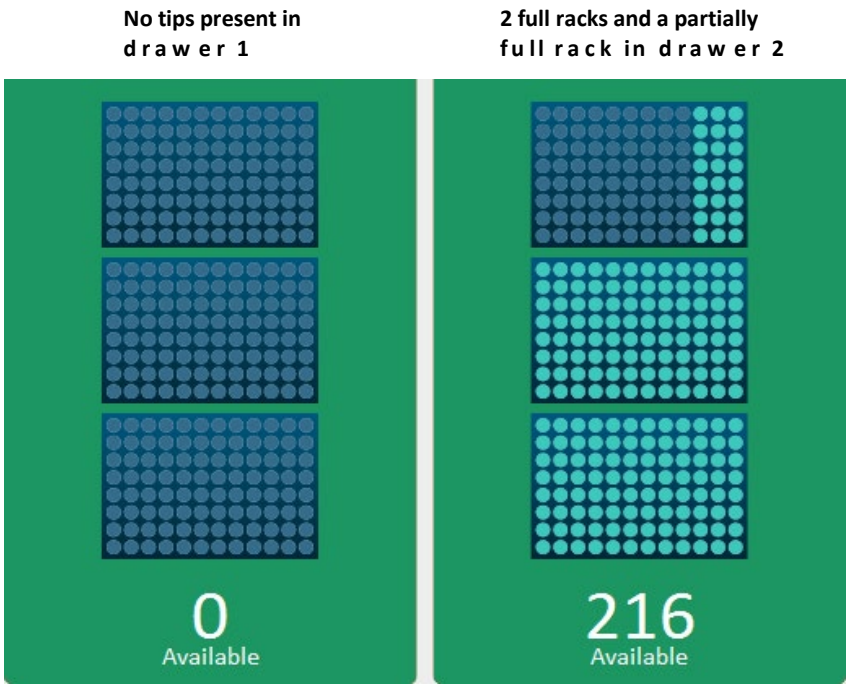
1. Open the System Resources drawers > remove empty tip racks.

2 Remove the cover from new tip racks and place them in the drawer. Fill all rack positions.



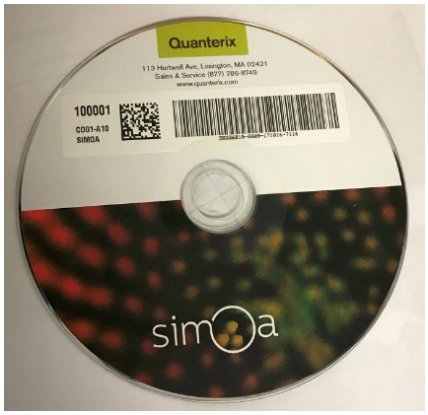
3 In the System Resources tab, touch one of the tip diagrams to open the Solid Resources screen.

4 In the Solid Resources screen, tap twice in the positions where you loaded new tip racks. The tip positions in the rack diagram turn light blue.



Loading Simoa Discs

Simoa Discs are loaded onto mounting poles at the rear of the System Resources drawers. One disc stack contains 16 discs. The maximum disc capacity is 32.



When you load a stack of discs, you scan the package barcode, which tells the software that you have loaded 16 discs. The instrument counts down from this number every time you consume a disc and notifies you if you need to load more discs.

CAUTION! Only load whole packages of Simoa Discs. Do not attempt to top off a partially used stack with new discs. To prevent disc contamination, do not remove the plastic wrapper from a stack of discs before you load the stack into the instrument.

❖ To calculate the number of discs needed for a run

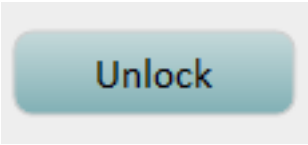
Each disc has 24 arrays, and one array is required per job. Therefore, divide the number of jobs in the run by 24 and then round up to the nearest whole disc. For example: 96 jobs/24 = 4 discs; 78 jobs/24 = 3.25, round up to 4 discs.

To calculate the number of jobs in the run, multiply the number of samples by the number of replicates per sample.

1. Touch the System Resources tab > touch one of the disc diagrams to view the Solid Resources screen (for information on reading the Solid Resources screen, see page 131).
2. View the Solid Resources screen to determine if there are enough discs for your run.

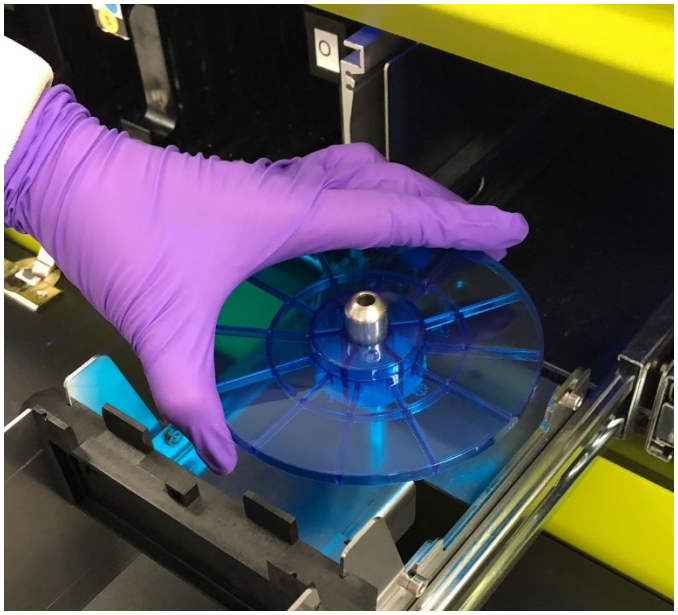
❖ To load Simoa Discs onto an empty disc pole

1. Obtain one package of discs.
2. When the Solid Resources screen indicates there are zero discs available, touch **Unlock** to open the appropriate System Resource drawer.



3. Pull out the System Resources drawer.

4 Remove the blue base plate from the disc pole. This plate sits at the bottom of every package of discs.



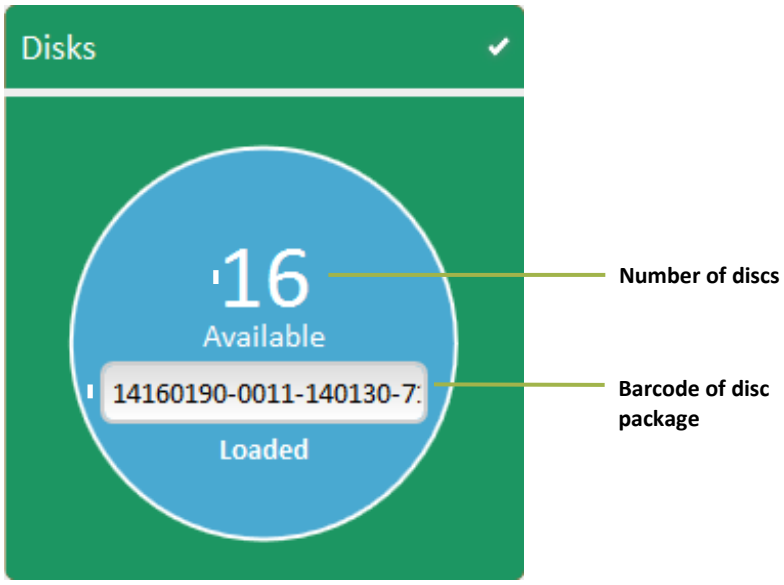
5 Load one package of discs:

Note! Only load a complete package of discs onto an empty disc pole. Do not add discs from the other stack.

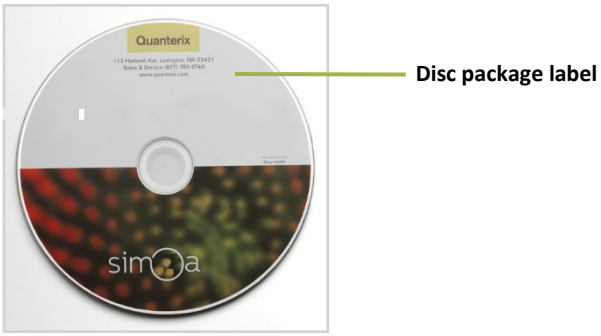
a Tap the disc barcode box.

b If an existing barcode appears in the box, delete it.

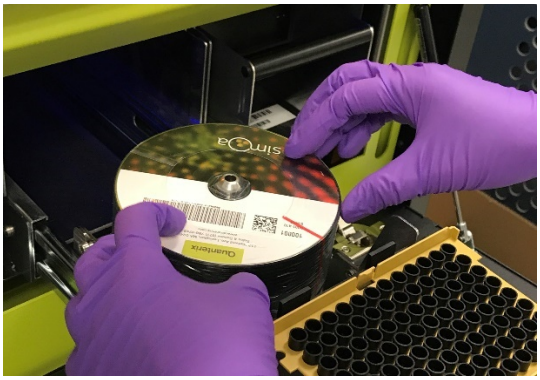
c Scan the package barcode with the barcode scanner. The barcode and the number of discs in the package (16) appear in the disc diagram.



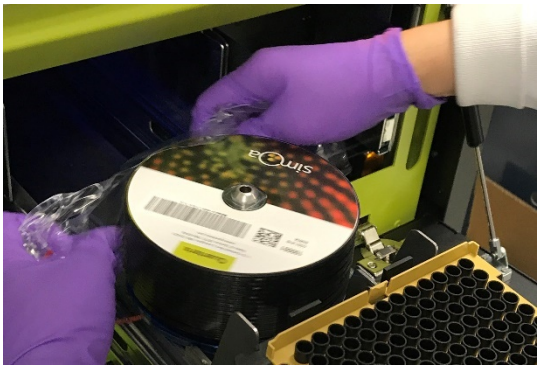
d Press the package onto the mounting pole. The package label should be on top.



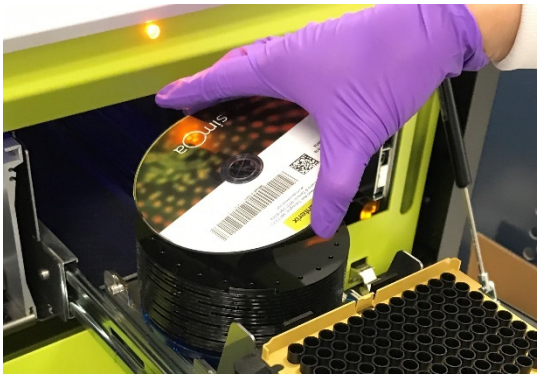
e Line up the perforation with the top of the empty disc pole, slide the disc stack onto the pole, and orient the red pull tab toward the front.



f Pull the red tear strip and remove the plastic disc covering completely.



g Remove the top disc imprinted with the Simoa logo from the spindle.



Note! Take care not to shift discs against each other as they are very sensitive to scratching, which can affect image quality and data.

6 Push the drawer into the instrument until it catches.

7 Verify that the Resources screen reports the correct number of discs in both drawers.

8 Repeat “To load Simoa discs onto an empty disc pole” on page 42 if you need to load a second package of discs.

Refilling the System Fluid Containers

Refill the secondary system fluid containers for wash buffer 1 and deionized water and the system fluid container for wash buffer 2 before the first run of the day and whenever the container alert icons at the bottom of the software screen turn red.

“System Liquid” is deionized water

Note that for wash buffer 1 and the deionized water, you only need to fill the secondary containers, not the primary containers. The primary containers passively refill themselves from the secondary containers. Wash buffer 2 only has one container, and it must be refilled.

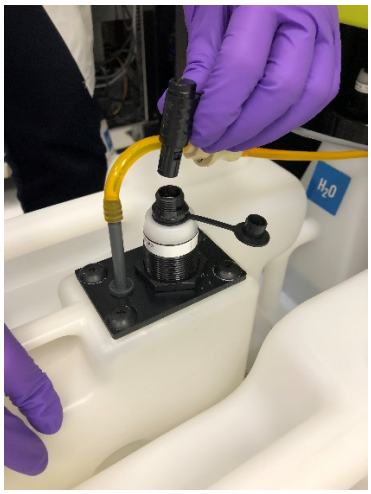
CAUTION! Be sure to refill the system fluid containers with the correct solution. Filling with the wrong solution may require multiple flushes with water and/or the appropriate fluid in order to clean the lines.

To refill a secondary system fluid container or wash buffer 2 container

1 Remove the container as described below.

- To remove the secondary containers for the deionized water and the system wash buffer 1, lift the containers out of their seats. As you lift the front end of the container, the plug connecting it to the primary container disconnects.

- To remove the system wash buffer 2 container, disconnect the sensor and fluid port and lift the container.


2 Unscrew the cap and refill the container with the correct fluid. Screw the container cap back on when you are finished.

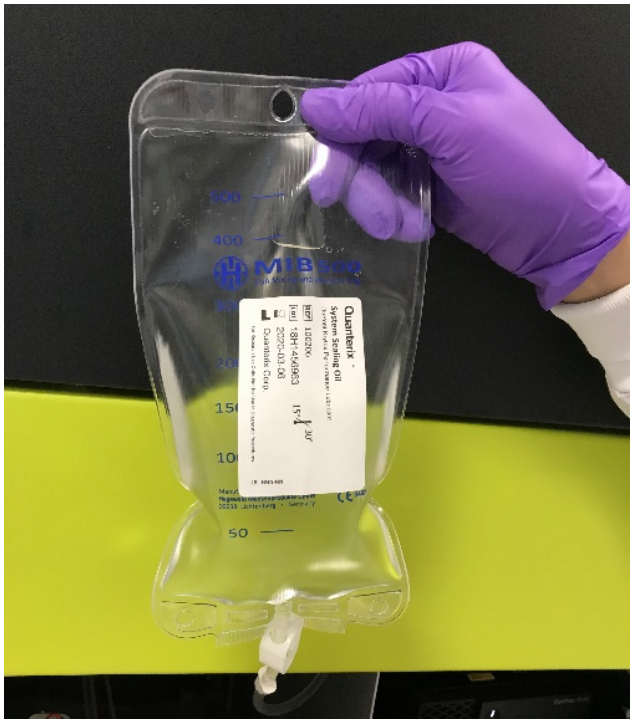
3 Replace the container, as described below. **Do not force a container into its seat.** The connectors should mate easily. If you experience resistance, remove the container and try again.

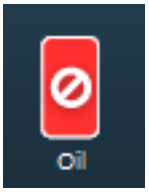
- To replace the deionized water and the system wash buffer 1 secondary containers, tip the connector end of the container down slightly and insert it into the seat.
- To replace the system wash buffer 2 container, place it in the seat between the secondary deionized water and secondary wash buffer 1 containers. Reconnect the sensor and fluid port.

Changing the Sealing Oil Bag

The sealing oil bag hangs from the ceiling of the lower system bay and connects to the instrument by tubing. Change the bag when the sealing oil icon at the bottom of the software screen is red.

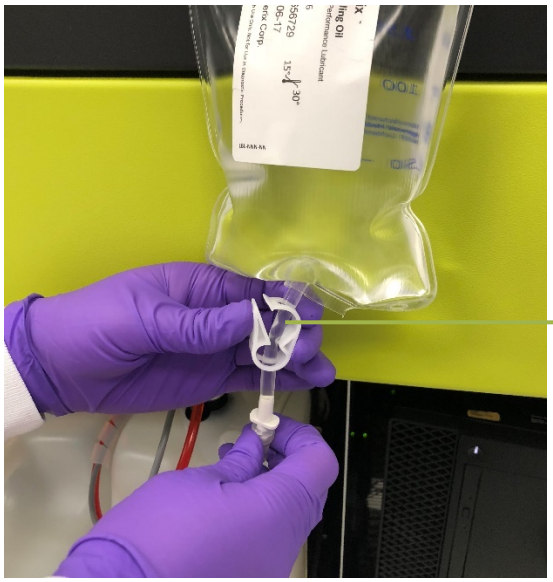
Note! Prior to running the Start of Day maintenance task, the sealing oil icon will appear red. An oil prime is performed as part of the Start of Day maintenance and the sealing oil icon status will be updated accordingly.



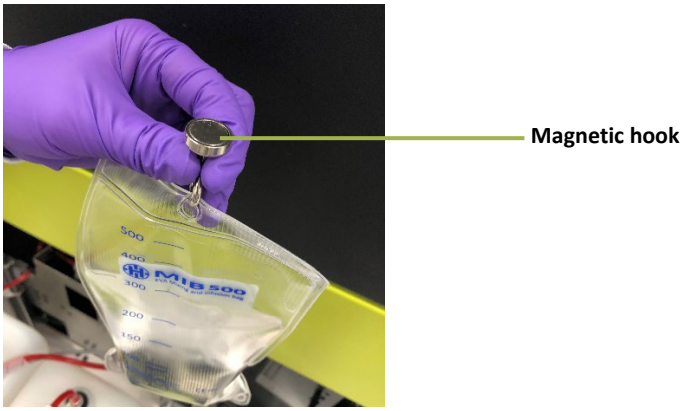


To change the sealing oil bag

1. Close the tubing clamp just below the bag.



2. Unscrew the oil tubing from the bag.
3. Unhook the bag and remove from the system bay. Dispose of the bag according to the waste disposal policies of your organization.
4. Connect the hook to the new bag by piercing through the perforated circle at the top of the bag. The magnetic hook may be temporarily removed from the system bay ceiling to accomplish this.



5 Confirm that the tubing clamp is closed on the new bag, then unscrew the yellow cap.

6 Connect the oil tubing to the new bag. Before connecting, twist the connectors in the opposite direction to allow "self-threading."

7 Open the tubing clamp that is below the bag.

8 Touch the Maintenance tab > touch the Replenish Oil task checkbox > touch **Run Task**. The task takes approximately 15 minutes to complete. During the task, the instrument software is inactive. When the software screen reactivates, the task is complete.

Emptying the Solid Waste Container

Empty the solid waste container whenever the solid waste alert icon at the bottom of the software screen turns yellow or red.

The solid waste container holds discarded cuvettes, disposable pipettor tips, and Simoa Discs.

To empty the solid waste container

1 Pull the solid waste drawer out of the system bay.

CAUTION! Do not place your hand in the opening in the shelf above the solid waste container when you pull the container drawer open. As the drawer slides out, it carries the container with it. If your hand is in the opening, your fingers or hand could be pinched between the edge of the opening and the rear top edge of the container.

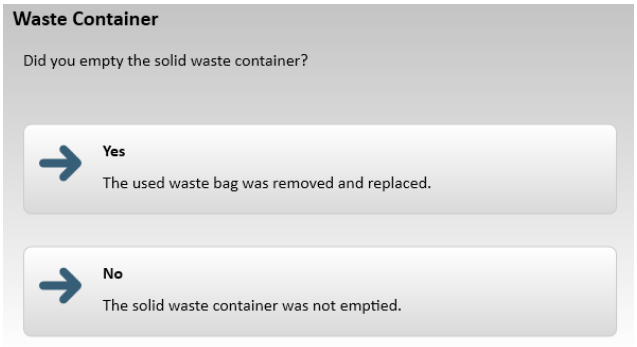
2 Remove the waste bag > insert a new waste bag. Dispose of the waste bag according to the waste disposal policies of your organization.

CAUTION! The bag contains biohazard waste materials and should be handled with care.

3 Touch the System Resources tab > touch one of the disc diagrams to view the Solid Resources screen (for information on reading the Solid Resources screen, see page 131).

4 Push the solid waste drawer into the system bay.

5 Select **"Yes"** when you see the following dialog box to confirm that you emptied the solid waste container.



Emptying the Liquid Waste Containers

The instrument discards liquids into only one liquid waste container at a time. When a container is in use, a light in its cap turns on.

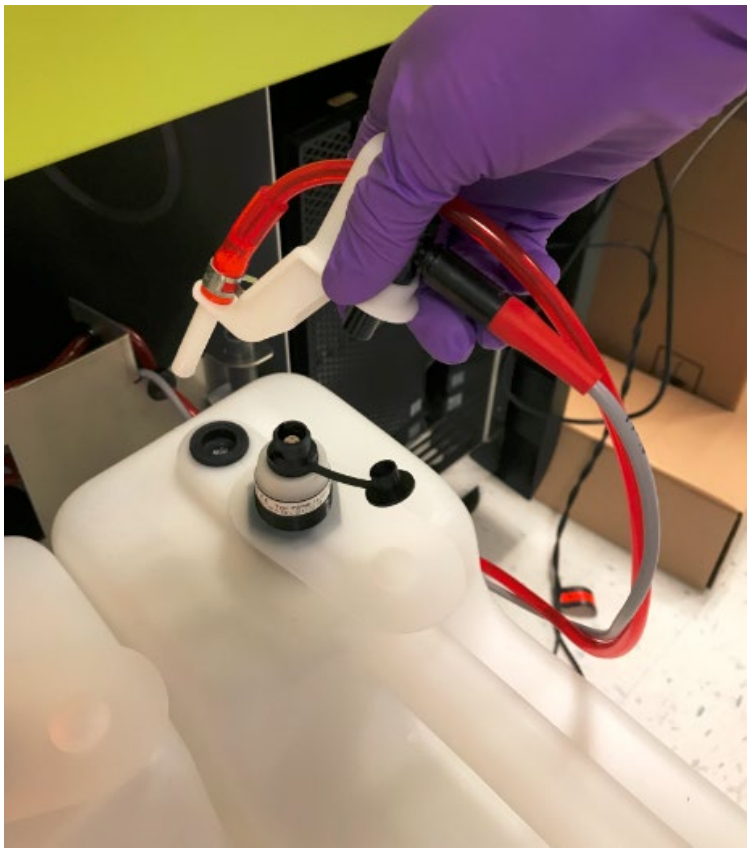
Empty both liquid waste containers before the first run of the day.

During a run, the instrument senses the fluid level in the containers with a floating sensor inside the containers.

To ensure decontamination of liquid waste, follow the instructions below to clean the waste container with the cleaner specified in your in-house decontamination procedures.

❖ To empty the liquid waste container

1 Disconnect the fluid tubing and the container sensor by pulling them straight up.



2 Remove the container and dispose of the liquid waste according to the waste disposal policies of your organization.

CAUTION! The liquid waste container holds biohazard waste and should be handled with care.

3 Place cleaner into the container as specified by your in-house cleaning procedures and place it back in the system bay.

4 Reconnect the tubing and the container sensor. When done correctly, you will hear a click when you reinsert each sensor.

Monitoring the Instrument's Internal Temperature

The instrument's internal temperature is displayed in the upper left corner of the System Resources screen. See Understanding the System Resources Screen on page 130 for more information.

The recommended internal temperature range is between 18.0°C (64.4°F) and 25.0°C (77.0°F). The system will notify you if the temperature falls outside of this range. If this happens, read the notification and follow the instructions on the screen to restore the instrument to an acceptable temperature.

CAUTION! Do not start a run while the internal instrument temperature is above or below the recommended range. Assay data may be compromised and any jobs processed while the temperature is out of range will be flagged.

6 Importing Assay Definitions

Understanding Assay Definitions

The system software provides a range of pre-defined 1-, 2-, and 3-step incubation protocols that specify reagents, calibrator levels and concentrations, reagent volumes, and incubation times. (For information on the protocol steps, see the instructions in your assay kit.)

An **assay definition** is an XML document that specifies the pre-defined protocol that a given Simoa® assay uses and all of the settings for the protocol. It also includes information on curve fitting and concentration result generation.

To perform a Simoa assay, you must import an assay definition in the system software.

To use Homebrew assays, you will create custom assays for your experiments (see Chapter 7 on page 63).

Multiplex Assay Definitions

You can perform singleplex and multiplex assays with the Simoa HD-X Analyzer. In multiplex assays, multiple subpopulations of beads, each with a unique fluorescent signature and specific antibody, are incubated together in the same sample. They are then imaged simultaneously on the same array.

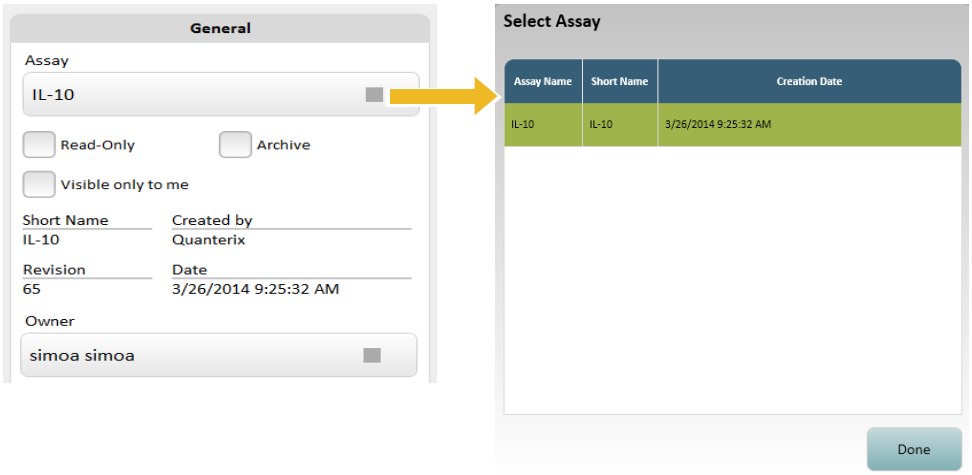
You can define your own Homebrew multiplex assays (see Chapter 7 on page 63) or use Quanterix®-supplied multiplex assays with premixed beads and multiplex assay definitions. You cannot import an assay definition or create a Homebrew assay definition that includes more plexes than your software configuration allows. The process for setting up and running multiplex assays is the same as the singleplex process with the exception of the acquisition channel settings.

Checking Imported Assay Definitions

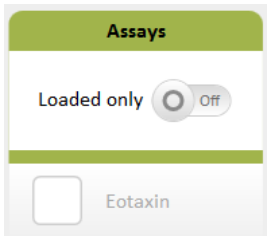
Before you set up a run, make sure that the assay definition you need has been imported on your Simoa HD-X Analyzer.

To check imported assay definitions

- Touch the **Custom Assay tab** > touch the **Assay** field under **General**. The **Select Assay** dialog opens, showing all assays that have been defined for the system.



- Alternatively, you can check imported assay definitions in the **Setup Run tab**. Touch the **Setup Run tab** > turn off the **Assays Loaded only** toggle to display all assay definitions that have already been imported into the system. Note that the **Loaded only** toggle is set to off by default.



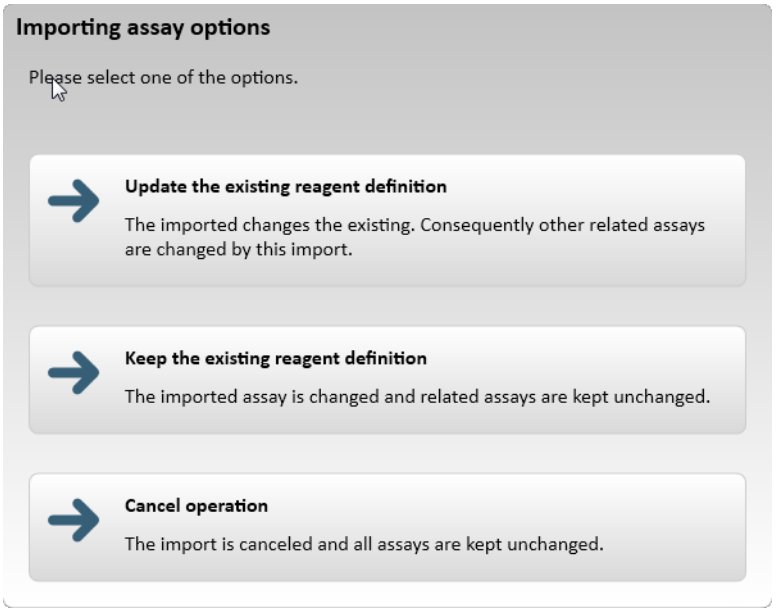
Importing an Assay Definition

You can download assay definitions from the Quanterix Customer Support website (http://portal.quanterix.com).

If you are importing an assay definition that shares reagents with existing assays, you can replace the existing reagent definition with the imported assay definition, or you can leave the existing definition for those shared reagents unchanged.

To import an assay definition

1. Touch the Custom Assay tab.
2. In the Assay Overview screen, touch **Import**.
3. Navigate to the assay definition you want to import and touch **Open**.
4. Choose the importing options:
   a. Select **Update the existing reagent definition** to replace existing shared reagent definitions with the values specified in the imported assay definition.
   b. Select **Keep the existing reagent definition** to leave existing shared reagent definitions unchanged. The imported assay uses the values of the previously specified reagents instead of the ones in the imported assay definition.
   c. Select **Cancel** to cancel the import.



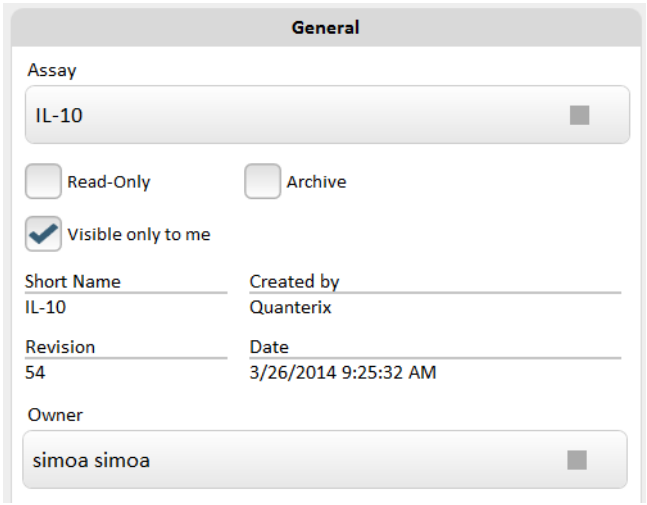
5 Optionally, edit the Assay Name or the Assay Short Name.

6 Touch **Save.**

Configure Assay Settings

You can specify the following assay settings that control how assays are displayed and accessed:

- Read-Only
- Visible Only to Me
- Archive



Read-Only

Depending on how roles and features are configured for your system, you may or may not see a Read-Only button on the Custom Assay screen.

Typically, administrators hide this button from users with non-administrator roles.

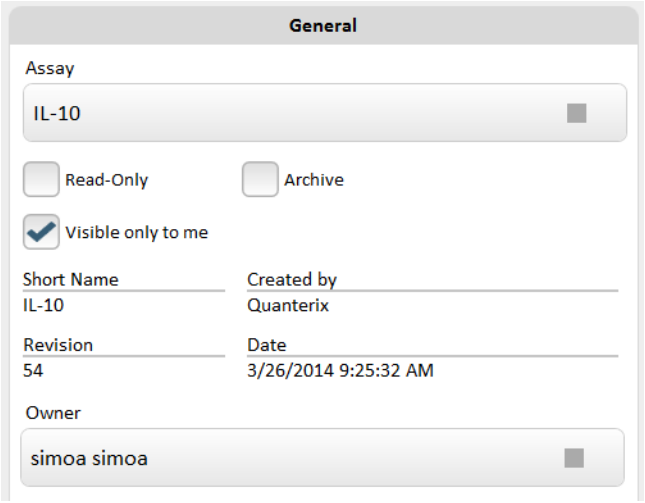
By default, imported assays are read-only and cannot be edited. Administrators can disable the Read-Only button to allow editing of assay definition values.

For more information, see “Managing Roles and Features” on page 179.

Visible Only to Me

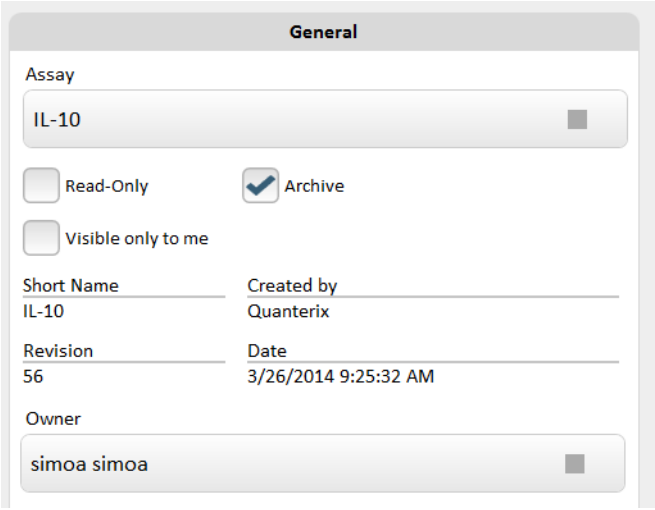
Your assay definitions are associated with your user profile to prevent other users from accidentally modifying them and to limit the number of assays displayed on the Setup Run tab.

To protect your assays, check the Visible Only to Me checkbox.



When you log in, you will see all assays that you defined and all assays created by others, except for those configured as Visible Only to Me when created by other users.

Archive assays that are not currently in use to limit the number of assays displayed in the other tabs. Check the **Archive** checkbox to hide an assay or uncheck it to display it again. When you archive an assay, it is stored in the database but is not visible in the **Setup Run** tab under **Assays**.



7 Developing Homebrew Assays

Overview of Homebrew Assay Development

Homebrew assays are custom Simoa® assays that you develop for your Homebrew reagents. You have complete freedom to develop and modify Homebrew assays in terms of reagents, calibrators, base protocol, incubation times, and volumes.

Note! This chapter explains how to use the Simoa HD-X Analyzer to perform a Homebrew assay run. For detailed instructions on Homebrew assay protocols, see the *Simoa Bead-Based Homebrew Assay Development Guide*.

You develop Homebrew assays in the Custom Assay tab of the Simoa software, in a four-step process:

Step 1: Define assay reagents

Name reagents, define their characteristics, and specify the rack positions they may occupy and the reagent bay or sample lanes in which they can be placed.

Step 2: Define the assay

Name the assay, choose a protocol for it, match your assay reagents to the protocol, and specify sample dilutions.

Step 3: (Optional) Add a dilution to the assay

All assays begin with no dilution (expressed as Neat or 1x), but you can add extra dilutions.

Step 4: Define a plex

Define the bead acquisition channels and the data analysis settings.

You can also modify reagent volumes and incubation times to optimize a Homebrew assay.

You can develop Homebrew assays remotely on any Windows computer on which you have installed the Simoa offline mode software. After developing an assay remotely, you must import it into the Simoa software on the instrument before you can use it.

For information on protocol steps, see the instructions in your assay kit.

Understanding Sample Dilutions

By default, Homebrew assays have no dilutions. In the Simoa software, this default is expressed as Neat or 1x. You can add any number of additional dilutions to a Homebrew assay.

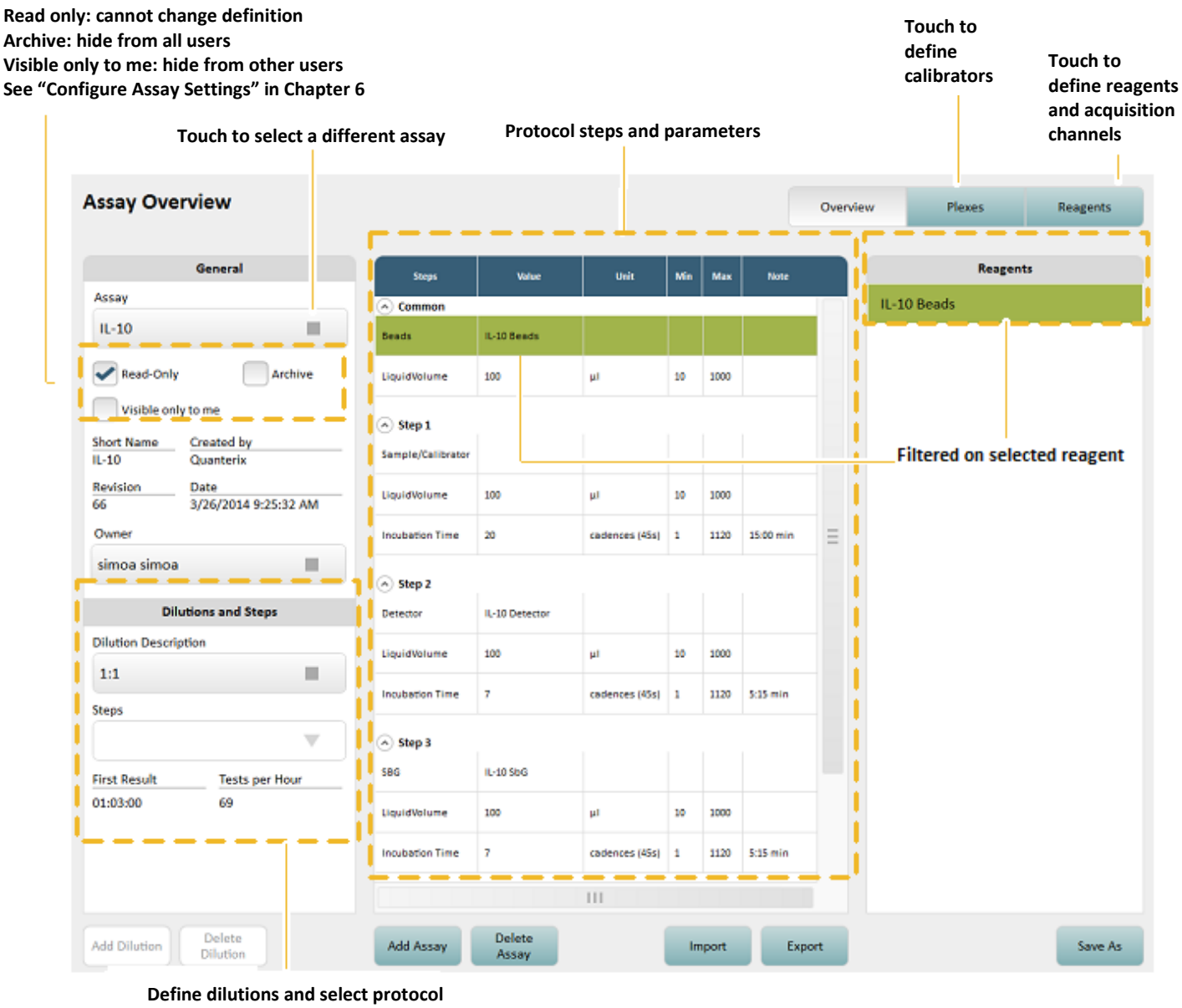
Setting up dilutions involves selecting a protocol that has a dilution step (for example, 2-step with dilution) and specifying the volumes of samples and diluents that the instrument will pipette into cuvettes.

To conserve samples, Quanterix® recommends that you set up dilutions to consume a maximum of 152 µL total of sample and volume, for example:

- 4x dilution: Specify 38 µL sample and 114 µL diluent = 152 µL total volume
- 10x dilution: Specify 15 µL sample and 135 µL diluent = 150 µL total volume

Understanding the Custom Assay Screens

Assay Overview Screen



The below table describes the options available under the Steps field shown on the Assay Overview and Add Assay Screens.

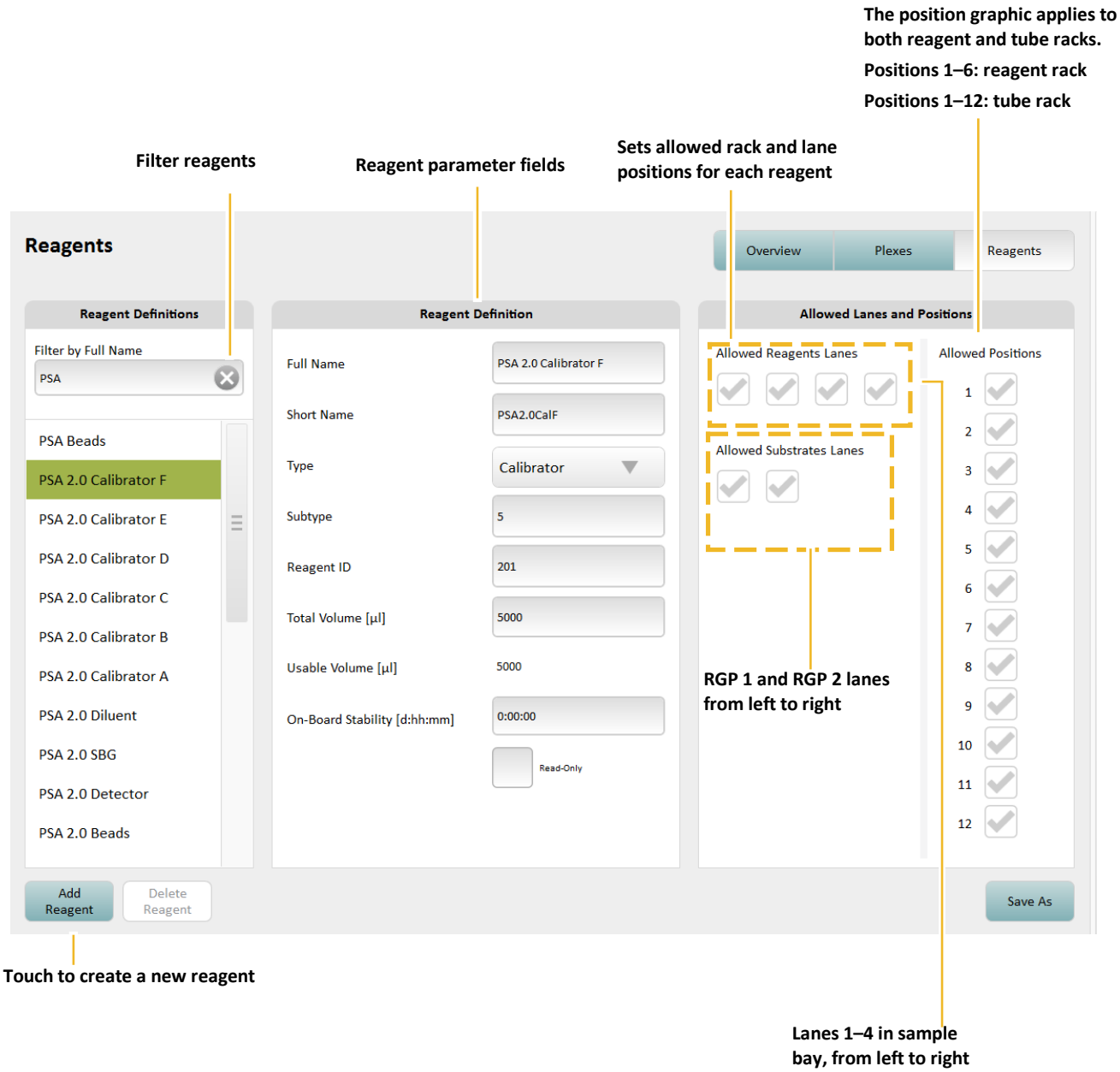
Steps Protocol	Protocol Description
1-Step	In a 1-step protocol, assay beads are combined with sample, detector antibody and streptavidin-ß-galactosidase (SBG) in a single step.
2-Step	In a 2-step protocol, assay beads are combined with sample and detector antibody in the same incubation (Step 1). Following a wash, streptavidin-ß-galactosidase (SBG) is mixed with the beads (Step 2).
3-Step	In a 3-step protocol, assay beads are combined and incubated with sample alone (Step 1). After washing, biotinylated detector antibodies are mixed and incubated with the beads (Step 2). Following another wash, streptavidin-ß-galactosidase (SBG) is mixed with the beads (Step 3).
Service Test	The Service Test protocol is used by Quanterix Support Team to evaluate system performance and is not suitable for routine assay work.

Each of the above protocols exists with a combination of the modifiers described below. For example, a “2-step assay with dilution” specifies a protocol where the assay is carried out in two steps in which diluted sample, assay beads and detector antibody are added in the first incubation step of the assay.

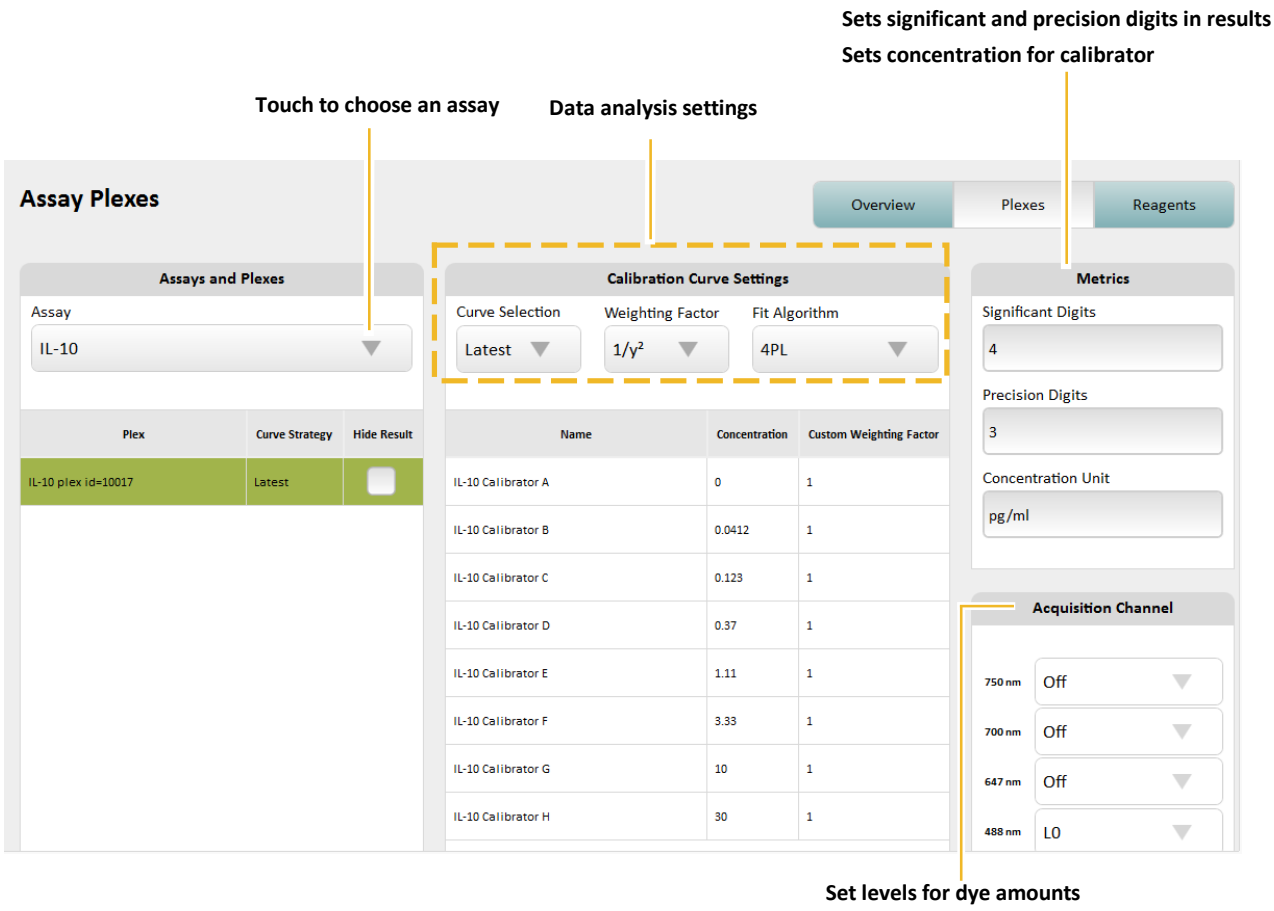
Steps Modifiers	Description
Neat	*Neat* definitions specify that samples are not diluted as part of the assay steps.
With Dilution	*With Dilution* definitions specify that samples are diluted on-board as part of the assay steps.

Assay Reagents Screen

This screen shows all reagents that have been defined on the instrument. These reagents may be selected for any assay.



Assay Plexes Screen



Note! Significant Digits and Precision Digits are predefined in Quanterix-supplied assays. However, you can change the default settings for Homebrew Assays to control how the numbers are displayed.

Defining Assay Reagents

The first step in defining a Homebrew assay is to define its reagents in the Assay Reagents screen (for information on this screen, see page 68). Reagents include capture beads, detector, SBG, RGP, sample diluent, and all calibrator levels.

You only need to define assay reagents once. Thereafter, you may use the definitions in any Homebrew assay. The Assay Reagents screen lists all reagents that have been defined for the software.

Note! Be sure to define all the reagents that you will need before you begin defining a Homebrew assay. You cannot add reagents as you set up the definition.

To define assay reagents

1. Touch the Custom Assay tab > touch **Reagents.**
2. Touch **Add Reagent**.
3. In the Reagent Definition box, select a type from the Type drop-down list.

4 Enter information into the other fields in the Reagent Definition box.

Field	Definition
Full Name	Name of the reagent (you may enter any name). **Examples:** Magnetic beads, Calibrator A
Short Name	Abbreviated form of the full name. The short name must be different from the full name. **Examples:** Beads, CalA
Subtype	If you have multiple reagents of a given type for a given assay, enter a Subtype for each. Start with 0 and increment by 1 for each reagent. For example, assign Subtype 0 to Detector 1 and Subtype 1 to Detector 2. Enter calibrator values as follows: **Calibrator** — **Enter this** Calibrator A — 0 Calibrator B — 1 Calibrator C — 2 Calibrator D — 3 Calibrator E — 4 Calibrator F — 5 Calibrator G — 6 Calibrator H — 7 Calibrator I — 8 Calibrator J — 9
Reagent ID	Reagent ID must be blank for Homebrew assays. Do not enter a value of any kind, including zero.
Total Volume µL	**Required field**. Enter the available volume for a new reagent bottle. The instrument uses this value to calculate the running total of Usable Volume. You will be putting bead, detector, and SBG reagent into the bottles provided in the Homebrew Kit. You can specify volume up to 18 mL for reagents and diluent. • If you always use the same fill volume, enter the value in µL units in the Total Volume µL field. • If fill volumes vary, enter 600 (the same as the dead volume). On the Load Reagents screen, enter the usable volume (fill volume minus dead volume) in place of 0 (zero) in the Available Volume field. For Calibrators A-J, enter any value greater than zero. (placeholder for future feature).

Field	Definition
Usable Volume µL	Initially, displays the Total Volume minus the dead volume value of 600. Recalculates as reagent is used.
On-Board Stability	Enter an estimated value, in the form (days:hours:minutes).

5 Specify the lanes and rack positions in which the reagent may be placed. To do this, touch checkboxes in the Allowed Lanes and Positions boxes.

Beads must be confined to positions 1, 2, or 3 in reagent rack.

6 Touch **Add Reagent**.

7 Repeat steps 2 to 6 to define all reagents. When you have finished defining reagents, touch **Overview** to return to the Assay Overview screen.

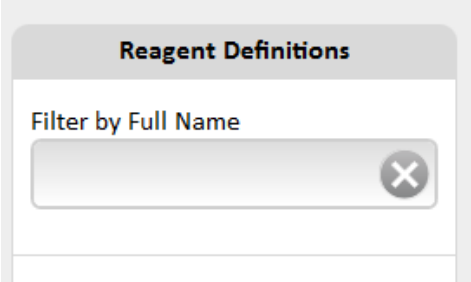
Note! You do not need to save the screen. All information that you enter is immediately entered into the Simoa software database.

Filtering Reagents

You can limit the list of reagents that appear on the screen to those of immediate interest with a filter. Type in any part of the reagent full name. The list of reagents is limited to those that contain the text that you typed anywhere in the reagent name.

❖ To filter the reagent display

Type a reagent name in the Filter by Full Name box. You only need to type enough of the name to create a unique filter.



Defining a Homebrew Assay

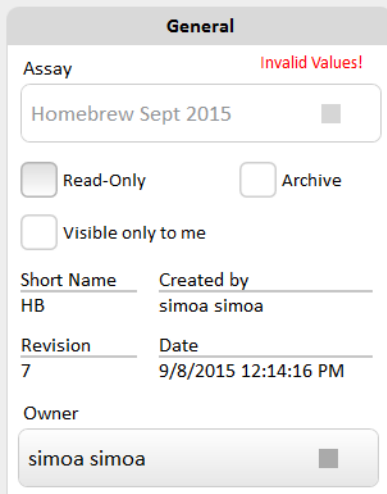
You will define the assay in the Assay Overview screen (for information on this screen, see page 66).

By default, assays do not have any dilutions. You can add dilutions to the assay after you create it (see “Adding a Dilution to a Homebrew Assay” on page 80).

❖ To define a Homebrew assay

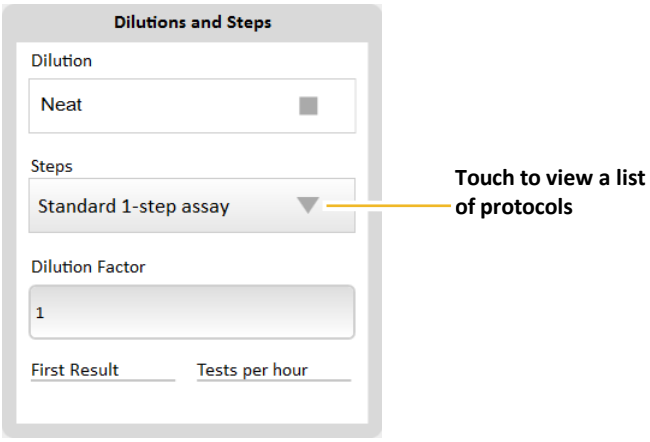
1 In the Assay Overview screen, touch **Add Assay**.

2 Enter an assay name > touch **Save**. The name appears in the General box along with the Invalid Values! flag. This flag disappears when you complete step 6.



3 In the Dilutions and Steps box, touch the dropdown arrow > select a protocol from the list. For information on protocol steps, see the instructions in your assay kit.

If you plan to set up extra dilutions, be sure to choose a protocol that includes the word "dilution" in its name (for example, "Standard 2-Step Dilution").

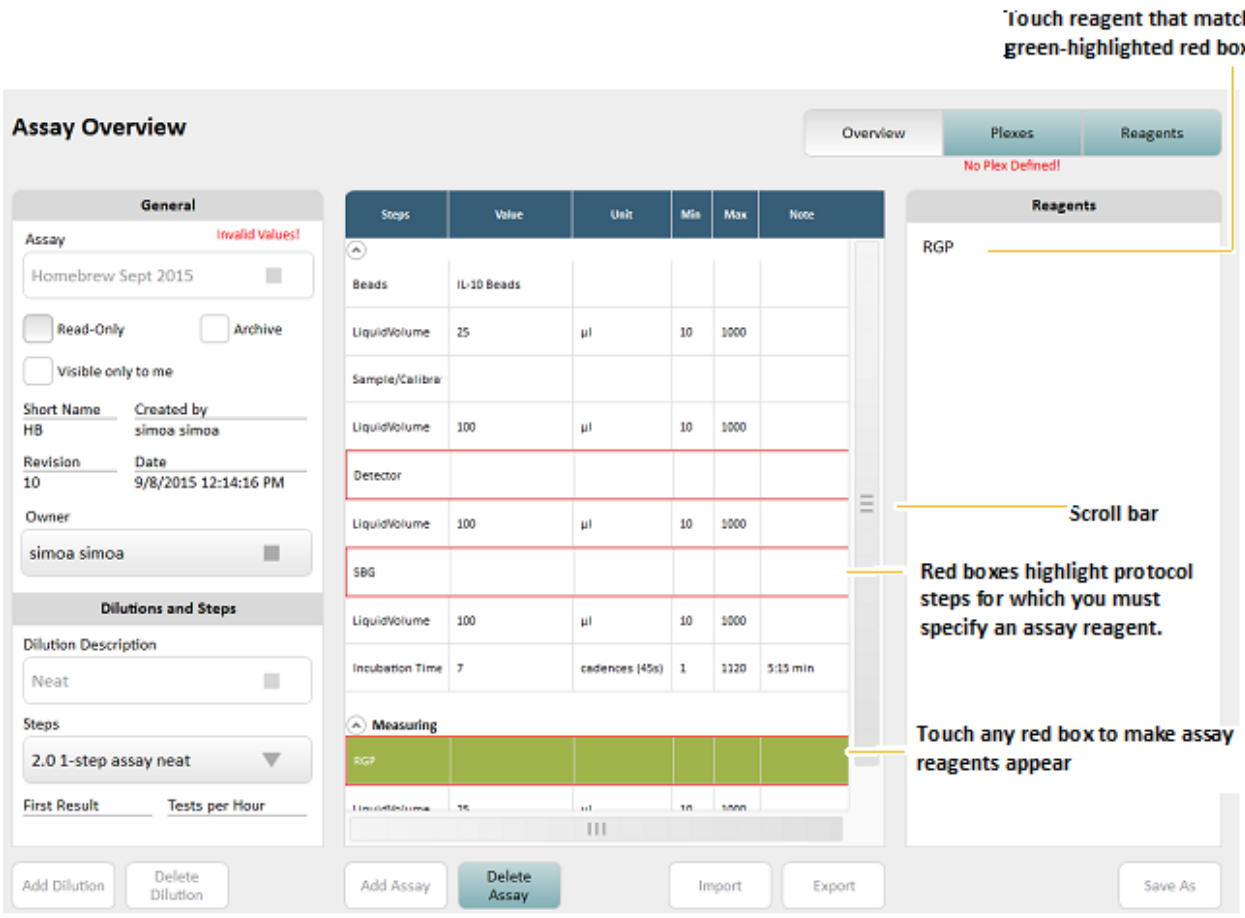


Note! The default Dilution Factor is 1. The Dilution name (in the first text box) may appear as 1x or Neat. Quanterix recommends using Neat to maintain naming consistency with predefined assays.

4 If the calibrators will use a dilution other than Neat, follow the instructions in "Adding a Dilution to a Homebrew Assay" on page 85, select the dilution, and continue with step 5.

5 When you select a protocol, its steps appear in the central box in the screen. A red border highlights each reagent addition step. You must associate an assay reagent with each red-highlighted step.

To view the assay reagents, touch any red box in the protocol. The box is highlighted in green and all of the defined assay reagents appear under Saved Reagents.



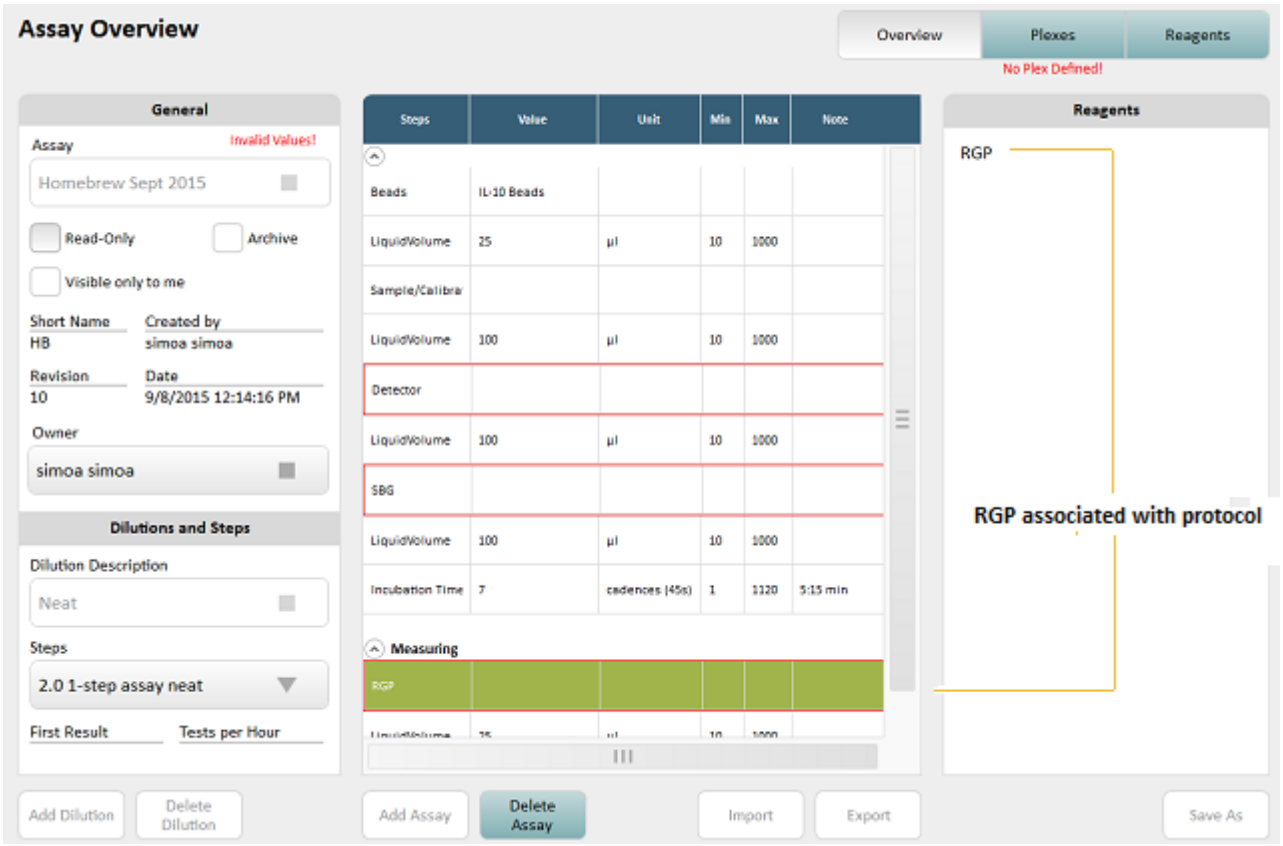
6 Associate each reagent in a red box with an assay reagent:

a Touch the first red box in the protocol to highlight it.

b Touch the reagent in the Assay Reagents box that matches the reagent named in the highlighted red box. For example, if you touch the box that says "Beads," you would touch a bead assay reagent.

c Repeat step b for all other reagents in red boxes. You are finished when "Invalid values!" disappears from the Assay box.

In this example, RGP has been associated with the protocol.



7 If necessary, modify the specimen or calibrator volumes by touching the number in the Value column in the LiquidVolume row you want to change and typing a new number.

8 When you have matched all protocol reagents with assay reagents, define calibrators for the assay (see page 76).

Defining Homebrew Assay Calibrators

You define calibrators on the Assay Plexes screen (for information on this screen, see page 69). You can define up to 10 calibrators for an assay.

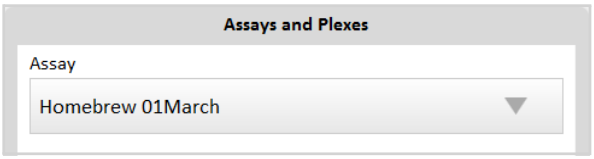
When you define calibrators, you also specify data analysis parameters. You can change these parameters when you analyze assay results.

The instructions in this section explain how to set up singleplex and multiplex assays. If you are setting up a multiplex assay, repeat the calibration definition steps for each plex.

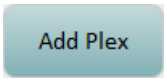
With the exception of the acquisition channel specification, the procedure for setting up mutiplexes and singleplexes is the same. When you set up a multiplex, make sure that the selected acquisition channels are unique for each plex in the assay (see step 9).

To define calibrators for a Homebrew assay

1. In the Custom Assay tab, touch **Plexes**.
2. Make sure that the new custom assay appears in the Assay field.

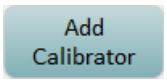


3. Touch **Add Plex**.

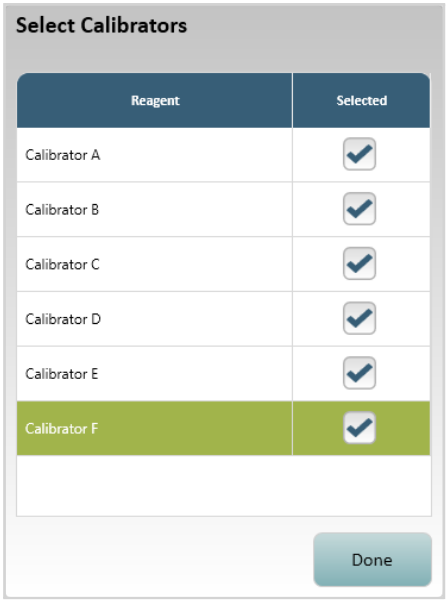


4. Edit the plex name.

5. Add calibrators to the plex:
   a. Touch **Add Calibrator**.



b In the Select Calibrators screen, touch the checkboxes for the calibrators that you want to add > touch **Done**. The Assay Plex screen shows the calibrators.



6 Enter a concentration for each calibrator:

a Double-touch **NaN** next to a calibrator.

b Enter a concentration (for example, 0, 0.1, 10).

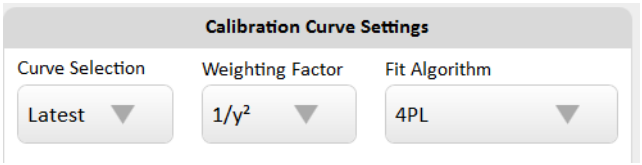
Note! Non-zero concentration values must be at least 0.01. If the lowest non-zero calibrator falls below 0.01, change the Concentration Unit (see step 8). For example, if your concentration is 0.001 pg/mL, specify the value in fg/mL (1 fg/mL in this example).

Name	Concentration	Custom Weighting Factor
Calibrator A	0	1
Calibrator B	0.1	1
Calibrator C	NaN	1
Calibrator D	NaN	1
Calibrator E	NaN	1
Calibrator F	NaN	1

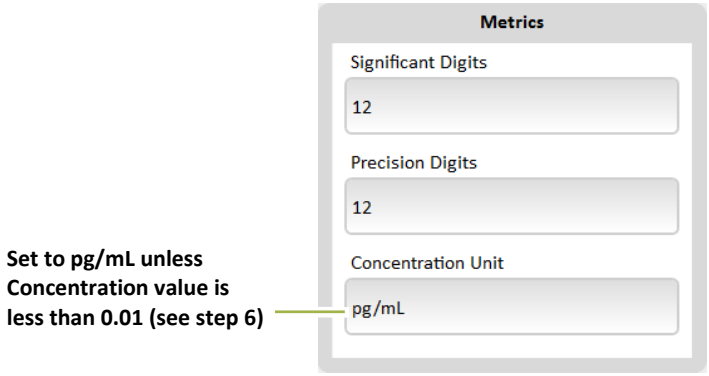
7 Select parameters in the Calibration Curve Settings box.

The two options for Under Curve Selection have the following meanings:

- **None:** No curve selection strategy will be used.
- **Latest:** The assay will use the most recently used strategy.

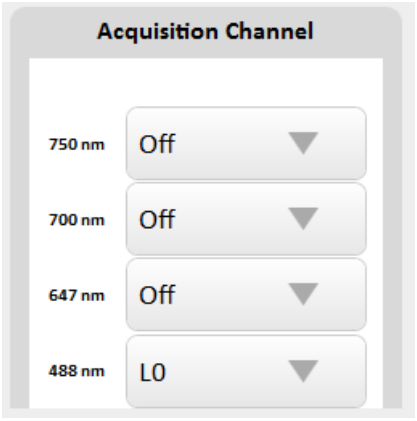


8 In the Metrics box, specify the number of significant digits and precision digits to be used in assay results and the unit of measure for the calibrators.



9 Specify the levels for the dyes in the Acquisition Channel box:

- For a singleplex assay using unencoded (dye-free) singleplex beads, set the 488 channel to L0 and leave all other channels set to Off.
- For a multiplex assay or a singleplex assay using dye-encoded multiplex beads, set the channels as described in Appendix 5 on page 213.



Adding a Dilution to a Homebrew Assay

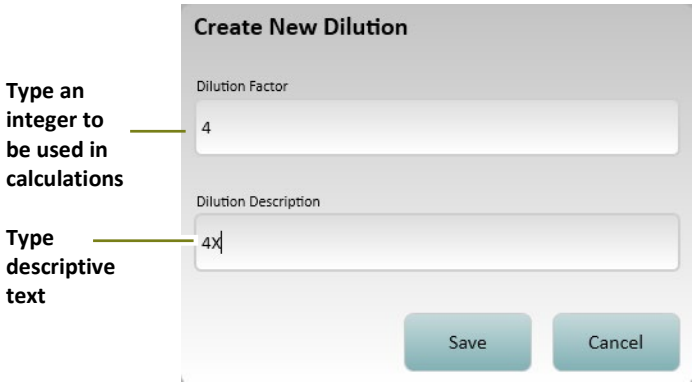
The process of adding a dilution is exactly like the process for creating a new assay, except that you must modify the volumes that the protocol specifies for samples and sample diluents.

For both samples and sample diluents, the protocol specifies the liquid volume parameter, which is the volume of sample or sample diluent that the instrument will pipette into cuvettes during the assay.

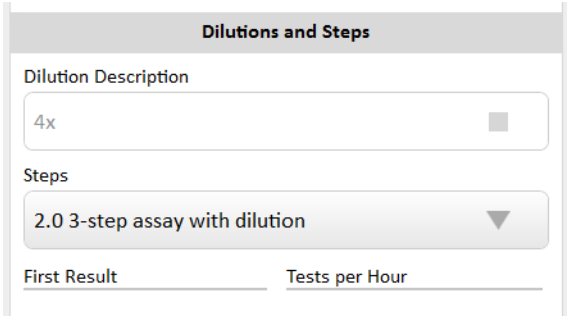
Before performing this procedure, be sure to read “Understanding Sample Dilutions” on page 70.

To add a dilution

1. Calculate the sample and sample diluent volumes.
2. In the Assay Overview screen, Touch **Add Dilution.**
3. In the Create New Dilution screen, type an integer value in the Dilution Factor box and type a description in the Dilution Description box.
4. Click **Save**.
5. The protocol steps display again, but assay reagents are no longer associated to them.



6 In the Dilutions and Steps box, touch the dropdown arrow to view a list of all protocols > select the protocol that you selected when you created the assay.



Note! This protocol must have the word "dilution" in its name. If it does not, you cannot add the dilution. Re-define the assay to use a protocol that permits you to add dilutions.

7 Associate the reagents in the red boxes with assay reagents, as described in step 6 of the procedure for defining an assay (see page 78).

8 Modify the volumes:

a Scroll through the protocol to locate the Sample/Calibrator step.

b Double-touch the LiquidVolume number for the Sample/Calibrator step > enter the sample volume for the dilution. For example, if you are following the recommendation to keep total volume to 100 µL, enter 25 to set up a 4x dilution, and enter 10 to set up a 10x dilution.

c Locate the Diluent step and repeat steps b and c to modify sample diluent volumes. For example, to finish setting up the example 4x dilution, enter 75 in the LiquidVolume field. To finish the 10x dilution, enter 90 in the LiquidVolume field.

This example shows completed volume modifications for a 4x dilution, with a total volume of 100 µL.

Sample/Calibrator					
LiquidVolume	25	µl	10	1000	
Detector					
LiquidVolume	100	µl	10	1000	

Modifying Incubation Times

Incubation times must be converted from minutes to "instrument cadences." Cadence refers to the instrument's fundamental timing. One cadence is 45 seconds. Use the following formula to convert minutes to cadences:

$$\frac{\text{X minutes} \times 60}{45}$$

If your result is not a whole number, round up the results.

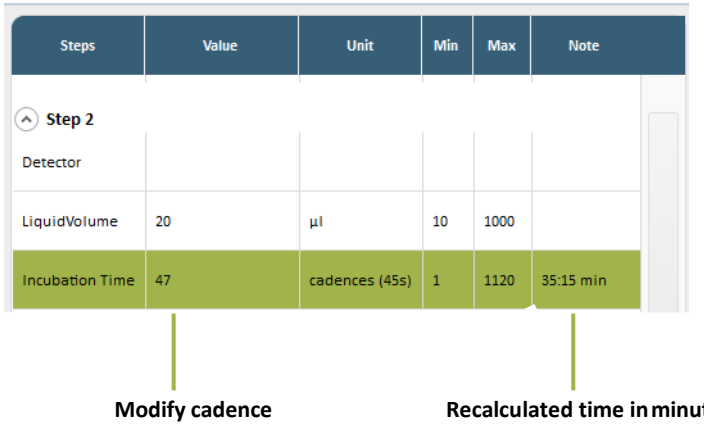
When you change the incubation cadence in the Value column, the time in minutes is automatically recalculated.

The following protocols have been tested and approved. If you are using untested protocols, unexpected results may occur.

Protocol	Incubation Time in Minutes	Incubation Time in Cadences
3-step	15–5–5	20–7–7
	30–5–5	40–7–7
2-step	35–5	47–7
	30–5	40–7
	9–3	12–4
	9–4	12–5
	12–3	16–4
1-step	Any timing will run properly.	

To modify incubation times

1. Select the assay to modify, as described in “Viewing Settings for a Homebrew Assay” on page 88.
2. Type the value for each reagent.

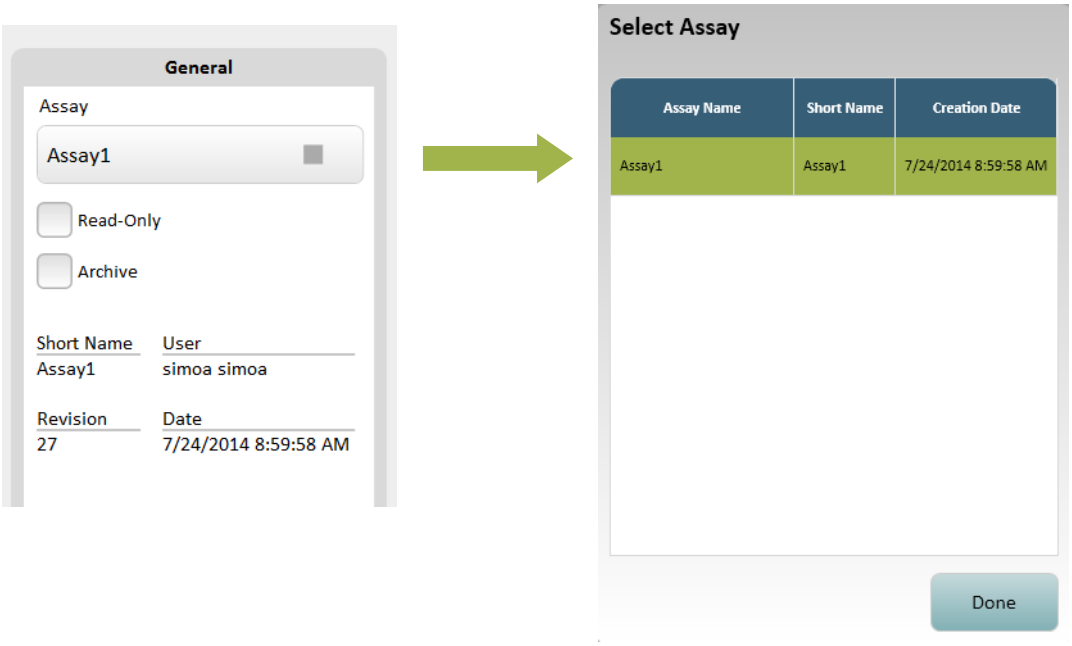


Viewing Settings for a Homebrew Assay

Assay settings are divided into the Assay Overview and Assay Plexes screens. The Assay Reagents screen shows all reagents that have been defined for the instrument; it is not assay-specific.

To view assay settings

1 In the Assay Overview screen, touch the square gray icon. A list of all assays that have been defined for the instrument appears.



2 Touch an assay in the Select Assay dialog > touch **Done**. The Assay Overview screen shows the protocol and dilution settings. To view a sample Assay Overview screen, see page 66.

3 To view calibrator settings, touch **Plexes**. To view a sample Assay Plexes screen, see page 69.

Viewing the List of Assay Reagents

The Assay Reagents screen shows all assay reagents that have been defined for the instrument.

To view assay reagents

In the Assay Overview screen, touch **Reagents**. To view a sample Assay Reagents screen, see page 68.

Deleting an Assay Reagent

You cannot delete an assay reagent if it is used in an assay definition that has been run on the instrument at least one time.

To delete an assay reagent

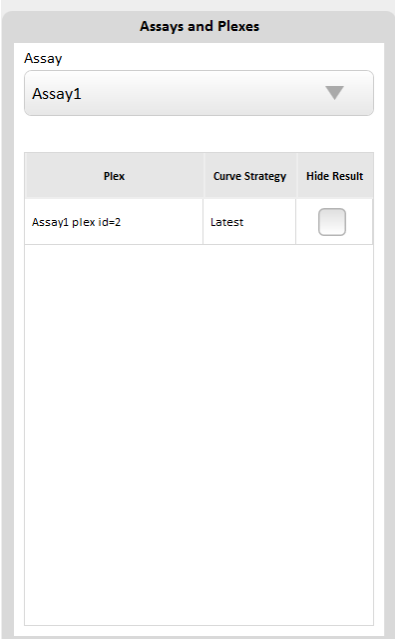
1. Touch the Custom Assay tab > touch **Reagents.**
2. Touch a reagent in the Reagent Definitions list.
3. Touch **Delete Reagent**.
4. Touch **Yes** to confirm the deletion.

Viewing the List of Assay Plexes

The Assay Plexes screen shows all assay plexes that have been defined for each assay. To view a sample Assay Plexes screen, see page 69.

To view assay plexes

1. Touch the Custom Assay tab > touch **Plexes.**
2. Select an assay from the Assay pull-down list. A list of plexes appears in Assays and Plexes.



Deleting an Assay Plex

To delete an assay plex

1. Touch the Custom Assay tab > touch **Plexes.**
2. Select an assay from Assay pull-down list.
3. Select a plex in Assays and Plexes.
4. Touch **Delete Plex**.
5. Touch **Yes** to confirm the deletion.

Deleting a Homebrew Assay

The Simoa software stores assays in its database. Assays can be deleted if there are no associated results. Results are regularly cleared out through the Database Clean task, so definitions that are unused can be deleted. Once an assay has been run (and has results associated with it), you cannot delete it from the database.

To delete an assay

1. Touch the Custom Assay tab > touch **Overview**.
2. Select the assay that you want to delete from the Assay drop-down list > touch **Done**.
3. Touch **Delete Assay**.
4. Touch **Yes** to confirm the deletion.

Developing Homebrew Assays Remotely

To develop assays remotely, install a copy of the software on a computer and then create assays on it, as described in this chapter. When you have finished creating the assays, use an external storage device to export the assays from your computer and import them into the instrument computer.

You can also export assays from the instrument computer and import them into your local copy of the software.

To export an assay

1. Touch the Custom Assay tab > select an assay from the Assay drop-down list.

2 Touch **Export**.

3 Navigate to an export directory > touch **Save**. The software copies the assay XML file to the directory.

❖ To import an assay into the instrument software

1 Open the System Bay drawer and connect the external storage device to one of the USB ports on the front panel of the instrument computer.

2 Touch the Custom Assay tab > touch **Import**.

3 Navigate to the assay XML file on the USB stick > touch **Open**.

4 Optionally, change the Assay Name and Assay Short Name.

5 Touch **Save** to import the assay. The Simoa software imports the assay into its database, and the protocol appears in the Assay drop-down list on the Custom Assay screen.

8 Performing an Assay Run

Steps in an Assay Run

All Simoa® assays—singleplex, multiplex, and Homebrew—are performed as described in this chapter. Although the examples in this chapter show plates of samples and calibrators, the instructions apply to tubes as well.

Performing a Simoa assay run involves the following general steps:

1. Prepare reagents, samples, and calibrators (if you will be running them).
2. Select Rack or Plate mode for samples. Prepare the instrument for the run.
3. Place bead, detector, and SBG reagents into a reagent rack, identify the reagents by position and barcode in the software, and load the rack into the instrument.
4. Place one or more vials of RGP into an RGP rack, identify the vials by position and barcode in the software, and load the rack into the instrument.
5. Place samples, calibrators, and controls in plate or tube racks, select an assay to run on them, name the run, name the rack or plate, identify the samples, calibrators, and controls in the software, and load the racks into the instrument.
6. Start the run and monitor it.

When the run finishes, the results appear in the Run History table in the History & Reports tab. For information on analyzing results, see “Analyzing Run Data” on page 135.

The Role of Barcodes in an Assay Run

The labels on all assay reagents (including Homebrew reagents) and Simoa Discs have barcodes that provide information such as reagent or item name, fill volume (as specified in the reagent definition), lot number, and expiration date. When you load barcoded reagents or RGP into the instrument, you can scan them with the handheld scanner or use the on-board scanner. When loading barcoded Simoa Discs, you must manually scan their barcodes using the handheld device.

The software uses the scanned barcode data to:

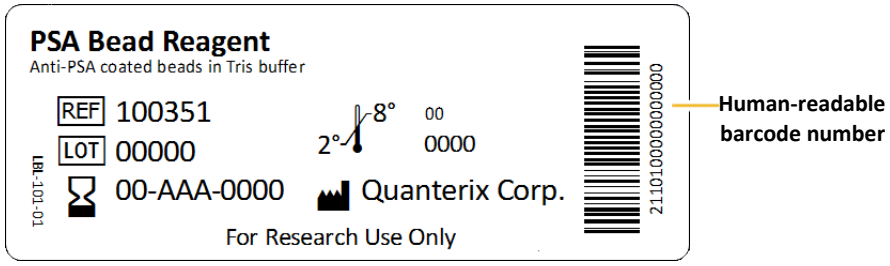
- Identify loaded reagents in screens.
- Monitor expiration dates.
- Calculate reagent and consumable consumption during an assay run and notify you when more reagents or consumables should be loaded.

Be sure to follow instructions in this guide regarding barcode scanning. If barcodes are not scanned, the instrument will not be able to perform the assay.

Note that you must apply the supplied barcoded Homebrew labels to your Homebrew reagents. Other Simoa assays are pre-labeled.

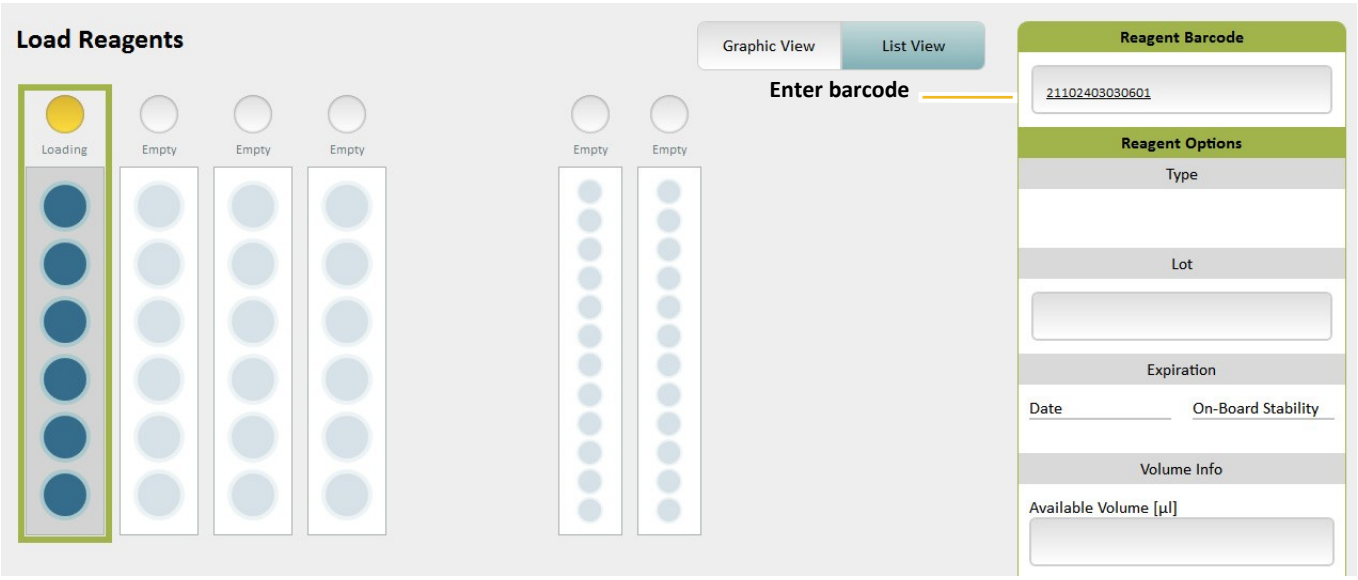
Manually Entering a Reagent Barcode

In the event that a reagent label is damaged and cannot be scanned, you will need to type the barcode number into the software. The human-readable version of the barcode is printed beneath the barcode.



To manually enter a reagent barcode

In the Load Reagents tab, touch the Reagent Barcode field > type the number > touch **Enter**. For information on loading reagents, see “Loading Reagents and RGP into the Instrument” on page 95.



Preparing Reagents, Calibrators, Controls, and Samples

Before preparing any assay materials or performing an assay on the instrument, read the assay kit instructions. The kit instructions contain important safety information and details about the assay procedure that are vital to performing the assay successfully.

Use Approved Sample Plates and Sample Tubes

Use only Quanterix®-supplied sample plates. These plates have been verified to be nonreactive with Simoa assay reagents and to be correctly sized to work with the instrument pipettors. For information on ordering plates, see page 2.

Please contact Quanterix Technical Support for information regarding which sample tubes you may use with your HD-X instrument.

CAUTION! Quanterix cannot guarantee system performance if you use any type of plate other than the ones supplied by Quanterix.

Preparing Reagents

Follow the assay kit instructions to prepare reagents.

If you are performing a Homebrew assay, you will need to prepare bead and detector reagents using your own diluents. See the *Simoa Bead-Based Homebrew Assay Development Guide* for more information.

Preparing Calibrators and Samples

Follow the assay kit instructions to prepare calibrators and samples. Note that the instrument does not perform calibrator dilutions.

Calibrators and samples may be placed in tubes or Quanterix-supplied plates. Contact Quanterix Technical Support for information regarding which tubes you may use. Calibrators and samples may be placed in the same plate.

After you pipette calibrators and samples into plates or tubes, place them immediately into the plate racks or tube racks. This procedure allows you to quickly place the racks into the instrument when you begin to identify calibrators and samples in the software.

If there is enough sample volume to spare, you can do multiple calibrator replicates in a single well. Pipette each set of replicates into the plate, in ascending or descending order. This allows you to rapidly identify them in the software by either column or row (see “Assigning Calibrators” on page 113).

Preparing the Instrument for the Run

For information on how to load reagent racks, plate and tube racks, and RGP racks, see pages 30 to 35.

To prepare the instrument

1. If this is the first run of the day, perform the instrument startup procedure (see page 27).
2. Load Simoa cuvettes, Simoa disposable pipettor tips, and Simoa Discs into the instrument as needed. For information on how to load these consumables, see page 36.
3. Set up the software for sample plates or sample tubes:
   a. Touch the Settings icon at the top right of the software to open the System Settings screen.

   b. Touch **Plate Mode** or **Rack Mode** > touch **Done**.
4. Obtain enough reagent racks, RGP racks, and either tube racks or plate racks to perform the assay.
5. Make sure that the necessary assay definition is imported on the instrument. For information on checking this, see page 58.

Loading Reagents and RGP into the Instrument

Loading reagents and RGP involves:

- Placing reagent bottles into a reagent rack.
- Enabling the on-board barcode scanner or scanning reagent label barcodes with the handheld scanner.
- Inserting the rack.

Load as follows:

- Insert the rack into any lane of the reagent bay.
- Load detector and SBG reagents into any position in the rack.
- Load beads into the first three (shaking) positions.
- Load RGP into lanes labeled RGP 1 or RGP 2.

Using the Barcode Scanners

You can use the on-board or handheld scanners to scan reagent and RGP barcodes. When the on-board barcode scanner is enabled, it automatically reads the reagent/RGP barcodes and associates each barcoded bottle with its position in the rack. When the on-board scanner is turned off, you must identify the rack position of the reagent bottles in the Load Reagents screen and then scan their barcodes using the handheld scanner.

You can manually enter one or more reagent barcodes (either by using the handheld scanner or by typing it) and still use the built-in scanner for the remainder of reagents on the rack.

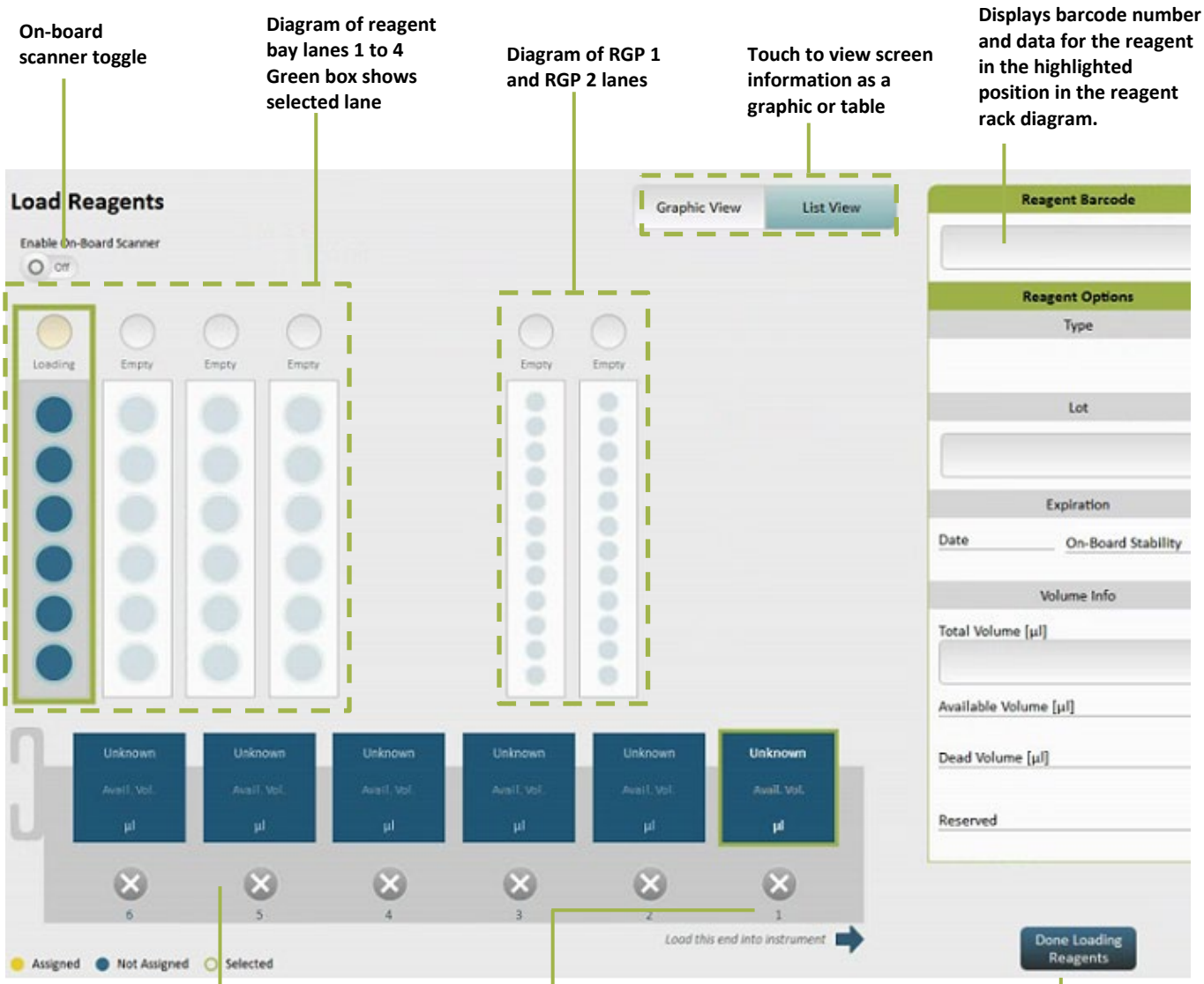
When the on-board scanner is turned on, it reads all reagent barcodes in the rack, including those that have already been scanned with the handheld device or typed in. If a barcode detected by the built-in scanner does not match the previously entered barcode, then the barcode and all associated reagent information will be overwritten by the on-board scanner.

The software associates the barcodes with their positions in the rack so that the instrument pipettors can be directed to the correct bottles in the loaded rack. The barcode data also allows the software to calculate running reagent volume and count down to reagent expiration dates.

Occasionally, a barcode may not be scannable because the label has been damaged in some way. When this happens, manually enter the barcode into the appropriate software field (see page 92).

Understanding the Load Reagents Screen

By default, the screen shows a reagent rack diagram, as shown below. When you touch one of the RGP lanes, the screen changes to show an RGP rack diagram. To view an example of the RGP rack diagram, see page 102.



Loading Reagents

Loading reagents involves placing the bead, detector, SBG, and sample diluent reagents in the reagent rack and inserting the rack into the reagent bay.

❖ To load reagents using the handheld barcode scanner

1. Mix the bead reagent according to instructions in the assay kit. If the bead reagent is left sitting stationary for more than five minutes before you load it, repeat this step.
2. Remove and set aside the caps from the reagent bottles.
3. Insert the bead reagent into reagent rack position 1, 2, or 3.

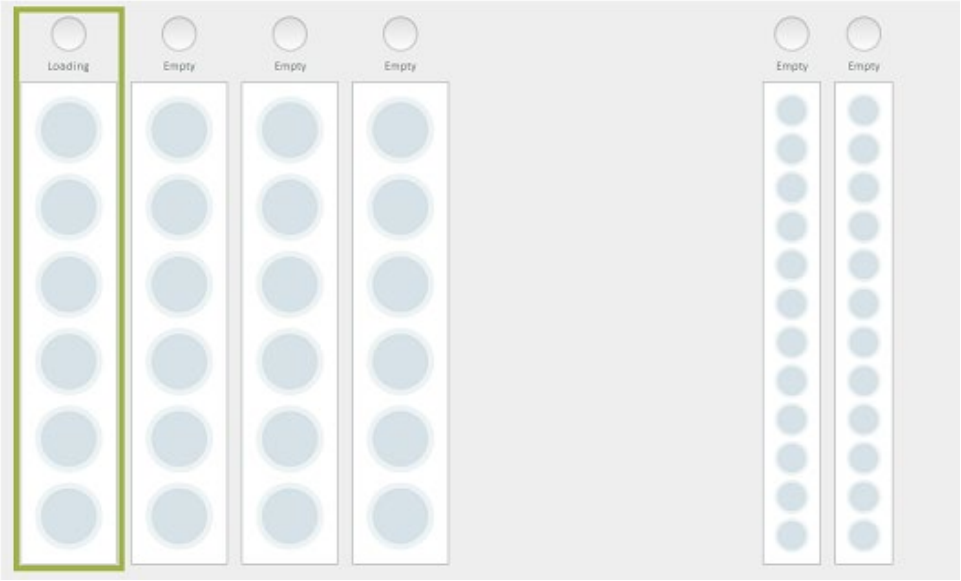
Note! You must insert the bead reagent in rack position 1, 2, or 3. These positions shake during the assay run to keep the beads suspended.

4. Insert the other reagents into any other reagent rack position, including unoccupied shaking positions (1, 2, or 3).

5 Turn the bottles so that the reagent label barcodes are centered in the notch of the bottle holder. The barcodes must be unobstructed so that they can be scanned accurately.

6 Touch the Load Reagents tab > touch a reagent bay lane in the screen. This step identifies the lane into which you will load the reagent rack.

Reagent Bay position 1 has been selected



7 Scan the bottle barcodes:

a In the reagent rack diagram, touch the position that corresponds to the position of the bead reagent bottle in the reagent rack.

b Touch the reagent barcode field and scan the bead reagent barcode with the handheld scanner. The barcode and data about the bead reagent appear in the green box in the screen. If a barcode does not scan, enter it manually (see “Manually Entering a Reagent Barcode” on page 92).

c Repeat these two steps for the rest of the reagents in the rack.

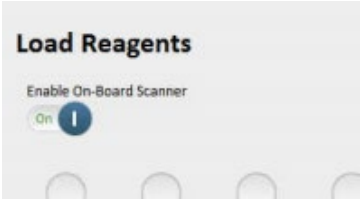
Note! If you have not inserted the bead reagent into position 1, 2, or 3, the software displays a warning, and you cannot proceed until you move the bottle into one of these positions.

8 Insert the rack in the reagent bay lane that you selected in step 6 until it clicks into place. The rack diagram updates to show the short name of each loaded reagent in its identified rack position. The lane diagram updates to show the positions of reagents in the selected lane. The Reagent Barcode box shows barcode data for the position that is highlighted in the reagent rack diagram.

❖ To load reagents using the on-board barcode scanner

1 Perform **Steps 1 through 5** as described in the previous section, “To load reagents using the handheld barcode scanner”.

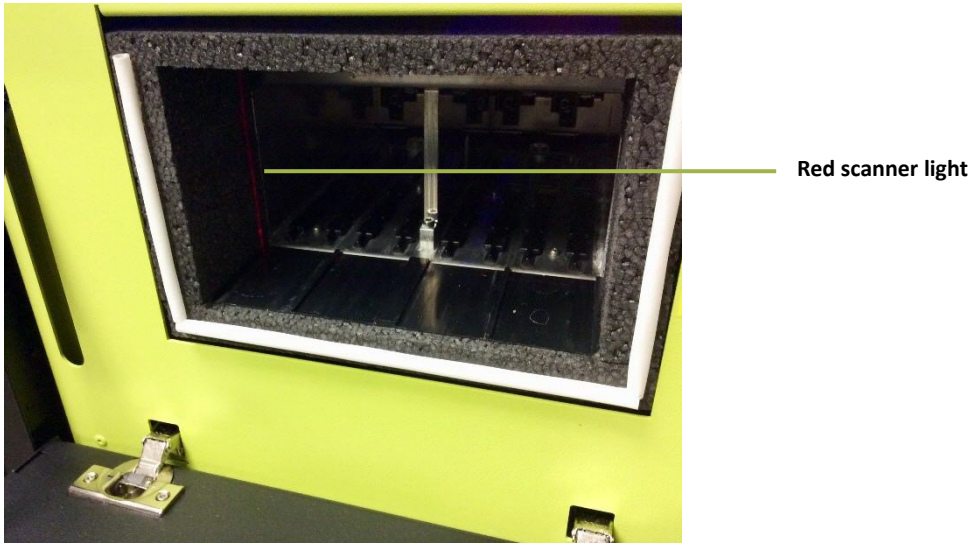
2 Touch the Load Reagents tab. On the Load Reagents screen, turn on the “Enable On-Board Scanner” toggle.



3 Touch a reagent bay lane in the Load Reagents screen. After you select a lane, the on-board scanner focuses its laser on the lane and a message appears to notify you that the barcode scanner is focusing. Wait until focusing completes and the message disappears before proceeding to the next step.

4 Insert the loaded rack in the reagent bay lane that you selected in step 3 until it clicks into place. As you load it, the on-board scanner reads the barcodes of each reagent bottle in the rack. The rack diagram updates to show the short name of each loaded reagent in its identified rack position. The lane diagram updates to show the positions of reagents in the selected lane. The Reagent Barcode box shows barcode data for the position that is highlighted in the reagent rack diagram.

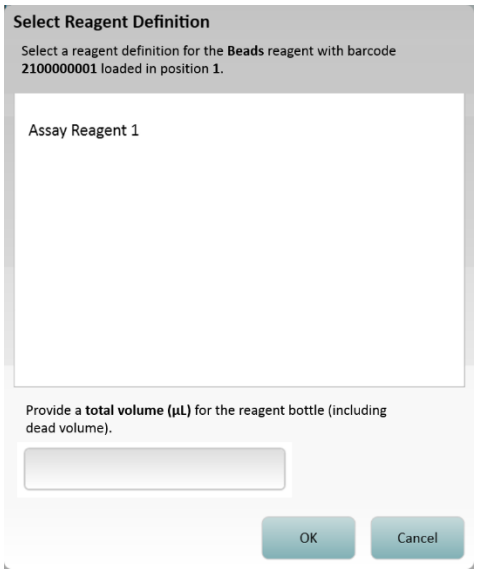
Note! The on-board scanner will only work if the red scanner light is visible while you are loading the reagent rack. It appears on the left side of the reagent bay when the scanner is turned on.



Loading Homebrew Reagents

To load Homebrew reagents, see the instructions under Loading Reagents on page 97. You may use the handheld or on-board barcode scanner.

When you scan a barcoded Homebrew reagent, you must select the corresponding reagent in the **Select Reagent Definition** window.



Make sure that the Homebrew reagent bottles are labeled, as described in the kit instructions, so that each bottle has the correct barcode.

- **When using the handheld barcode scanner:** The Select Reagent Definition window appears on the screen as you manually scan each bottle.
- **When using the on-board scanner:** The scanner detects Homebrew barcodes and the Select Reagent Definition window appears for each Homebrew reagent bottle in the order of rack position. For example, if position 1 contains a Homebrew beads bottle and position 2 contains a Homebrew detector bottle, the window appears first for the beads bottle. When you select the corresponding reagent definition and select OK, the window closes and a new one appears for the detector bottle.

When the **Select Reagent Definition** window appears:

1. Select the corresponding reagent.
2. Enter the total volume for the reagent bottle (including dead volume) in the field at the bottom of the window. The amount you enter here populates the Total Volume field in the Load Reagents screen.
3. Select **OK**.

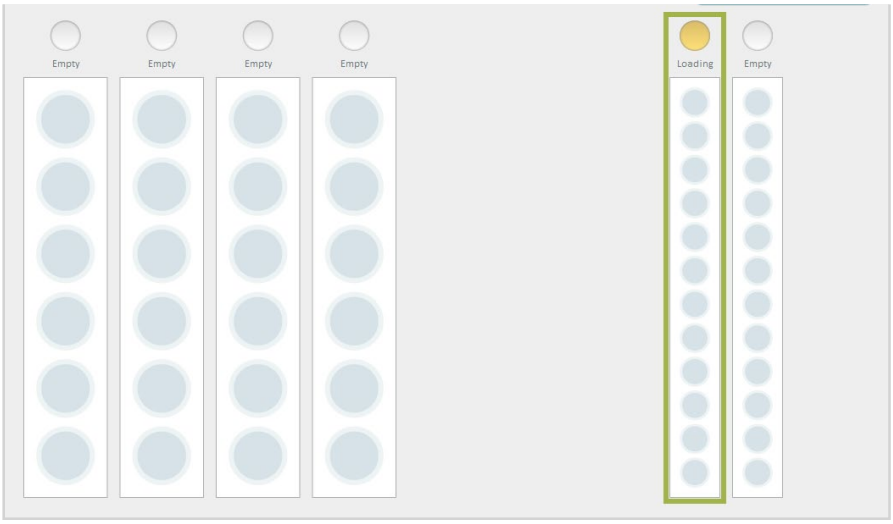
RGP must be loaded into an RGP rack and placed in sample bay RGP 1 or RGP 2 only (the substrate lanes).

The RGP racks are customized to hold the short RGP reagent vials at optimum position for the instrument pipettors.

CAUTION! Do not load RGP into a sample tube rack. Always use an RGP rack. If you load RGP into a sample rack, the pipettor will strike the bottom of the loaded vials, possibly damaging the instrument.

To load RGP using the handheld barcode scanner

1. Prepare RGP according to the assay kit instructions.
2. Remove the caps of one or more RGP vials > insert the RGP vials into the RGP rack.
3. Touch the Load Reagents tab > touch lane RGP 1 or RGP 2 in the screen. This step identifies the lane into which you load the RGP rack. The Load Reagents screen shows an RGP rack diagram.



Sample bay lane RGP 1 has been selected

4 Scan the vial barcodes:

a In the RGP rack diagram, touch the position that corresponds to the position of the RGP vial in the reagent rack.

b Scan an RGP vial barcode with the handheld scanner and insert the vial in position 1 of the rack. The barcode and data about the scanned vial appear in the Load Reagents screen.

c Repeat these two steps for the rest of the RGP vials.

5 Insert the rack into the lane that you selected in step 3 until it clicks into place. The RGP rack diagram updates to show each RGP vial in its identified rack position. The lane diagram updates to show the RGP vial positions in the selected lane.

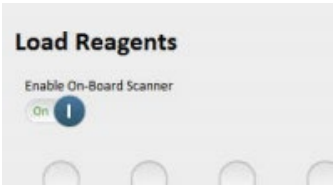
❖ To load RGP using the on-board barcode scanner

1 Prepare RGP according to the assay kit instructions.

2 Remove the caps from one or more RGP vials > insert the RGP vials into the RGP rack.

3 Turn the RGP vials in the rack so that the barcodes are centered in the notch of the bottle holder. The barcodes must be unobstructed so that they can be scanned accurately.

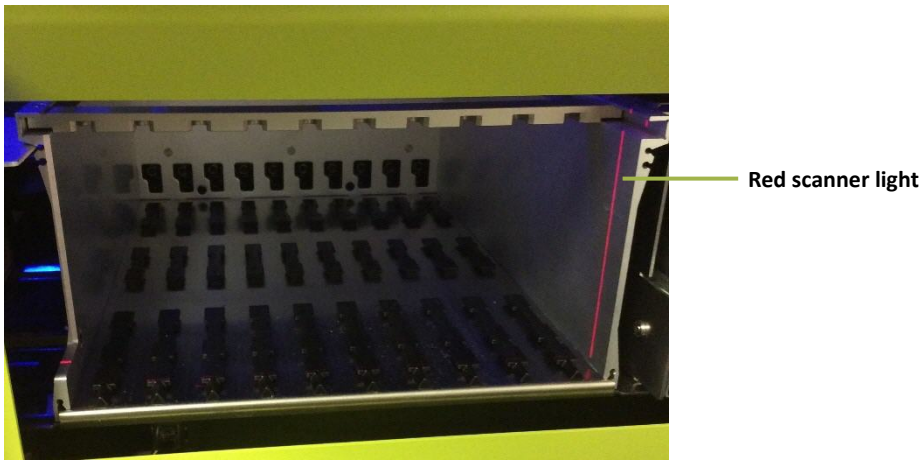
4 Touch the Load Reagents tab. Turn on the “Enable On-Board Scanner” toggle in the upper left part of the screen.



5 Touch lane RGP 1 or RGP 2 in the Load Reagents screen. Once you have selected an RGP lane, the on-board barcode scanner focuses its laser on the lane and a message appears to notify you that the scanner is focusing. Wait until the message disappears before proceeding to the next step.

6 Insert the rack into the lane that you selected in step 4 until it clicks into place. As you insert the rack, the on-board barcode scanner reads the barcode for each RGP bottle. The RGP rack diagram updates to show each RGP vial in its identified rack position. The lane diagram updates to show the RGP vial positions in the selected lane.

Note! The on-board scanner will only work if the red scanner light is visible while you load the RGP rack. The red light appears on the right side of the sample bay when the scanner is turned on.



Setting Up a Run

Setting up the run involves identifying calibrators and samples in the Setup Run tab. In this tab, you can toggle between the Assign Calibrators and Assign Samples screen.

In this chapter, the examples describe a single plate that contains all of the calibrators. You can also set up your assay calibrators on multiple plates.

Once you have set up a run, you can export the assignments to a file and import the mappings the next time you run the assay.

If an experiment requires multiple assay runs, you can assign a **Batch Name** to all the plates or tube racks that you run for the experiment. This procedure allows you to group the experiment's results in the Run History screen.

Assigning calibrators and samples involves:

- Selecting an assay.
- Identifying in the software the physical location of calibrators and samples in the plates or tubes that you will be loading into the instrument.
- Associating each calibrator with one of the calibrator concentrations in the assay definition.
- Selecting the number of replicates to run. See "Preparing Calibrators and Samples" on page 93 for a complete explanation.
- Naming each sample, selecting the number of replicates to run, and, depending on the assay, selecting a dilution factor for the sample.
- Selecting the sample processing order (Rows or Columns).

Note! You will find it easier to assign calibrators and samples if you put them into plate racks or tube racks and then place the racks in the sample bay before you begin. Do not fully insert the racks; simply place them into the bay. The procedures for assigning calibrators and samples include directions to do this.

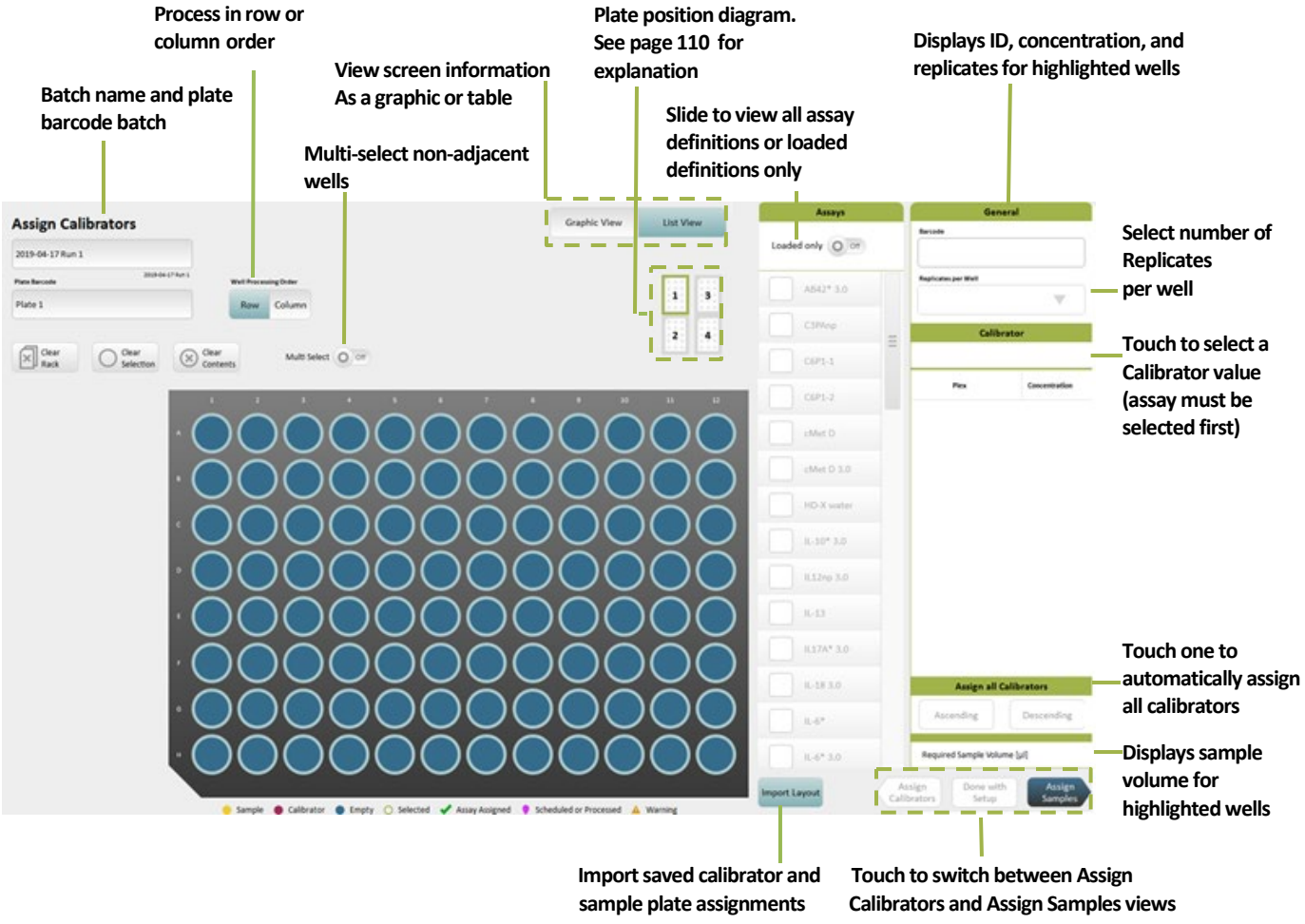
Understanding the Assign Calibrators Screen

The Assign Calibrators screen shows a plate diagram or a tube diagram depending on how you have set up the instrument (see “Preparing the Instrument for the Run” on page 94).

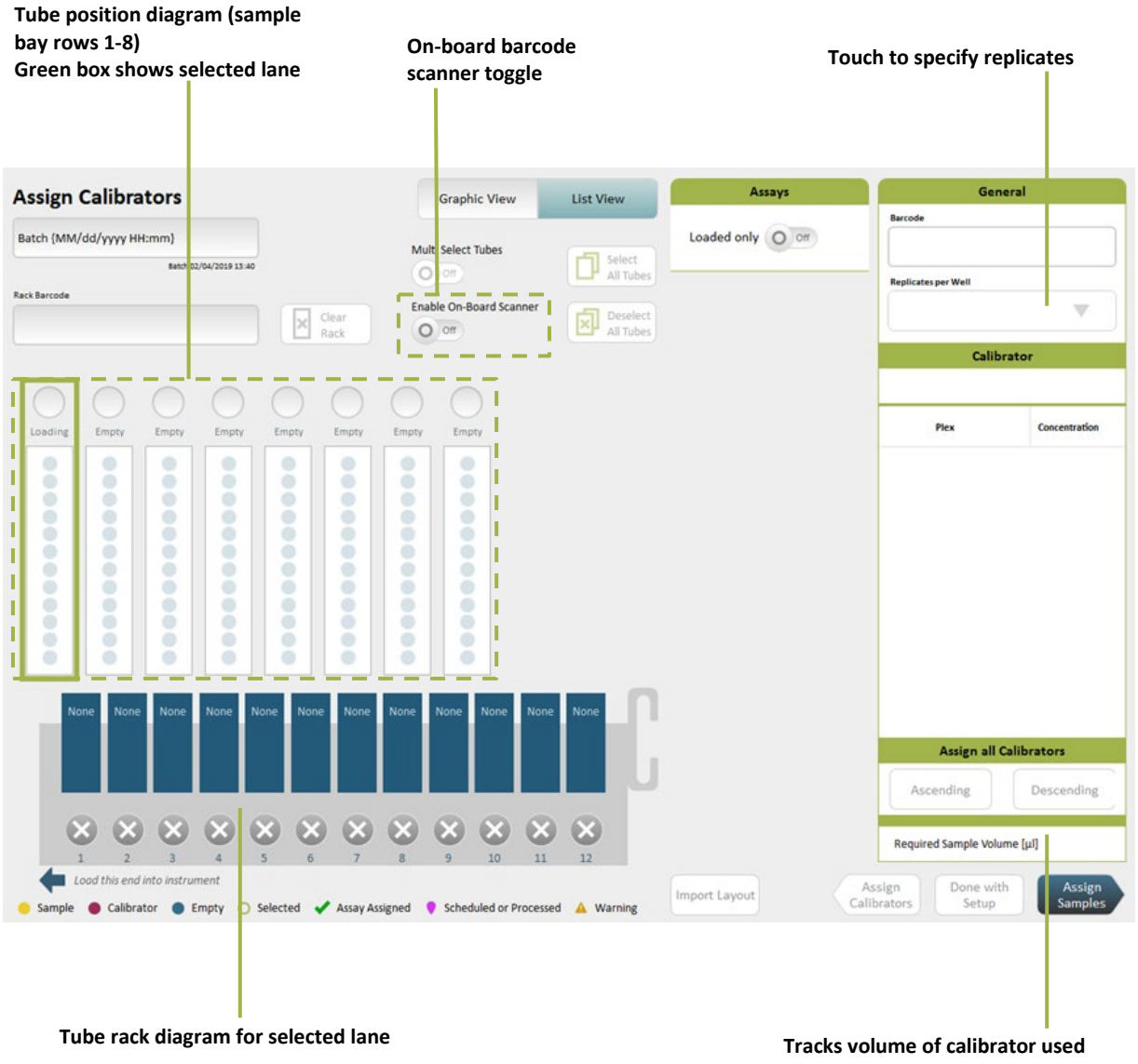
For information on using the well selection tools, see “Selecting Plate Wells and Removing Assignments” on page 110.

Use Multi Select to assign replicates for multiple calibrators.

The Assign Calibrators screen in plate view appears below.

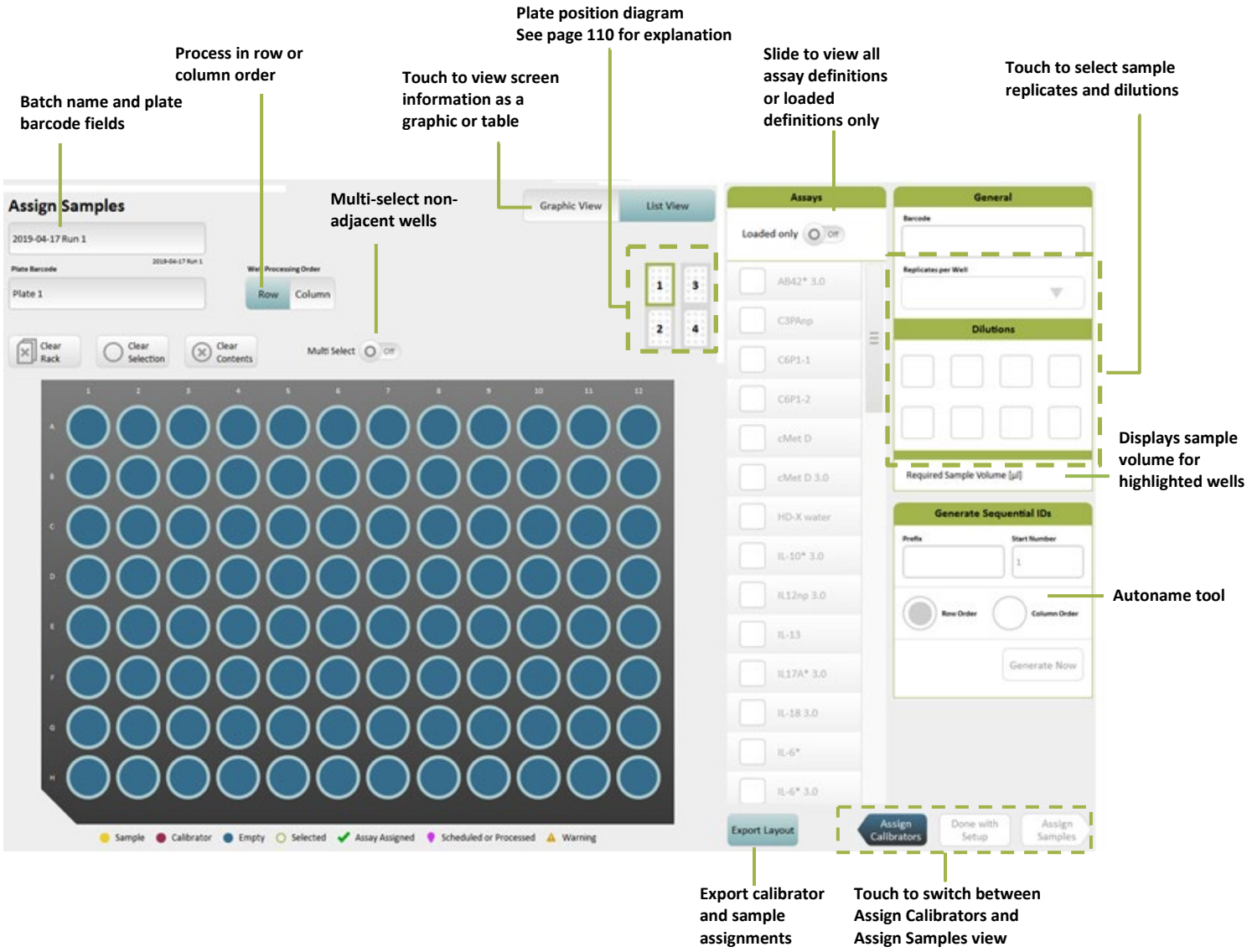


The Assign Calibrators screen in tube rack view appears below. It is identical to the screen for plates except that it shows a tube rack diagram, a location diagram for the eight sample bay lanes, and the Enable On-Board Scanner toggle.

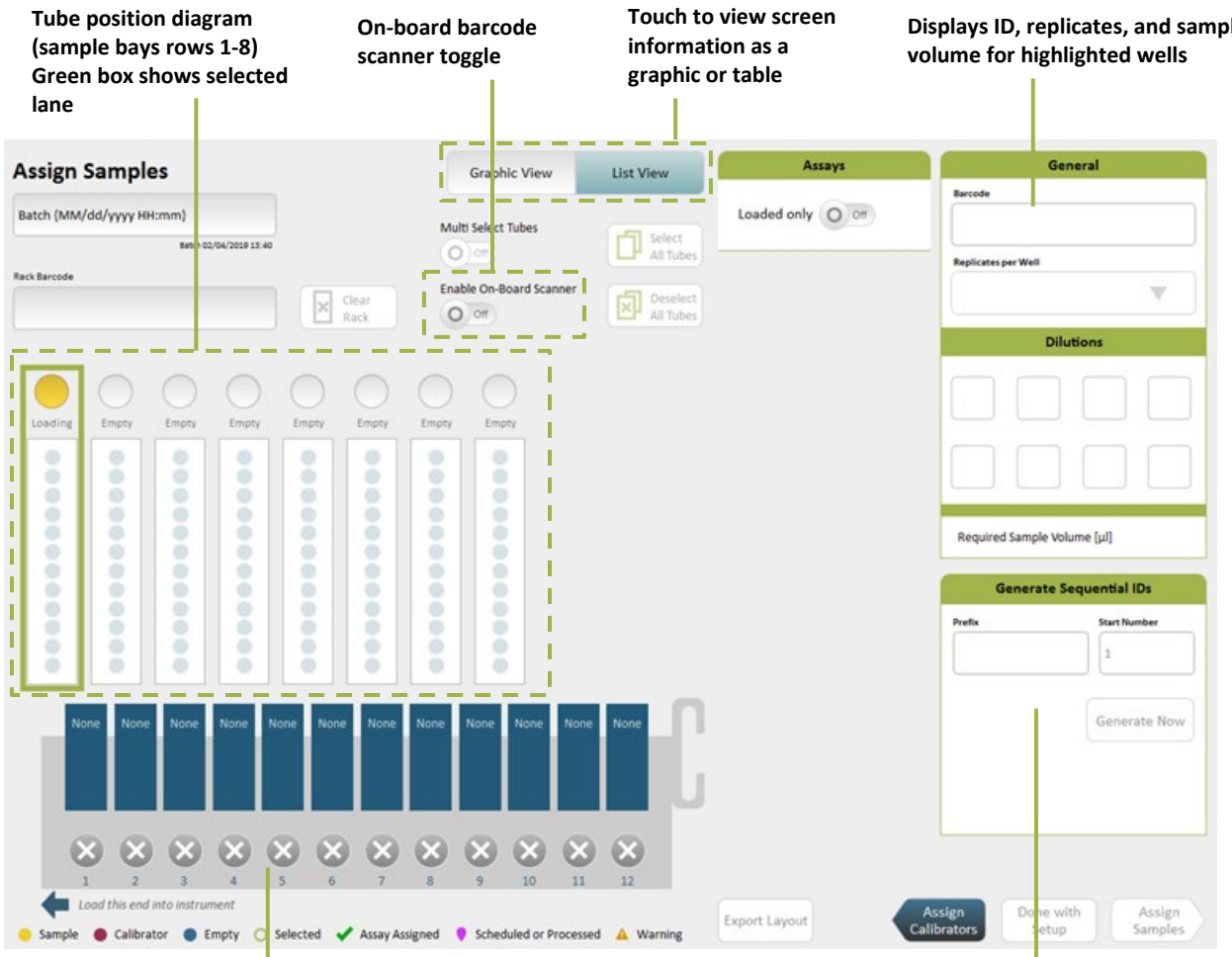


Understanding the Assign Samples Screen

The Assign Samples screen in **plate view** appears below.



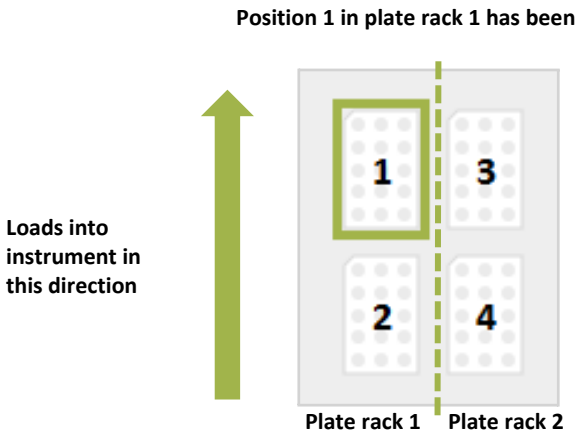
The Assign Samples screen in tube rack view appears below. It is identical to the Assign Samples screen for plates except that it shows a tube rack diagram, a location diagram for the eight sample bay lanes, and the Enable On-Board Scanner toggle.



Understanding the Plate Position Diagram

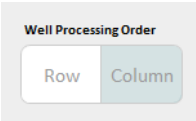
The plate position diagram identifies the lanes into which a plate is loaded and the position of a plate in those lanes. Plate rack 1 may only be loaded into lanes 1–4, and plate rack 2 into lanes 5–8. There are two plate positions for each plate rack.

Selections in the diagram apply to both the Assign Calibrators and the Assign Samples screens. For example, if you select plate 2 in the Assign Calibrators screen and then switch to the Assign Samples screen, the plate remains selected.



Selecting Processing Order

You can process your calibrators and samples by Row (left to right) or Column (top to bottom). This is a batch-wide setting that applies to all jobs in a run, even if on separate plates. If you have multiple plates, they are processed in ascending numerical order from 1 to 4.



Selecting Plate Wells and Removing Assignments

You must select plate wells to assign calibrators or samples to them. After you have made assignments, you can deselect or select wells without changing their assignments.

- To select a block of wells, touch the plate and draw a marquee around the wells.
- To select individual, nonadjacent wells, turn on **Multi Select** > touch the wells.

- To remove specific selections, touch the plate and draw a marquee around the wells > touch **Clear Selected**.
- To remove all selections and assignments on a plate, touch **Clear Rack**.
- To remove assignments from specific wells, select the wells > touch **Clear Contents**.

Understanding the Required Sample Volume Field

The Required Sample Volume field reports the total amount of sample that will be consumed by all assignments that you have made. This field can help you monitor sample consumption so that you do not assign more sample volume than you have placed in a plate or a tube.

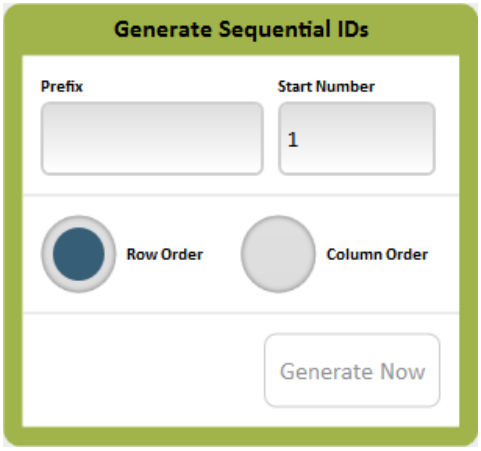
Understanding the Autoname Tool

Use the Autoname tool to quickly name a range of plated samples. This tool creates names of the following format:

[alphanumeric prefix up to nine characters long][first number in series]

For example: PSA02Mar1, PSA02Mar2, PSA02Mar3

Autonames are applied to a set of selected samples in row order or column order.



To generate sequential IDs

1. Enter a text or numeric prefix in the Prefix field.
2. Touch **Row Order** or **Column Order**.
3. Optionally, change the Start Number.
4. Touch **Generate Now**.

Scanning Sample Barcodes

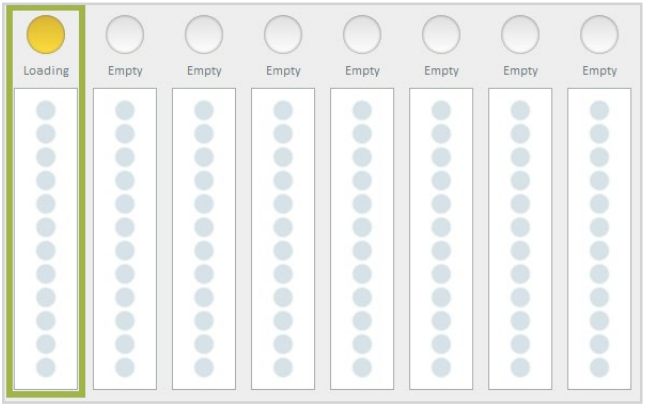
When loading samples in tube racks, you can use the handheld or on-board scanners to enter sample barcode information.

Note that if you are using the on-board scanner, you must assign all sample properties (including assay definition, replicates, and dilutions) prior to loading the barcoded sample tube rack. These properties cannot be modified once you insert the rack.

To scan samples with the handheld scanner

1. In the **Setup Run tab** > **Assign Samples** screen, select the lane into which you plan to load the sample tube rack.

Sample bay lane 1 has been selected



2. Touch the position of the sample tube in the tube rack diagram.

Tube 1 has been selected

3. Scan the sample barcode with the handheld scanner.

To scan samples with the on-board scanner

1. In the **Setup Run** tab > **Assign Samples** screen, turn on the **Enable On-Board Scanner** toggle.
2. Select the lane into which you plan to insert the tube rack.

3. Wait for the on-board scanner to focus its laser on the lane. You will see a notification informing you that the laser is focusing. Do not navigate to a new tab while the scanner is focusing, as this will halt the process.

4. Insert the rack into the sample bay lane that you selected. The on-board scanner will read the sample barcodes as you insert the rack.

Assigning Calibrators

To assign calibrators, you identify in the software the physical location of all calibrators in a plate or tube rack, and then you associate each calibrator with a calibrator concentration in the assay definition.

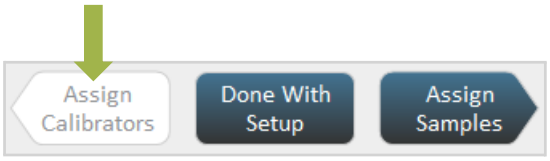
Note! You can import a saved map of calibrator and sample assignments. For information about this option, see “Importing and Exporting a Layout” on page 128. The Import button appears on the Assign Calibrators screen. The Export button appears on the Assign Samples screen.

Assigning Calibrators in Plates

Make sure that you have plated the calibrators correctly. You can assign multiple calibrators to a plate up to the capacity of the plate well. See “Preparing Calibrators and Samples” on page 93.

1 Open the Assign Calibrators screen:

- If you are in the Assign Samples screen, touch **Assign Calibrators**.
- If you are not in the Assign Samples screen, touch the Setup Run tab > touch **Assign Calibrators**.

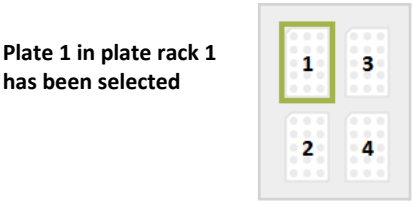


2 Recommended: Place prepared plates of calibrators and samples in plate racks and put the racks into the sample bay, but do not fully insert them.

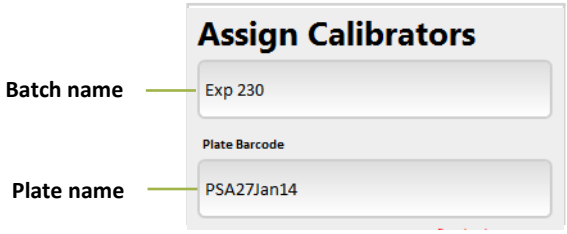
Note! If you choose to insert the plate racks after you finish assigning calibrators and samples, **you must make sure** that the plate containing the calibrators is loaded in the position that you identify for it in the next step of this procedure. Placing the racks into the sample bay now makes it easier to correctly identify plate position.

3 Name the plate that contains the calibrators:

a Touch the plate position that matches the location of the plate that contains the calibrators. For information on reading the plate position diagram, see page 110.



b Enter a name for the batch and a plate name > press **Enter**. You can use any combination of letters and numbers.



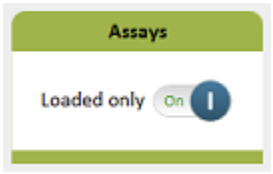
4 Perform the following steps to associate each set of calibrator replicates with the calibrator values in the assay definition, in ascending or descending order.

a Identify the first of the plated calibrators:

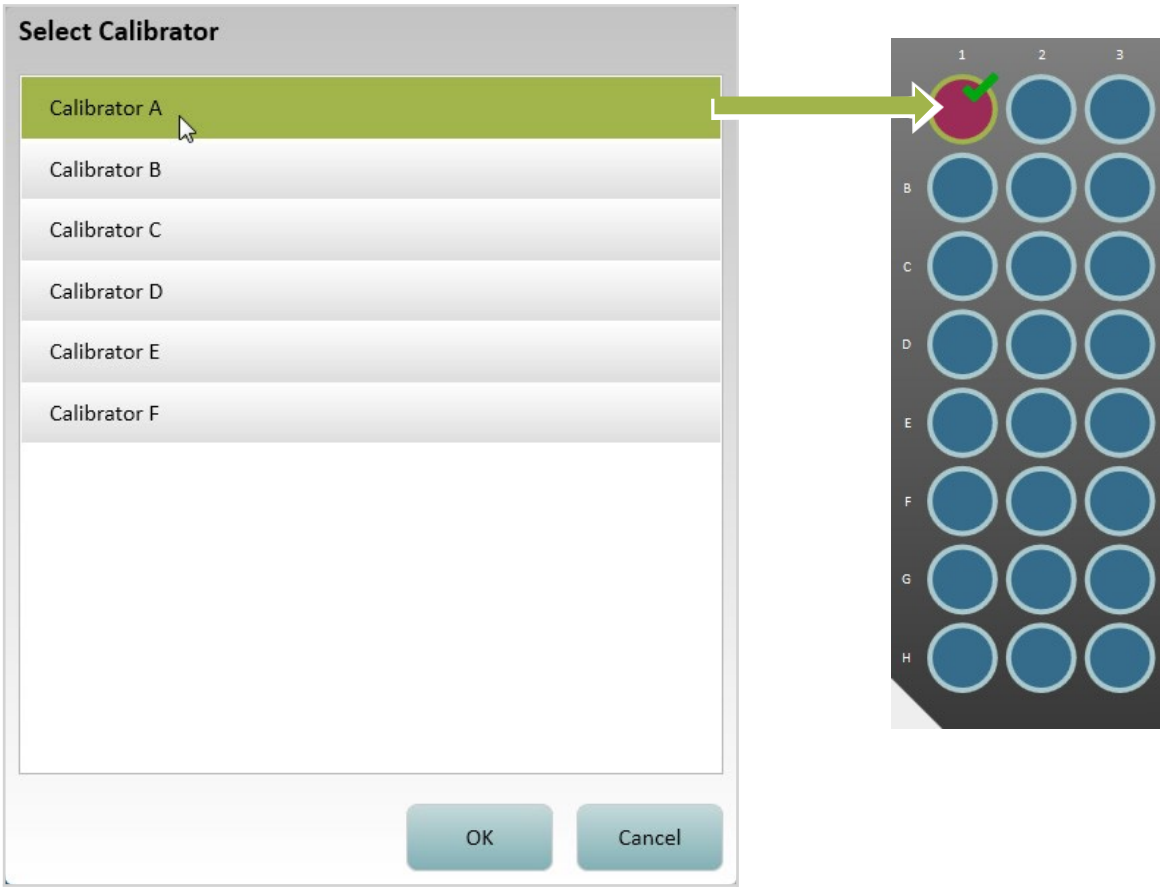
- **If you have plated only one set of calibrators:** Touch the well that contains the first calibrator in the set.
- **If you have plated two or more replicates:** Touch the first calibrator for one of the replicates. (You will repeat the entire assignment procedure to assign the remaining replicates.)

b Identify the assay:

- **If you have already loaded reagents:** Set the Assays Loaded only button to **On** so that the screen only displays assay definitions that contain the loaded reagents. Select your assay.

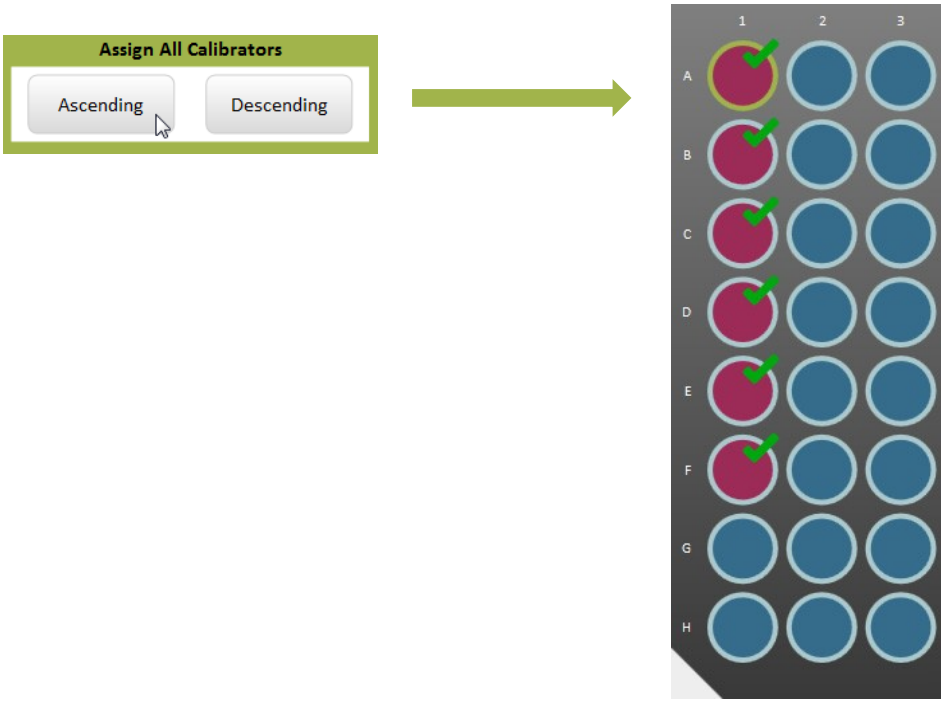


c Touch the highest or lowest calibrator > touch **OK**. The well that you selected turns red and a checkmark appears on it, signifying that an assay has been assigned to the well.



d Touch **Ascending** or **Descending** under Assign All Calibrators. The software assigns all calibrators that are defined for the assay in the specified order, beginning with the selected well. The plate diagram shows the assignments. To view the concentration that is assigned to a well, touch the well and view the Calibrator box.

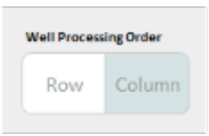
e Repeat to assign all sets of calibrator replicates.



5 Select the processing order for the plates. This is a batch-wide setting that applies to all jobs in the run, even if on separate plates.

- Select **Row** to process the calibrators from left to right
- Select **Column** to process the calibrators from top to bottom.

If you have multiple plates, they are processed in ascending numerical order from 1 to 4.

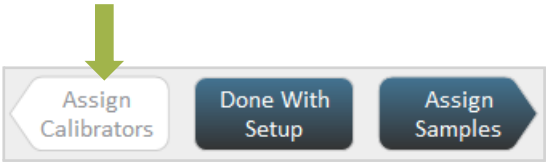


6 If the plates contain samples, assign them now. See “Assigning Samples in Plates” on page 121. If you are finished, see “Loading Calibrators/Samples into the Instrument” on page 127.

Assigning Calibrators in Tubes

1 Open the Assign Calibrators screen:

- If you are in the Assign Samples screen, touch **Assign Calibrators**.
- If you are not in the Assign Samples screen, touch the Setup Run tab > touch **Assign Calibrators**.

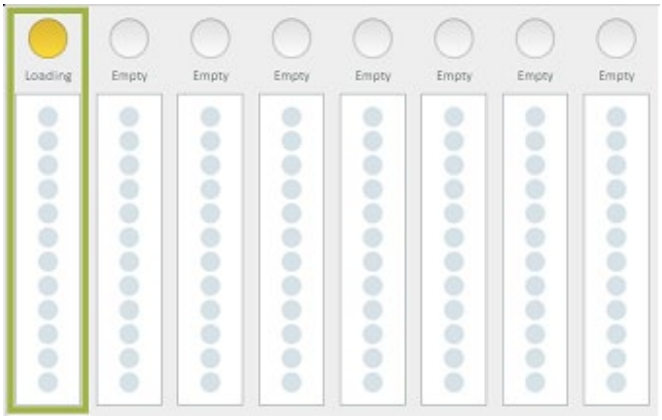


2 Recommended: Place tubes of prepared calibrators and samples in tube racks and put the racks into the sample bay, but do not fully insert them.

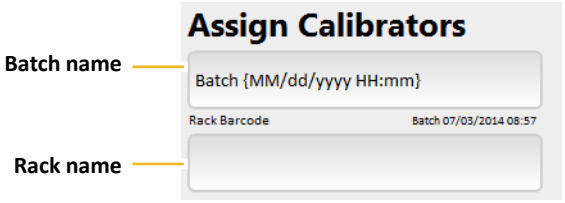
Note! If you choose to insert the tube racks after you finish assigning calibrators and samples, **you must make sure** that tubes containing the calibrators are loaded in the positions that you identify for them in the next step of this procedure. Placing the racks into the sample bay now makes it easier to correctly identify tube positions.

3 In the tube position diagram, touch the lane that contains the calibrators.

Sample bay lane 1 has been selected



4 Enter a batch name and a tube rack name > touch **Enter**. You can use any combination of letters and numbers.



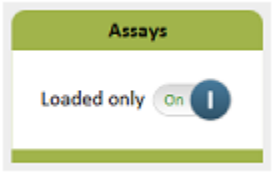
5 Perform the following steps to associate tubes of calibrators with the calibrator values in the assay definition, in ascending or descending order.

a In the tube rack diagram, touch the position that holds the first calibrator in the rack.

Tube 1 has been selected

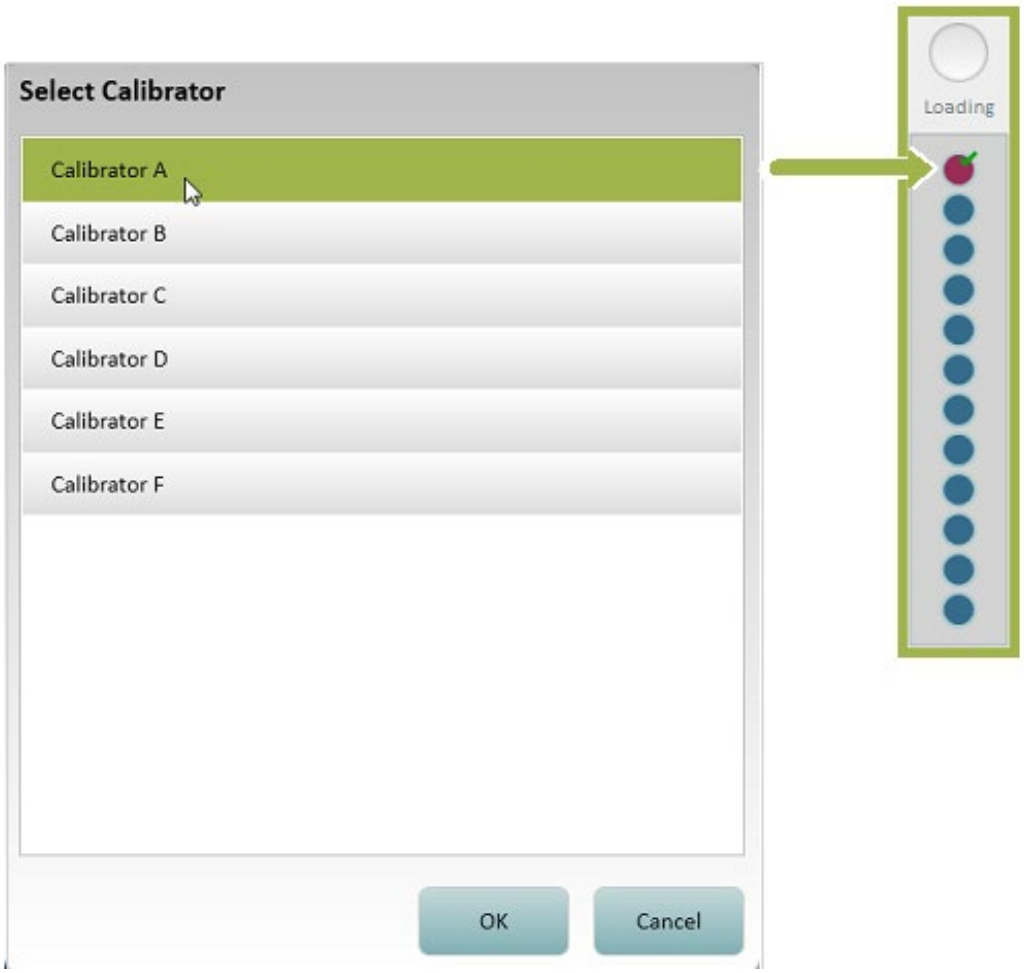
b Identify the assay:

- **If you have already loaded reagents:** Set the Assays Loaded only button to **On** so that the screen only displays assay definitions that contain the loaded reagents. Select your assay.



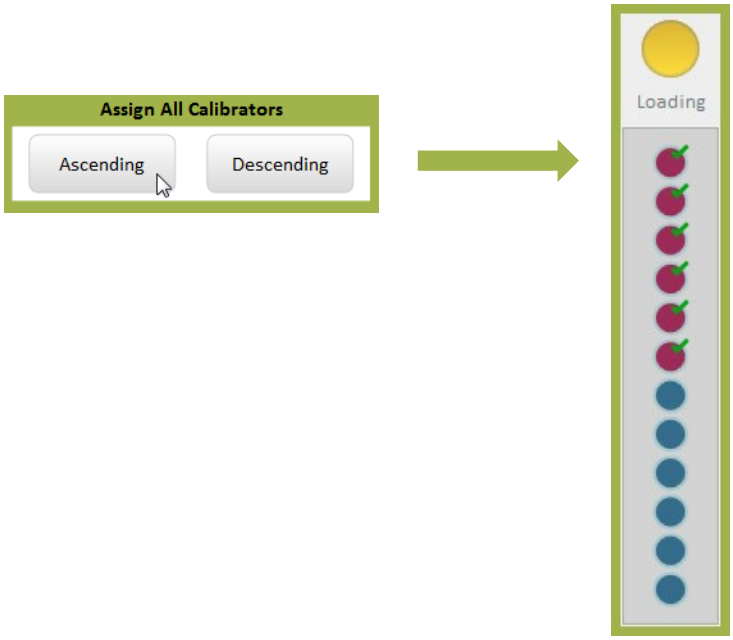
c In the Select Calibrator dialog, touch the position that contains the highest or lowest calibrator > touch **OK**.

In the rack position diagram, the tube position that you selected turns red and a checkmark appears on it, signifying that an assay has been assigned to the tube.



d Touch **Ascending** or **Descending** under Assign All Calibrators. The software assigns all calibrators that are defined for the assay in the specified order, beginning with the selected tube position. The tube position diagram shows the assignments.

To view a calibrator assignment, touch a tube in the tube rack diagram. The calibrator and concentration appear in the Calibrator box.



6 If you have loaded tubes of samples, assign them now. See “Assigning Samples in Tubes” on page 125. If you are finished, see “Loading Calibrators/Samples into the Instrument” on page 127.

Assigning Samples

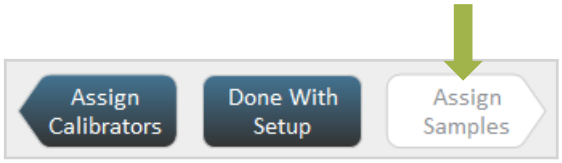
To assign samples, you identify the physical location of all samples in a plate or tube rack, and then you specify the number of replicates to run for each sample and dilutions (if the assay definition allows).

Note! You can also import a saved layout of sample (and calibrator) assignments. For information about this option, see "Importing and Exporting a Layout" on page 128.

Assigning Samples in Plates

1 Open the Assign Samples screen:

- If you are in the Assign Calibrators screen, touch **Assign Samples**.
- If you are not in the Assign Calibrators screen, touch the Setup Run tab > touch **Assign Samples**.



2 Recommended: Place prepared plates of calibrators and samples in plate racks and put the racks into the sample bay, but do not fully insert them.

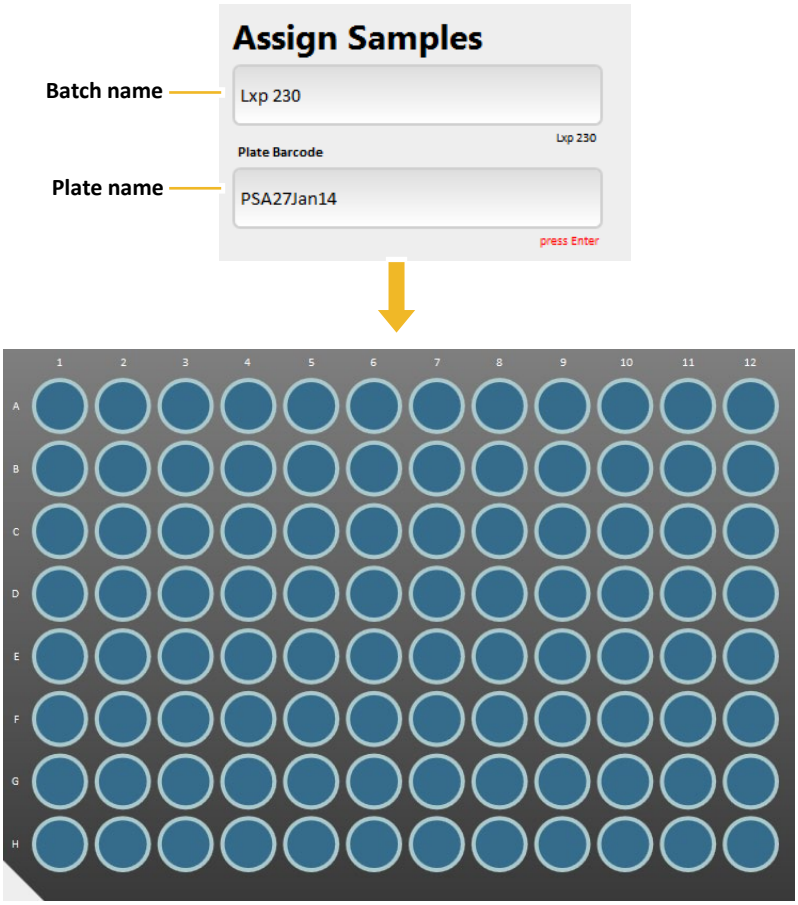
Note! If you choose to insert the plate racks after you finish assigning calibrators and samples, **you must make sure** that plates containing the samples are loaded in the positions that you identify for them in the next step of this procedure. Placing the racks into the sample bay now makes it easier to correctly identify plate positions.

3 In the plate position diagram, touch a plate that contains samples. (For information on reading the plate position diagram, see page 110.)

Plate 1 in plate rack 1 has been selected

a If a name appears in the Plate Barcode field, the plate is already named, and you can skip to step 4. This is true if you touch a plate that contains calibrators that you have previously assigned.

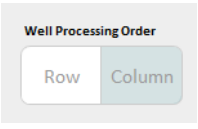
b If the Plate Barcode field is empty, enter a batch name and a plate name > touch **Enter**. You can use any combination of letters and numbers.



4 Select the processing order for the plates. This is a batch-wide setting that applies to all jobs in the run, even if on separate plates.

- Select **Row** to process the samples from left to right.
- Select **Column** to process the samples from top to bottom.

If you have multiple plates, they are processed in ascending numerical order from 1 to 4.



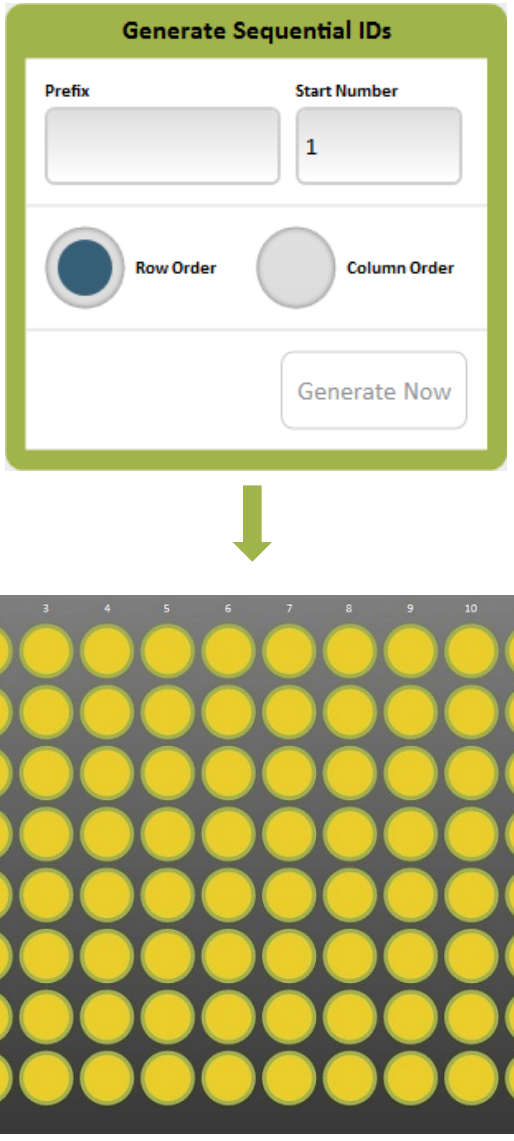
5 Assign an ID to the samples in the selected plate (see page 111 for information on autonames):

a Select a range of wells (for information on selecting wells, see page 110).

b In the Autoname tool, enter a prefix string, which will apply to every name, and a starting number.

c Touch **Row Order** or **Column Order**.

d Touch **Generate Now**. The software assigns a name to the selected wells and they turn yellow.

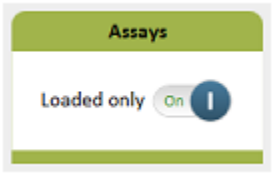


6 Specify an assay, replicates, and dilutions for samples:

a Select plate wells.

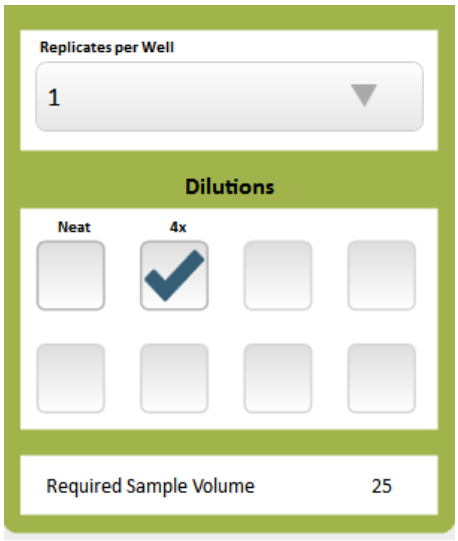
b Specify an assay (checkmarks appear on the selected wells after you do this):

- **If you have already loaded reagents:** Set the Assays Loaded only button to **On** so that the screen only displays assay definitions that contain the loaded reagents. Select your assay.



c Specify replicates and dilutions:

- Select a replicate number from the drop-down list.
- Touch the dilution that is specified in the assay kit instructions.
- Touch any other dilution that has a checkmark to remove the checkmark.



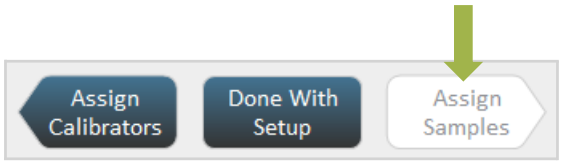
7 If you have more than one plate of samples, repeat steps 3 to 5 to assign all the samples.

8 If you need to assign calibrators, see “Assigning Calibrators in Plates” on page 113. If you are finished, see “Loading Calibrators/Samples into the Instrument” on page 127.

Assigning Samples in Tubes

1 Open the Assign Samples screen:

- If you are in the Assign Calibrators screen, touch **Assign Samples**.
- If you are not in the Assign Calibrators screen, touch the Setup Run tab > touch **Assign Samples**.

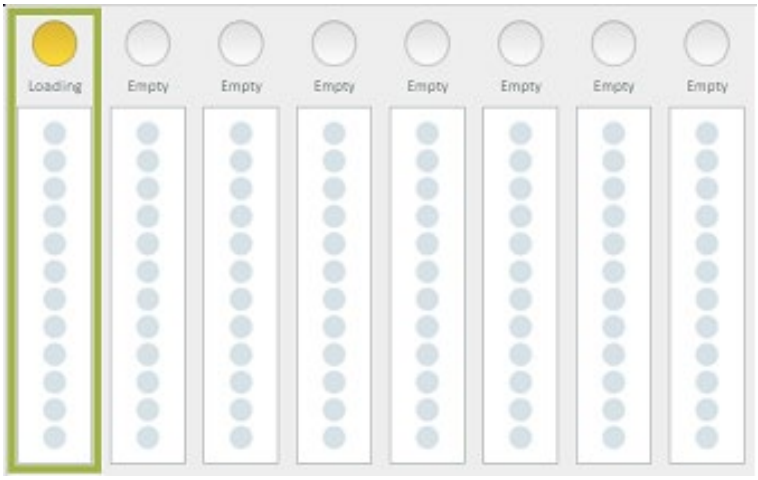


2 Recommended: Place prepared tubes of calibrators and samples in tube racks and put the racks into the sample bay, but do not fully insert them.

Note! If you choose to insert the tube racks after you finish assigning calibrators and samples, **you must make sure** that tubes containing the samples are loaded in the positions that you identify for them in the next step of this procedure. Placing the racks into the sample bay now makes it easier to correctly identify tube positions.

3 Touch a lane that holds a rack containing sample tubes.

Sample bay lane 1 has been selected



4 Assign an ID to the samples in the selected rack (see page 111 for information on autonaming):

a In the tube rack diagram, select a tube position.

Tube 1 has been selected

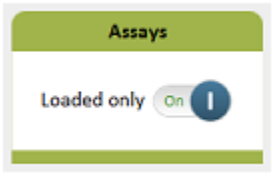
b Enter a Barcode ID for the tube. This step is not necessary if you already assigned an ID to the samples, as specified in Step 4 above.

c Physically load the rack. See “Loading Calibrators/Samples into the Instrument” on page 127.

d Repeat these steps to name all tubes in the rack.

5 Select an assay for the selected tube:

- **If you have already loaded reagents:** Set the Assays Loaded only button to **On** so that the screen only displays assay definitions that contain the loaded reagents. Select your assay.

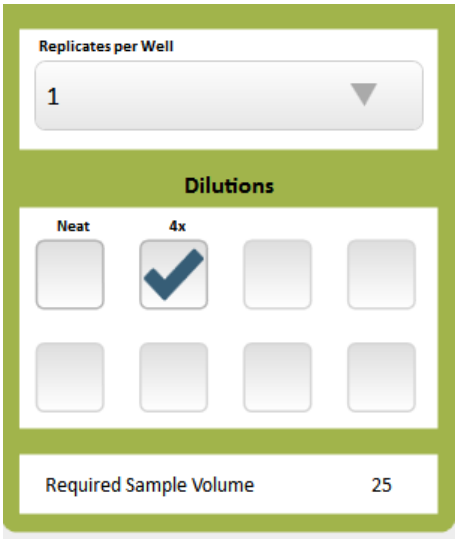


6 Specify replicates and dilutions for the selected tube:

a Select a replicate number from the drop-down list.

b Touch the dilution that is specified in the assay kit instructions.

c Touch any other dilution that has a checkmark to remove the checkmark.



7 Repeat steps 3 to 6 to assign all tubes of samples.

8 If you need to assign calibrators, assign them now. See “Assigning Calibrators in Tubes” on page 117. If you are finished, see “Loading Calibrators/Samples into the Instrument” on page 127.

Loading Calibrators/Samples into the Instrument

The final step in assigning calibrators and samples is to load them into the instrument so that the software accepts the assignments.

❖ To load assigned calibrators and samples

1 Optional: Save the layout. In the Assigned Samples screen, touch **Export Layout** > save the file in the Save As dialog.

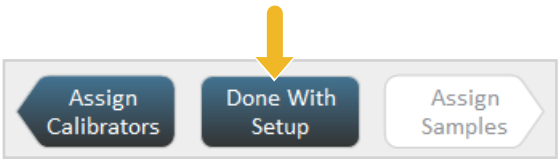
2 If you have not already done so, place the plate racks or tube racks into the sample bay.

Note! Place the racks in the sample bay according to the assignments that you have made. If you do not put plates or tubes in the positions that you identified for them, the instrument will not be able to locate the calibrators or samples correctly, and your assay results will be undefined.

3 Push the plate racks or tube racks into the sample bay until they latch into place.

4 Recommended: Touch **List View** to see a list of everything you have loaded. This allows you to check for the correct number of replicates, dilutions, and other consumables.

5 Touch **Done With Setup**. The All Resources screen in the System Resources tab opens. See “Starting a Run” on page 128 to initiate the run.



Importing and Exporting a Layout

You can export a finished layout of calibrator and sample assignments to a file and then re-use it the next time you run the assay.

❖ To export a layout

In the Assign Samples screen, touch **Export Layout** > name and save the file. Do not change the specified file extension (.csv).

❖ To import a layout

In the Assign Calibrators screen, touch **Import Layout** > open the file.

Starting a Run

You can begin a run immediately after you assign calibrators and samples if sufficient system resources are available and no mandatory maintenance tasks are pending.

When you finish assigning calibrators and samples, the software calculates the system resources needed to run the assay and posts resource status on the System Resources screen. If enough consumables are available to run the loaded calibrators and samples, the supplies of wash buffer are sufficient, the liquid waste container is not full, and no maintenance tasks are pending, you can start the run.

To start a run

1. Touch **Done With Setup** in the Assign Calibrators or Assign Samples screen. The System Resources tab opens.
2. If the button is active, touch **Start Run**.

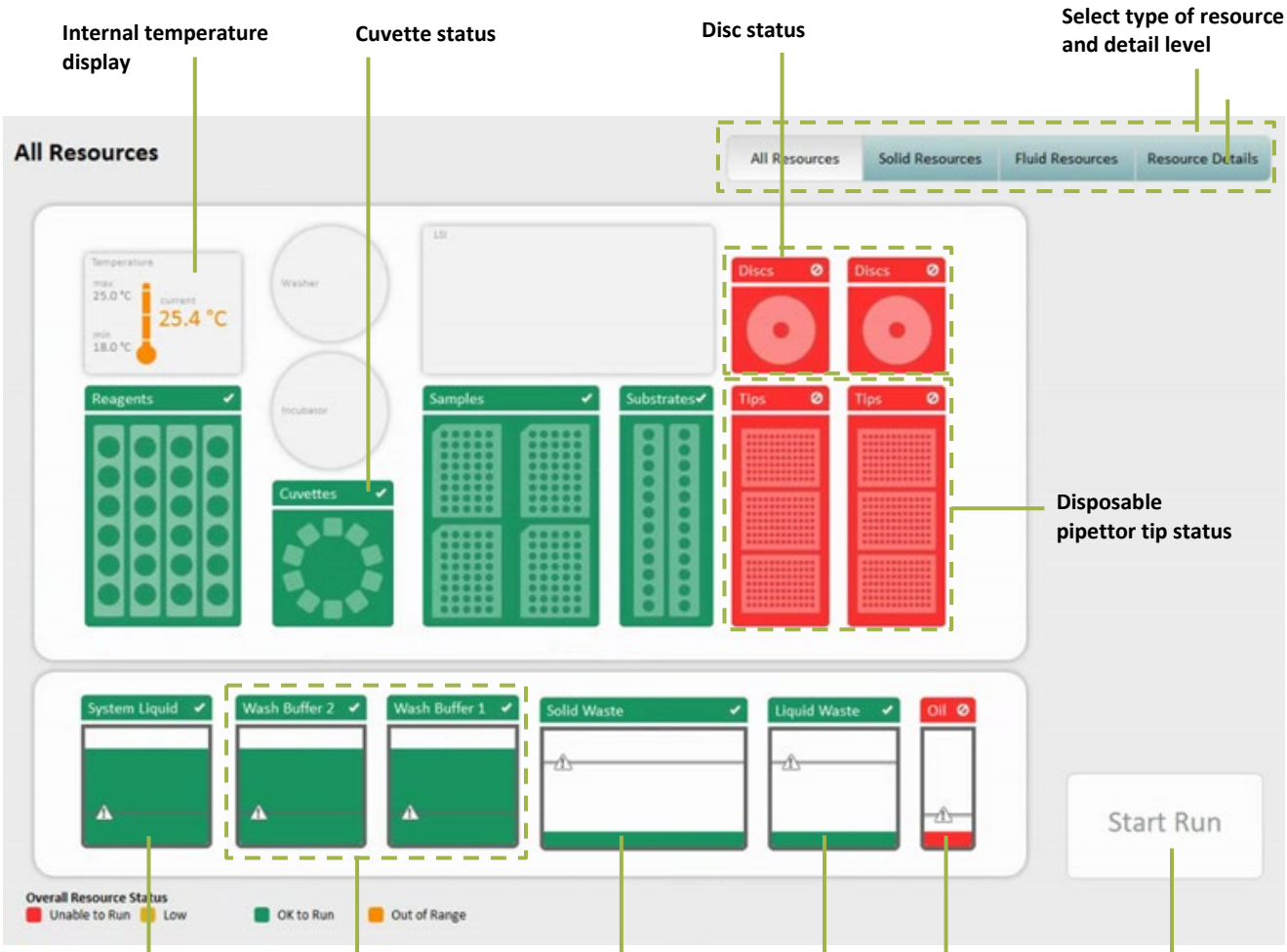
If the button is inactive, touch **Resource Details** to display a complete readiness status (including status of beads, detectors, SBG, and RGP). You may need to perform some of the following tasks to activate the **Start Run** button again:

- If the Maintenance task icon is red, touch the Maintenance icon and perform any pending mandatory tasks. See Chapter 11, “Maintaining the Simoa HD-X Analyzer,” on page 151.
- If the cuvettes, tips, or discs diagrams are red, add more of those resources. See “Loading Instrument Consumables” on page 36.
- If the system liquid diagram or either wash buffer diagrams are red, refill the container. See “Refilling the System Fluid Containers” on page 46.
- If the liquid waste diagram is red, empty the liquid waste container. See “Emptying the Liquid Waste Containers” on page 53.
- If the solid waste diagram is red, empty the solid waste container. See “Emptying the Solid Waste Container” on page 51.
- If the sealing oil diagram is red, replace the sealing oil bag. See “Changing the Sealing Oil Bag” on page 49.

Understanding the System Resources Screen

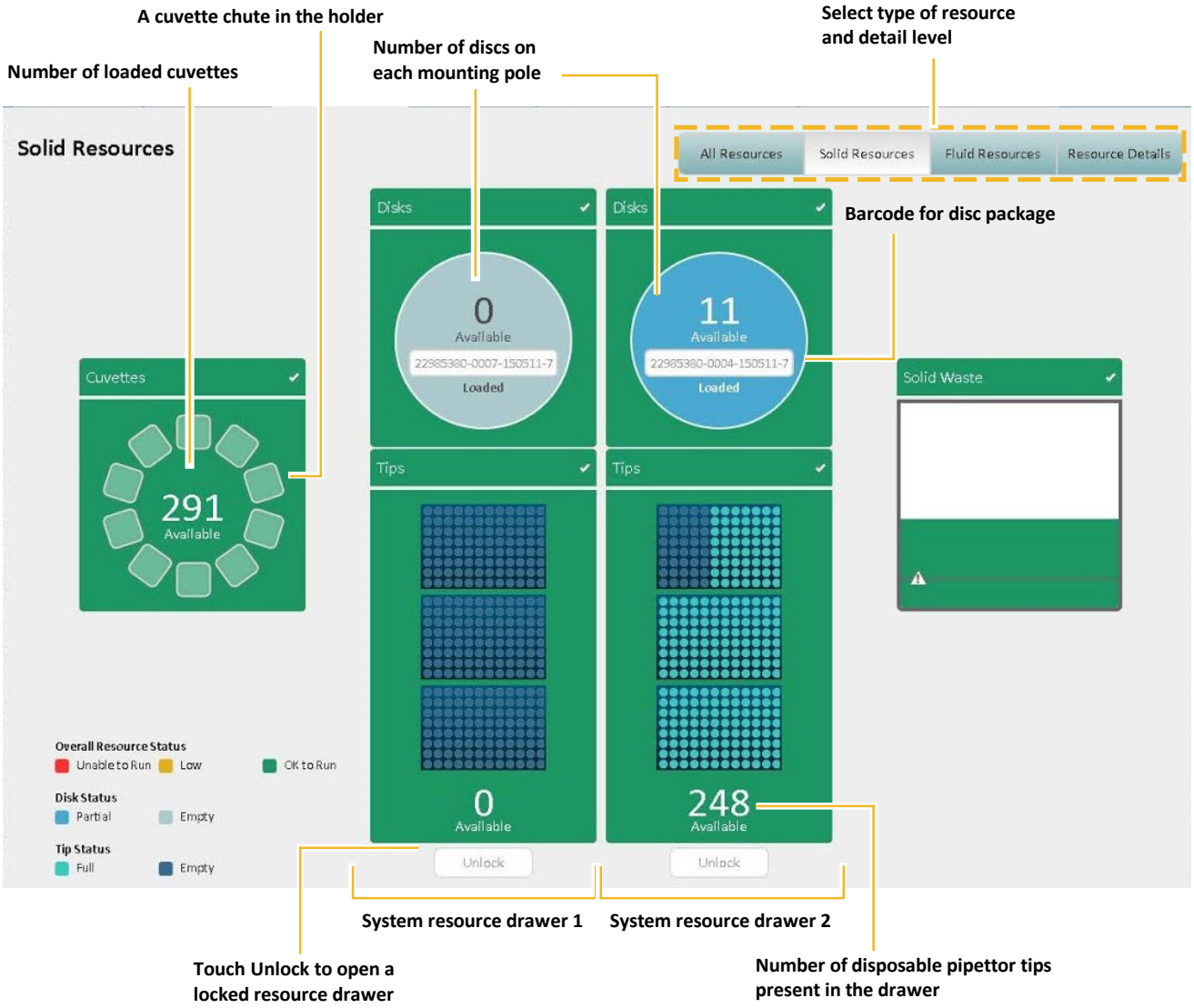
Diagram color indicates status:

- **Green:** Adequate amounts
- **Yellow:** Low amounts
- **Red:** Empty, must be refilled, or container is full and must be emptied.



Understanding the Solid Resources Screen

Touch the System Resources tab > touch the cuvette diagram or one of the disposable tip or disc diagrams. The Solid Resources screen opens.



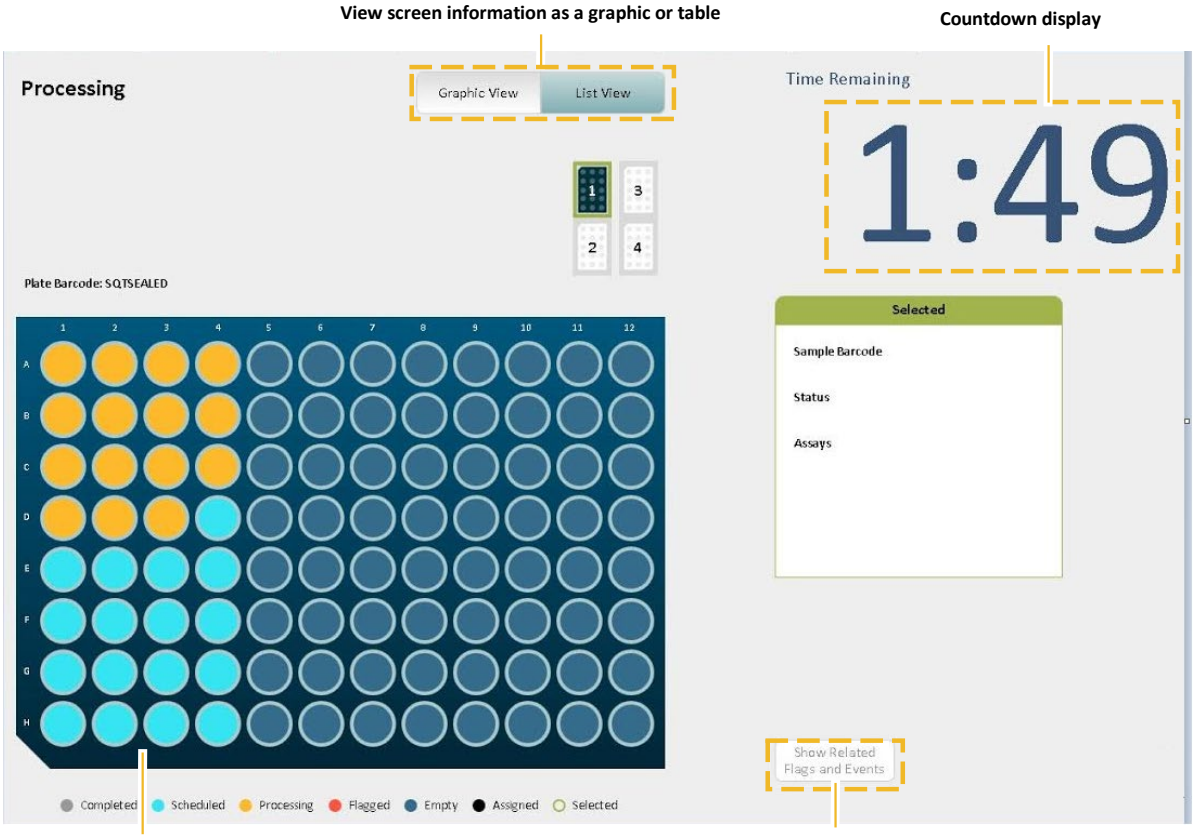
Monitoring a Run

Touch the Current Run tab to observe the status of sample processing during the run. Each loaded plate or tube is graphically represented on this tab. As samples are processed, they change color. See the color key at the bottom of the screen for an explanation of the meaning of each color.

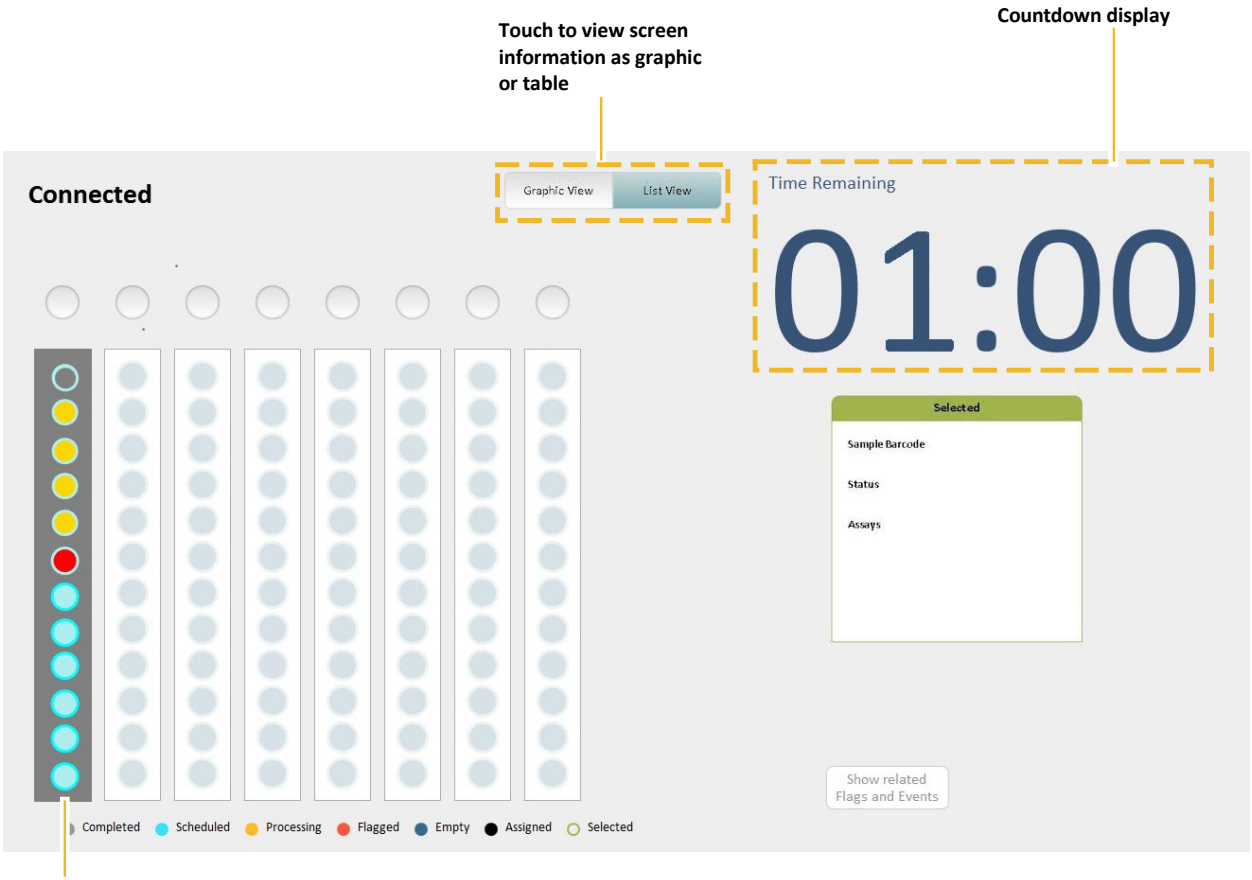
Sample In-Process Run screens for plates and tubes appear below.

CAUTION! Do not attempt to open the system resource drawers; remove or insert a reagent rack, a sample rack, or an RGP rack; or change the software settings once the run has begun. Doing any of these things may cause the instrument to halt the run, with subsequent loss of results. You can refill system liquids and empty one liquid waste container at a time during the run.

Understanding the In-Process Run Screen for Plates



Understanding the In-Process Run Screen for Tubes



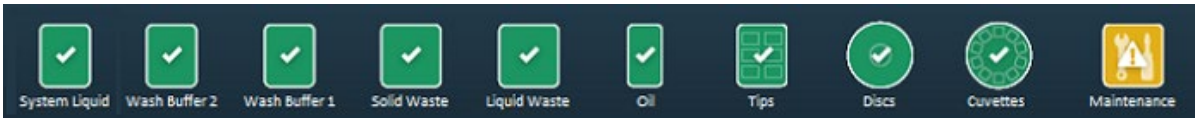
Checking Instrument Status

During the run, periodically check the instrument status indicators at the bottom of the software to determine whether you need to refill any system liquids or empty any system waste containers. The icons for system fluids, consumables, and instrument waste containers change color when any of these components need attention:

- **Green:** Adequate amounts
- **Yellow:** Low amounts
- **Red:** Empty, must be refilled, or container is full and must be emptied

For information about refilling system fluid containers or emptying system waste containers, see Chapter 11, “Maintaining the Simoa HD-X Analyzer,” on page 151.

When the run finishes, the countdown display says 00:00 and the instrument state notice changes to Ready.



Viewing the Event Log

Samples that cannot be processed for some reason are flagged red in the Current Run tab. To determine the reason for the flag, you can view the Event Log.

❖ To view the Event Log

Touch the History & Reports tab > touch **Event Log**. For more information, see Understanding the Event Log Screen on page 146.

9 Analyzing Run Data

Data Analysis Overview

When the assay run is complete, results appear on the Run History screen. You can perform data analysis on all of the results or a subset.

Results can be exported to an external analysis package or a LIMS system, or you can view and analyze results in the instrument's software. The following options are available:

- Export a CSV file for external analysis
- Export a PDF or Excel file for external analysis
- Analyze the results in the instrument's Data Reduction screen

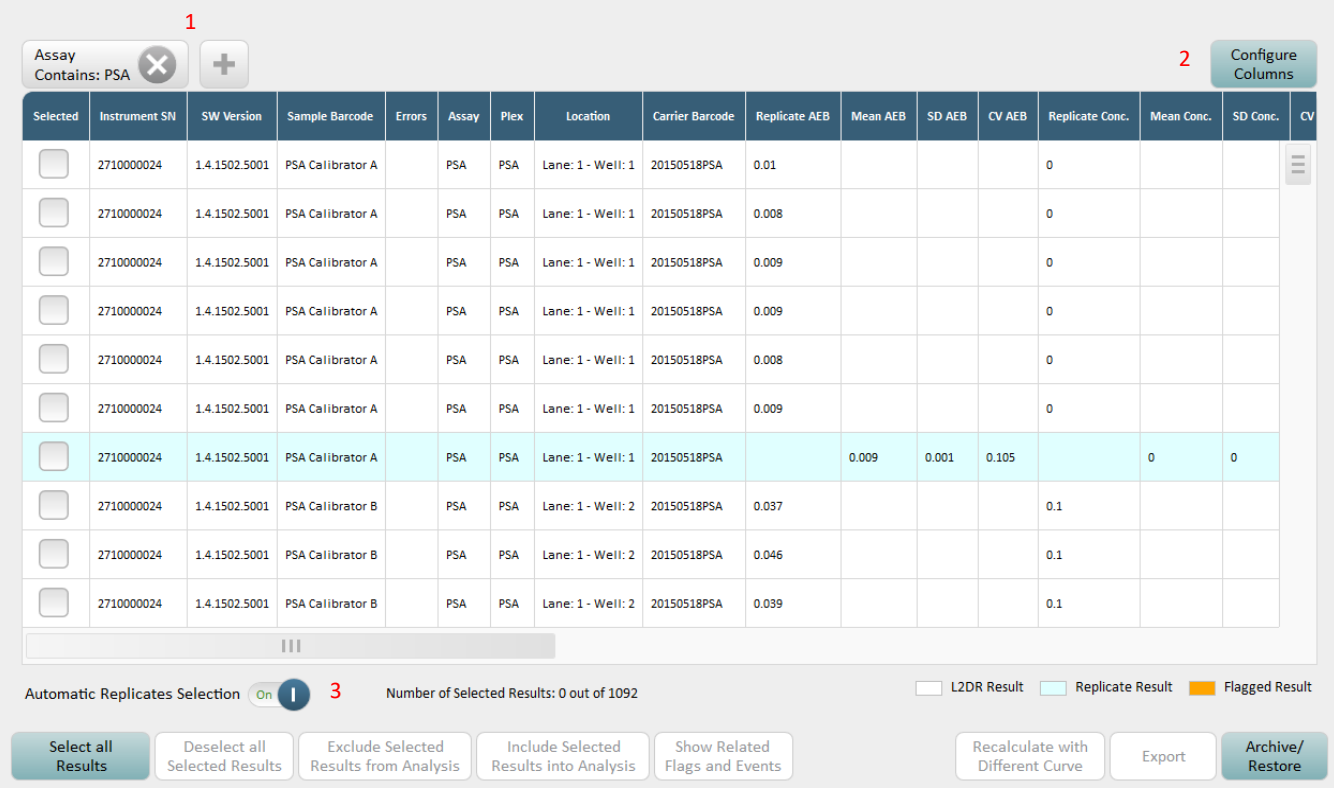
See *Simoa® HD-X Data Analysis Guide* for complete instructions on how to analyze data when your assay run is complete.

Log in to the Quanterix® portal and view a data analysis training video, *Training – Modifying Calibration and Curves and Recalculating Samples*, on the customer portal at http://portal.quanterix.com.

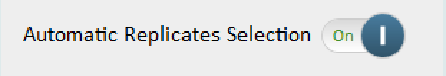
Working with Run History

The Run History screen provides options that allow you to select the data and columns to include in the analysis, export the data to a CSV file, and move analysis data between multiple instruments.

The Run History view calculates replicate statistics only for samples (including calibrators) that come from the same well or tube (for each group of replicates that originate from the same location).



The following table explains how to use the Run History screen.

Element	Definition
1 Add New Filter 	Adds filter criteria that determine what results appear in the table. Multiple filters are allowed. Touch the Add New Filter (+) button to add a filter. Touch the X on the filter button to remove the filter from the table. Loading a large amount of data into the results table can decrease responsiveness. To avoid this, add a new filter before removing unwanted ones.
2 Configure Columns	Opens the Configure Columns window that allows you to configure column display and ordering.
3 Automatic Replicates Selection	When enabled, the software automatically selects all related replicates when one of the replicate results is selected. 

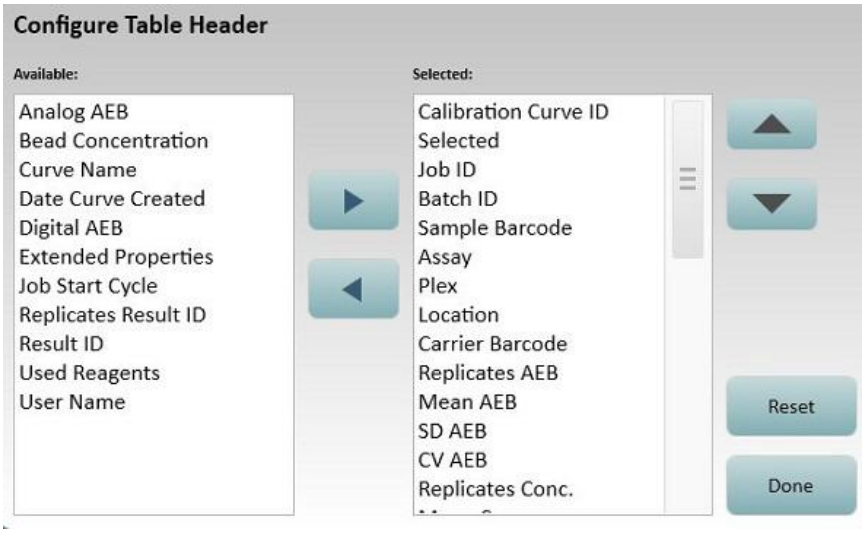
Element	Definition
4 Select all Results	Selects all currently displayed results, as determined by data filters, if any are in place.
5 Deselect all Selected Results	Clears all selections made on results in the table.
6 Exclude Selected Results from Analysis	Marks selected results for exclusion in analysis. Only results that have been automatically included in analysis can be excluded manually. Isingle is not recalculated when excluding results in the Run History table. **Note**: Ensure the Automatic Replicates Selection button is turned off > select the replicates to be excluded from analysis, then touch **Exclude Selected Results from Analysis** button for result.
7 Include Selected Results Into Analysis	Marks selected results for inclusion in analysis. Only results that have been excluded automatically from analysis can be included manually.
8 Show Related Flags and Events	Displays all flags and event messages for selected results.
9 Recalculate with Different Curve	Allows you to recalculate concentration results by manipulating the default calibration curve or to select a new calibration curve.
10 Export	Exports displayed results to a comma separated value (CSV) file. All columns displayed in the table are exported. If you have not selected a column with Configure Columns, it is not exported. Additionally, if you applied data filters to the table, only results matching the filter criteria are exported.
11 Archive/Restore	Provides ability to move result data between two computers running the Simoa HD-X software. Results archived (exported) to an XML file can be restored (imported) into the software running on another computer.

Exporting Results in CSV Format

Export a CSV file from the Run History screen to create a file for external analysis. To export data for troubleshooting analysis, include the Extended Properties column.

To export results in CSV format

1. In the History & Reports tab, touch **Run History**.
2. Clear all filters on the results table.
3. Touch the **Add New Filter** button > select a filter from the drop-down list > touch **Next.**
4. Specify the filter criteria. Some filters provide drop-down lists and others provide a text entry field that sets the filter.
5. Touch **OK**. The results are filtered and the filter name appears above the results table.
6. Touch **Configure Columns**.
7. Move columns between the Available and Selected lists and change their order with the up and down arrow key buttons. The columns you select identify the data that is exported See *Appendix A – Configure Columns Definitions* for more information about each column.
8. Touch **Done**.



9. Touch **Select All**.
10. Touch **Export** > specify a file name > touch **Save**.

Exporting Results in PDF or Microsoft Excel Format

Export a file in PDF or Microsoft Excel format from the Reports window to create a file for external analysis.

To export results in PDF or Microsoft Excel format

1. In the History & Reports tab, touch **Reports**.
2. Touch a report in the displayed list.
3. Select individual reports or touch **Select All** for all reports.
4. Touch **Preview Report** > touch **Export**.
5. Save the data in PDF or Excel format.

Analyzing Results in the Data Reduction Tab

The instrument plots a Calibration Curve representing the selected results on the Data Reduction tab.

Curves are batch-based. All the samples in the batch have the same calibration curve applied to them, regardless of processing order.

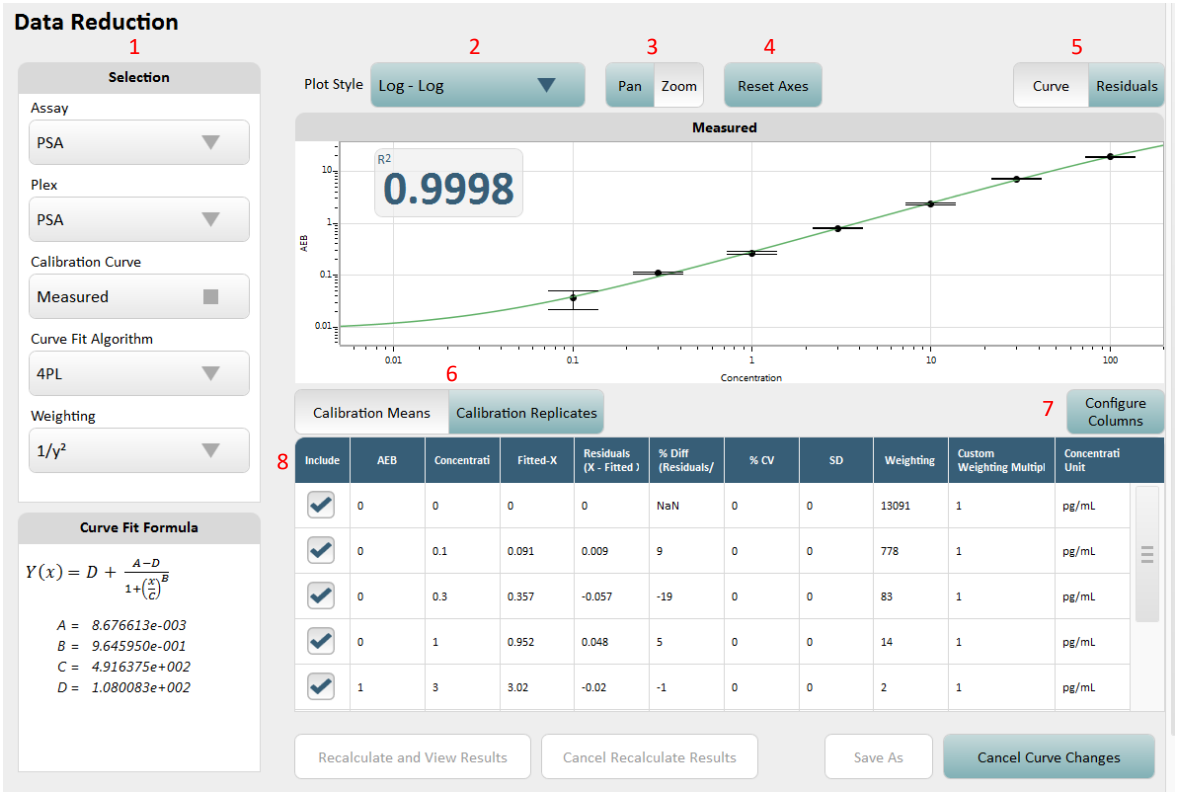
The calibration data table contains all calibration data points used in curve fitting. The table includes aggregated replicates data. If calibrators were run as replicates, their averages are used.

Curves are specific to the assay and the plex. All samples of the same assay, plex, and batch will initially have the same calibration curve, but they can be assigned to a different curve, if calibration data points are included in the batch or Curve Selection Strategy is set to None. Following is a sample Data Reduction tab and a table that explains its components.

To review results in the Data Reduction tab

1. In the Data Reduction tab, select the relevant fields in Assay, Plex, Calibration Curve, Curve Fit Algorithm, and Weighting.
2. Use the tools described in the following table to analyze the data.

The following screen shows a calibration curve calculated with the default settings. Some default settings, such as fitting algorithm, come from plex definition.



The following table explains how to use the Data Reduction tab.

Element	Definition
1 Selection	Controls which calibration curve is displayed and the fitting algorithm and weighting used to generate the curve. Below are the recommended minimum number of data points needed for the various curve formulas. **Points Needed** — **Curve Formula** 2 — Piecewise Linear, Linear, Exponential, Log-Log 3 — Quadratic 4 — Cubic, 4PL 5 — 5PL

Element	Definition
2 Plot Style	Controls how the data is plotted.
3 Pan/Zoom	Pan across the graph or zoom in on a selected area for more detail.
4 Reset Axes	Use this to reset the graph axes to their original values.
5 Curve/Residuals	Use the Curve and Residuals buttons to toggle between the calibration curve and fitting errors.
6 Calibration Means/ Calibration Replicates	**Calibration Means** shows the replicates' summarized information in the main table. **Calibration Replicates** shows individual replicate concentrations.
7 Configure Columns	Select the columns to include in the calibration data table.
8 Include	To exclude individual replicate results from analysis, uncheck the **Include** checkbox in the row you want to exclude. For detailed instructions, see *Simoa HD-X Data Analysis Guide*.
9 Recalculate and View Results	After selecting a different calibration curve, recalculates results and displays them in the Run History table.
10 Cancel Recalculate Results	Cancel recalculation and return to the History and Reports tab.
11 Save As	Saves the calibration curve. When you modify an existing curve, you must save it before it can be used to recalculate the results.
12 Cancel Curve Changes	Resets any changes made to the existing calibration curve.

10 Using Reports, Run History, and the Event Log

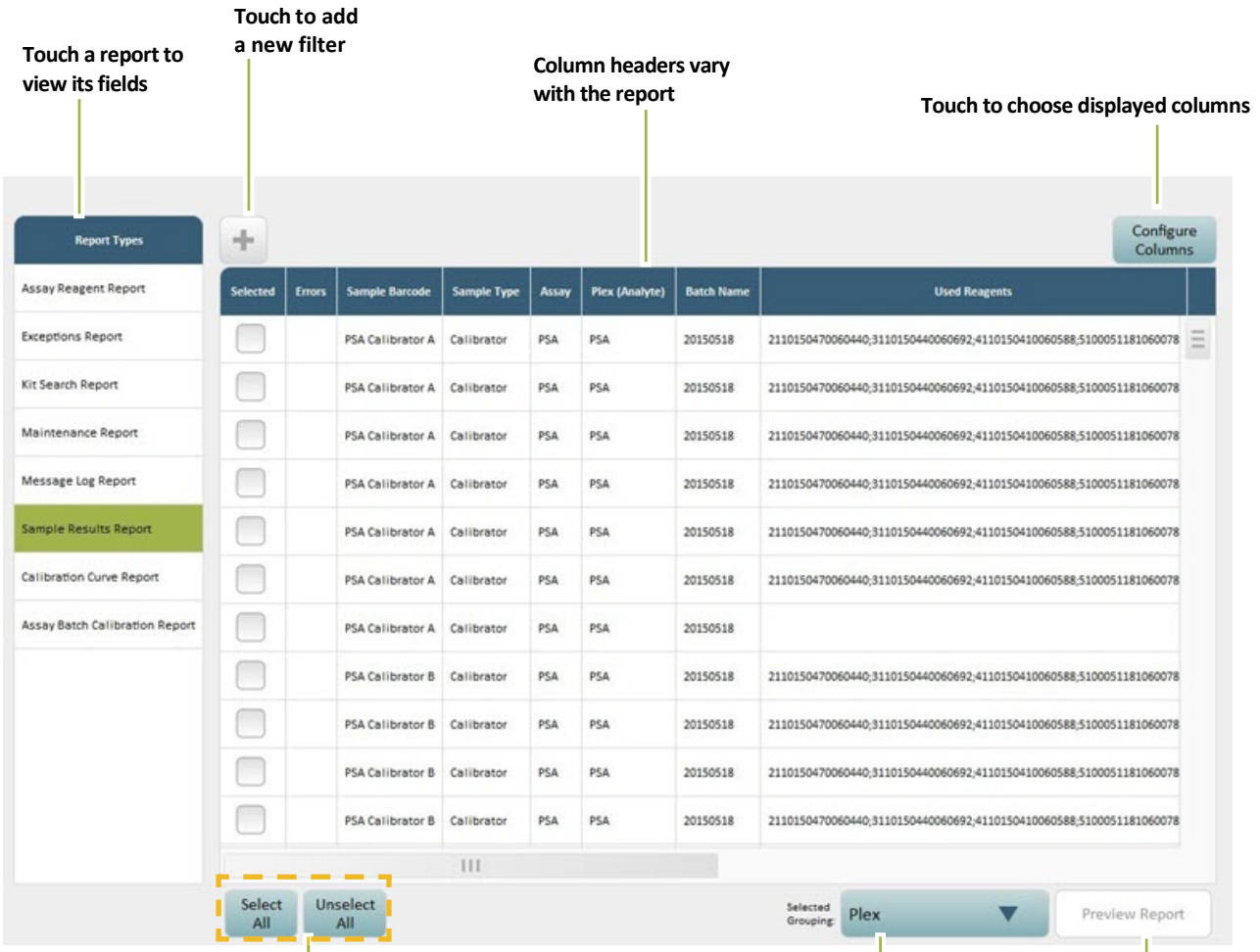
The History and Reports Tab

The History and Reports tab provides access to information about your assay runs from the following screens:

- Reports
- Run History
- Event Log

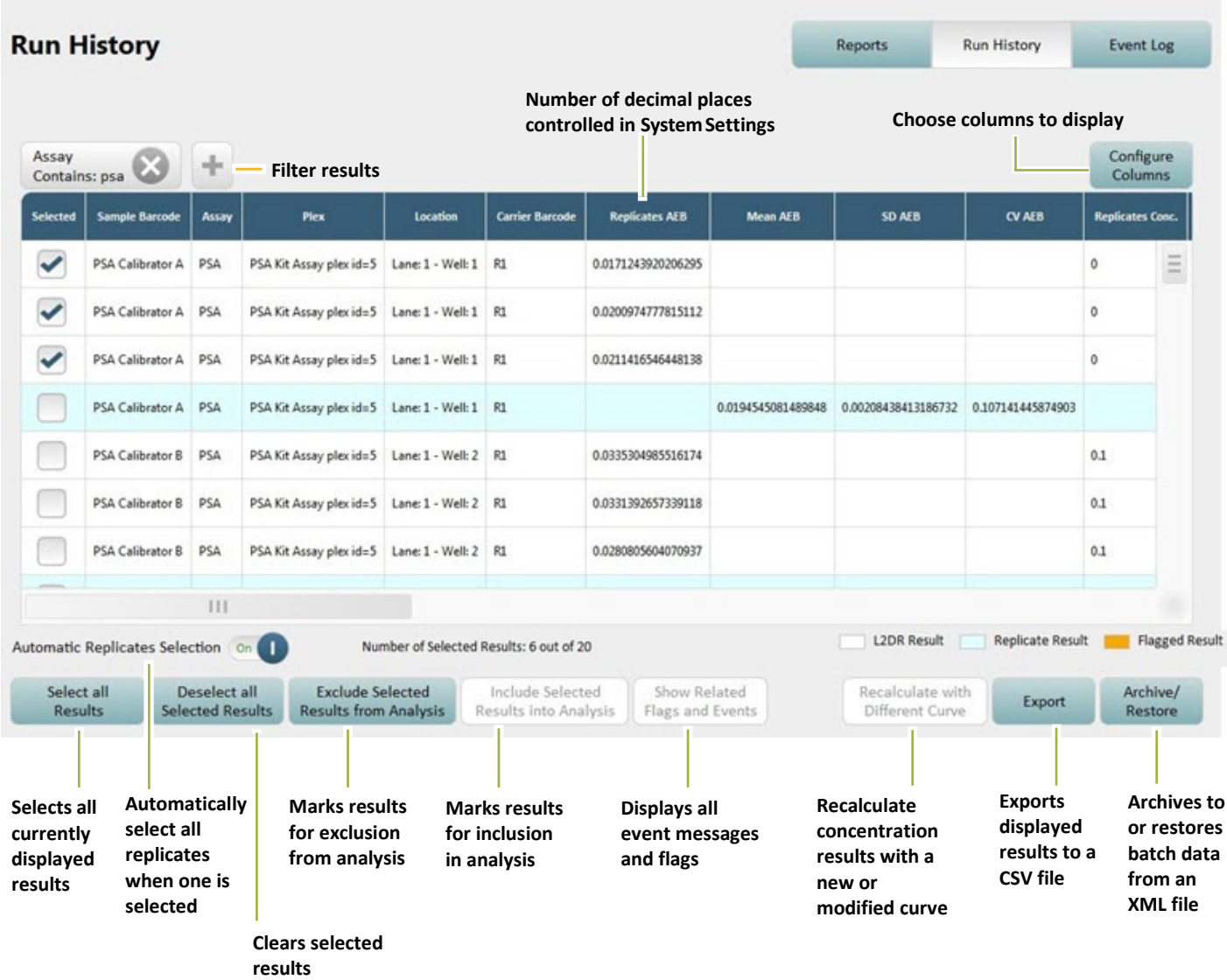
Understanding the Reports Screen

A report is a PDF file of run data that you can print or export to a computer. See “Creating a Report” on page 149 for an explanation of each report.



Understanding the Run History Screen

This tab is most frequently used to export the raw data for archival use or inclusion in the database. The Run History view calculates replicate statistics only for samples (including calibrators) that come from the same well or tube (for each group of replicates that originate from the same location).

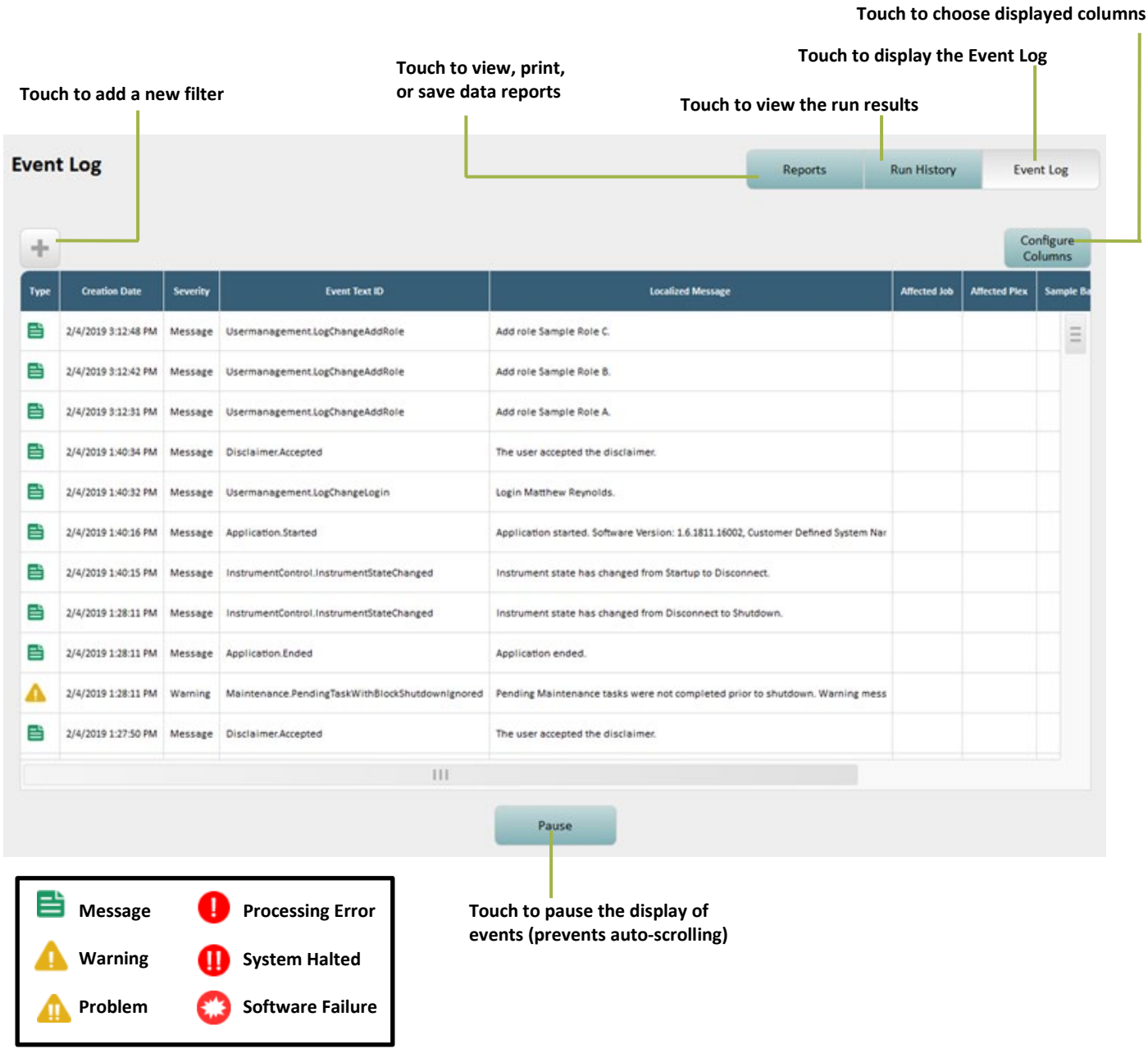


Understanding the Event Log Screen

The Event Log tracks significant events that occur during software operation. Event log information is helpful for troubleshooting problems.

Icons in the Type column specify the severity of the message. These icons also appear at the bottom of every tab in the software. A number in the center of these icons signifies how many messages of that type are listed in the log.

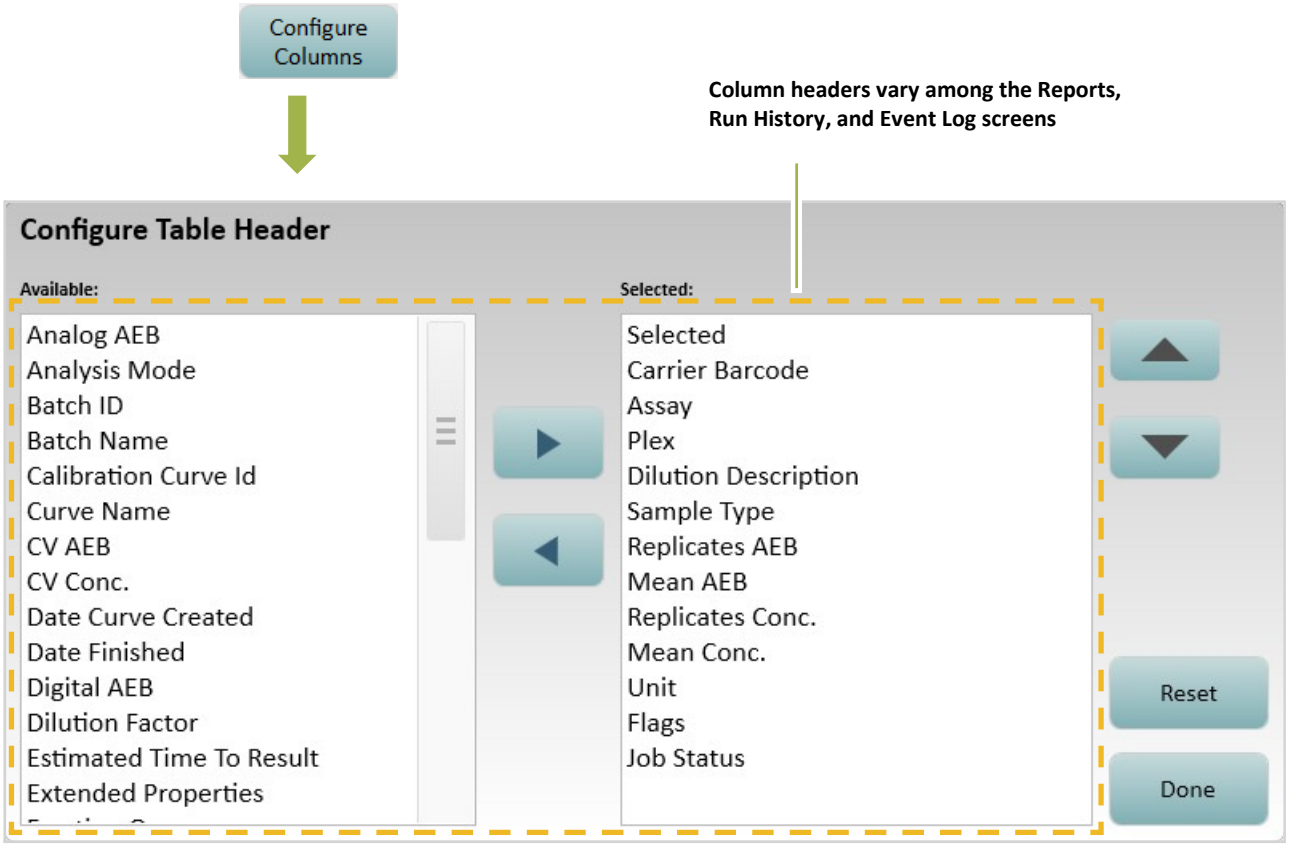
To view the Event Log, Touch the History & Reports tab > touch **Event Log**.



Customizing the Table View in the History & Reports Tab

Changing Columns

To change the columns for any table in the History & Report tab, touch **Configure Columns** > touch the arrows to move column names between the Available and the Selected boxes > touch **Done**. See Quanterix® document *Simoa® HD-X Data Analysis Guide* for an explanation of each column.



Changing Column Order

To move a column in any table in the History & Reports tab, use the up and down arrow keys, or touch the column header and drag it to the new position. In table view, you can also reorder the columns by dragging.

Sorting Rows

To sort any table in the History & Reports tab in ascending or descending alphabetical order for a specific column, touch the column header.

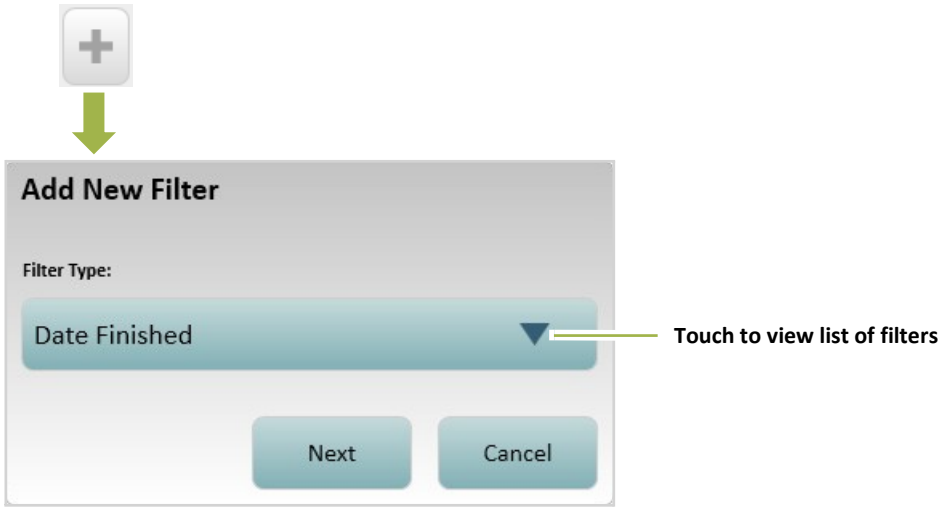
Adding a Filter

A filter modifies the contents of the Run History, the Event Log, or a report to show only data that matches the filter requirements. For example, you can filter the Event Log to show only warning messages.

You can add multiple filters to any table. Available filters vary with the table type.

❖ To add a filter

1. Touch the **Add New Filter** button > select a filter type from the drop-down list > touch **Next**.
2. Specify the filter criteria. Some filters provide drop-down lists for this, and others provide a text entry field in which you enter terms that set the filter.
3. Touch **OK**. The filter appears above the table.



❖ To remove a filter

Touch the large **X** in the filter.

Creating a Report

A report is a PDF file of run data that you can export to a computer or print. You can create the following types of reports:

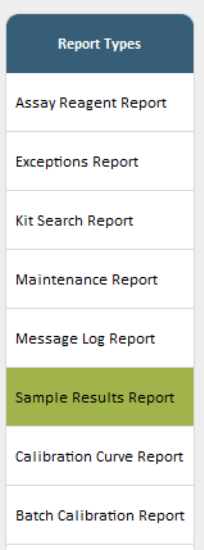
Assay Reagent Report	Reagents used in selected assay runs
Exceptions Report	List of exception (error) messages occurring during the selected runs
Kit Search Report	List of samples associated with a specific kit reagent lot
Maintenance Report	List of completed instrument maintenance tasks
Message Log Report	List of all messages generated by the software during a specified selected time interval
Sample Results Report	Results for selected assay runs
Calibration Curve Report	Calibration curve information for selected run (fitting algorithm, weighting factor, style, etc.)
Batch Calibration Report	Includes calibration curves for all assays and all samples processed for a selected batch. Organized by assay plexes.

To customize reports, change the report table (see “Customizing the Table View in the History & Reports Tab” on page 147.)

❖ To create a report

1 Touch the History & Reports tab > touch **Reports**.

2 Touch a report type.



Exporting or Printing a Report

You can preview a report, export it to a file, or print it.

❖ To export or print a report

1 Touch **Preview Report** to view the formatted report. After a few moments, the report appears.

2 Touch **Export** or **Print**.

- **Export:** Opens a Save As window. Enter a filename > select a file type > navigate to a storage location > touch **Save**. You can export a report as a PDF (.pdf) or an Excel file (.xls).
- **Print:** Sends the report to the printer that is connected to the instrument computer.

3 Touch **Done**.

Customizing the Logo for a Report

See “Managing Report Settings” on page 180.

11 Maintaining the Simoa® HD-X Analyzer

Performing Maintenance Tasks

Perform instrument maintenance tasks according to the following schedule table. These tasks are listed in the Maintenance tab.

In addition to the tasks listed below, delete all old unused assay definitions (on a monthly schedule) to improve instrument performance. This is especially important if you have multiple users who create numerous Homebrew assays.

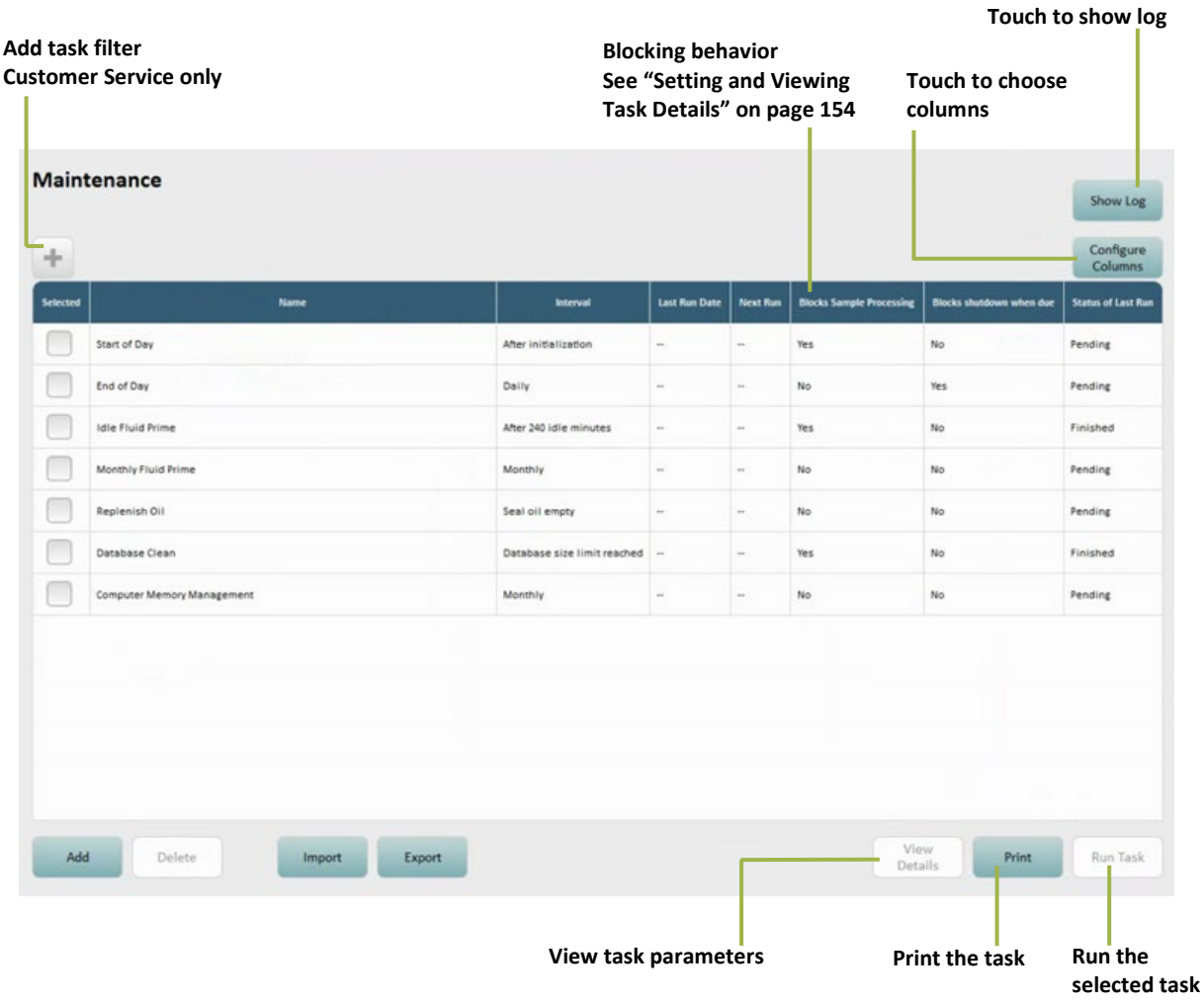
Note! Check the Quanterix® customer portal at http://portal.quanterix.com/ for updates to monthly maintenance procedures.

Task	Default Interval	Description
Start of Day	After initialization	Prepares instrument systems to start a run. See “Performing the Start of Day Task” on page 156.
End of Day	Daily after the last run of the day	Cleans the system at the end of the day.
Idle Fluid Prime	After 240 idle minutes	Primes the system fluids and resets the idle time counter.
Monthly Fluid Prime	Monthly	Primes the system fluids three times the normal length, approximately 30 minutes.
Replenish Oil	When the sealing oil is empty	Primes sealing oil through the entire line.
Database Clean	Database size limit reached, at least weekly	Cleans up the database. See “Performing the Database Clean Task” on page 159.
Computer Memory Management	Monthly	Removes temporary files and performs disk utility that improves data access speeds.

Note! The quality of system fluids may degrade over time due to limited shelf life, cross-contamination, salt buildup, etc. You must perform the monthly maintenance procedures described in this chapter to maintain optimal performance of the instrument. As a check, you can run the Simoa Qualification Test (SQT) to measure the precision of the system upon completion of the maintenance tasks.

Understanding the Maintenance Screen

The Maintenance screen shows status information about each maintenance task.



Determining Whether a Maintenance Task Was Performed

The Maintenance screen shows status information about each maintenance task. Two methods are available to do this:

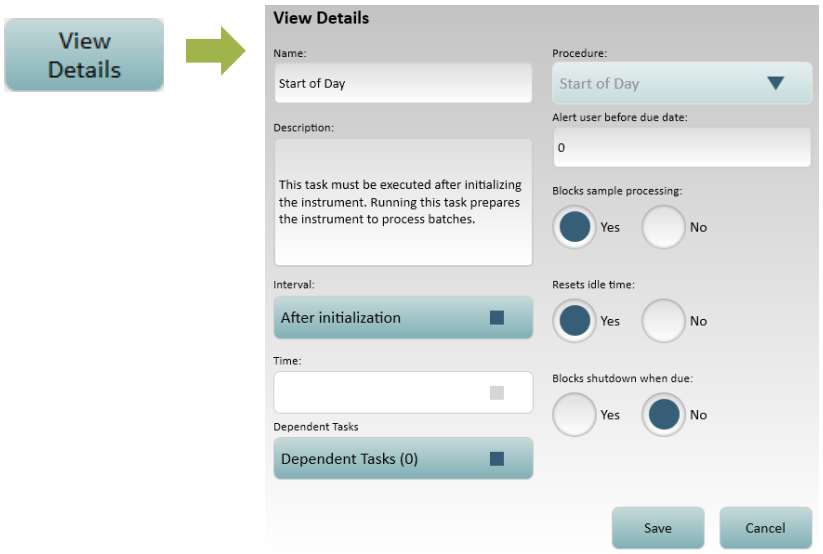
- Check the Last Run column in the Maintenance tab, which reports the date and time when a task was last performed.
- In the Maintenance tab, touch **Show Log** to view a log that lists all completed maintenance tasks.

Setting and Viewing Task Details

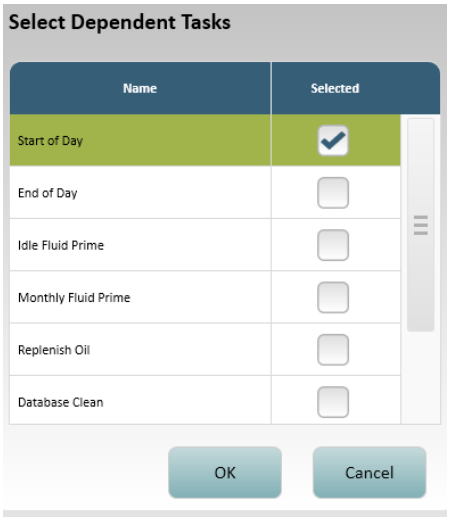
Use the Task Details screen to set and display additional task settings.

To view task details

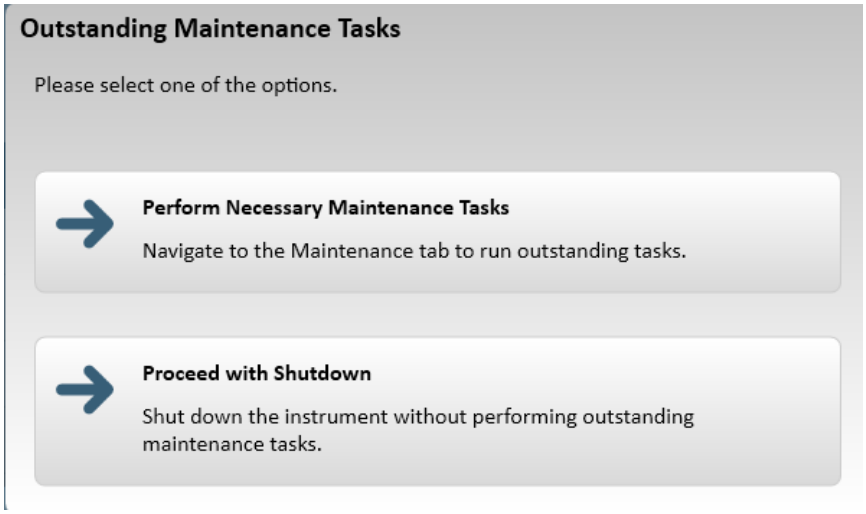
Touch the checkbox for a task > touch **View Details**.



- **Interval** – The frequency of the task.
- **Time** – Time of day the task is performed.
- **Dependent Tasks** – Run specified tasks immediately after completion of the current task.

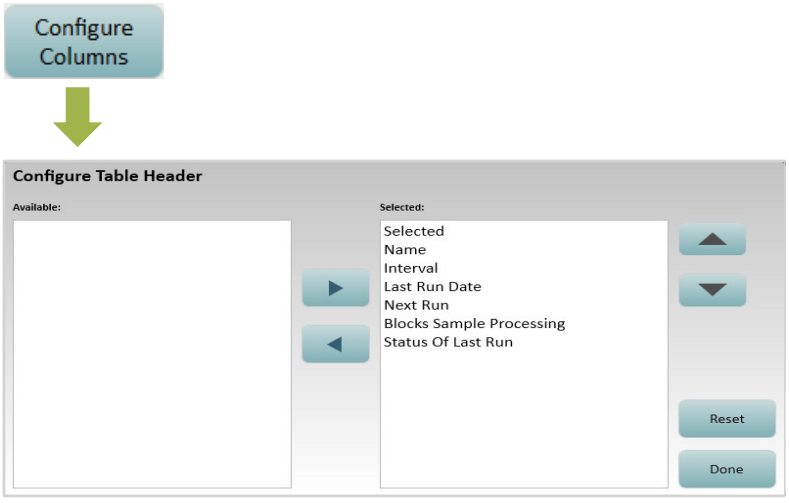


- **Alert user before due date** – Number of days prior to the due date that the software provides a notification prompting you to complete the task. This is only available for the Monthly Fluid Prime and Computer Memory Management tasks.
- **Blocks sample processing** – Prevents/allows running assays when the task is not complete.
- **Resets idle time** – Resets/does not reset counter after task is performed.
- **Blocks shutdown when due** – When enabled, the following prompt appears when you shut down the software.



Changing Columns

To change the table columns, touch **Configure Columns** > touch the arrows to move column names between the Available and the Selected boxes.



Performing the Start of Day Task

This task sets up the instrument for a new assay run as follows:

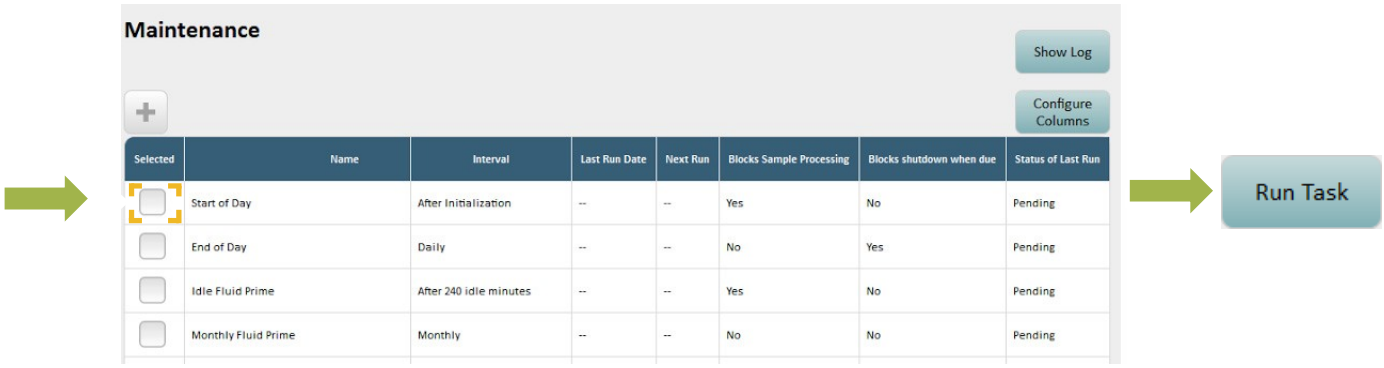
- Primes oil line.
- Flushes the pipettor with deionized water, cleans it with system wash buffer 1, and flushes it again with deionized water.
- Primes tubes with wash buffer fluid, then discards the cuvettes.
- Loads 10 cuvettes into the Wash/Incubator rings.

After you perform the End of Day Task, you must perform the Start of Day Task before performing a run.

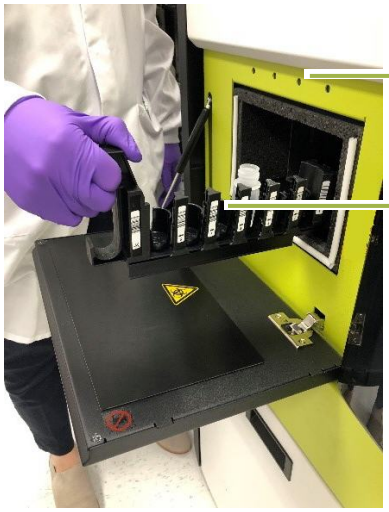
To perform the Start of Day task

a. Fill a clean reagent bottle a little more than half way with system wash buffer 1. Make sure there are no bubbles in the bottle.

b. Touch the Maintenance Tab > touch the Start of Day task checkbox > touch **Run Task**.



c When prompted, place the reagent bottle into position 3 on a reagent rack (fourth position from the handle) and put the rack in the rightmost reagent bay position (lane 4).



d After loading the reagent rack into the instrument, select “next step” to continue with the Start of Day task.

e Allow approximately 20 minutes for completion. When the task is complete, touch **Close**, remove the reagent rack and dispose of the reagent bottle.

Performing the End of Day Task

The End of Day task flushes the pipettor that aspirates reagents and all pipettor and system fluid tubings to prevent the buildup of salts, which can clog the pipettor and instrument tubings.

The task performs these actions:

- Flushes the pipettor with deionized water, cleans it with system wash buffer 1, and flushes it again with deionized water.
- Primes the tubing for system wash buffer 1 and 2 with deionized water.

After you perform the End of Day Task, you must perform the Start of Day Task before performing a run. Quanterix recommends setting a dependent task to automate this process, as described in Setting and Viewing Task Details.

❖ To perform the End of Day task

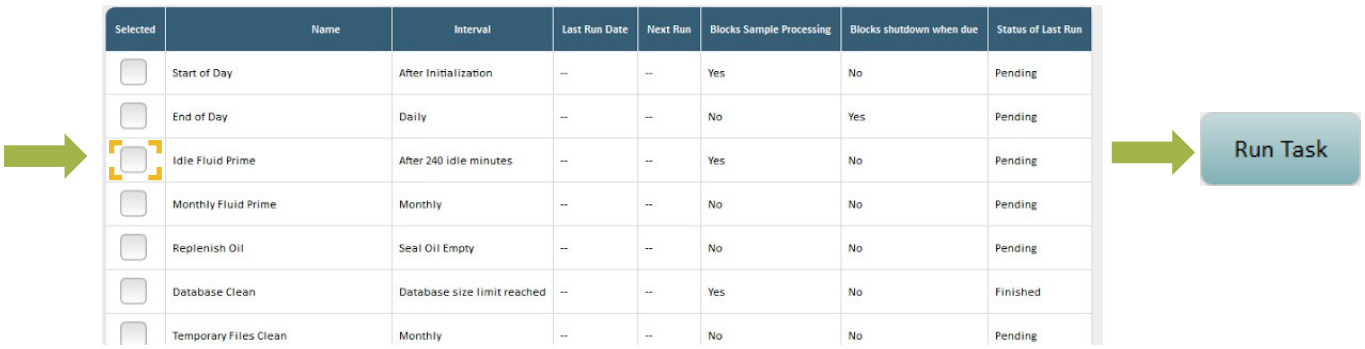
1. Fill a clean reagent bottle a little more than half way with system wash buffer 1. Make sure that there are no bubbles in the bottle.
2. Touch the Maintenance tab > touch the End of Day task checkbox > touch **Run Task.**

3. When prompted, place the bottle into position 3 on a reagent rack (fourth position from the handle) and put the rack in the rightmost reagent bay position (lane 4). See the photo in the section above.
4. Allow approximately 20 minutes for completion. When the task is complete, touch **Close**, and remove the reagent rack and dispose of the reagent bottle.

Performing the Idle Fluid Prime Task

Prime the system fluids after 240 idle minutes. Idle time is the amount of time since the instrument finished a run, or the time since a task that resets the idle counter was run.

1. In the Maintenance tab, touch the Idle Fluid Prime checkbox > touch **Run Task**.
2. The system fluids are primed and the idle time counter is set to zero.



Performing the Database Clean Task

The Simoa HD-X Analyzer generates a lot of data for each sample—approximately 115 MB per result—mostly consisting of raw camera images. For example, a batch with 96 samples results in approximately 11 GB of data. This data is stored in a database on the instrument computer.

Each *week*, you must perform the Database Clean task to purge old data so that new data can be stored. The task removes all data for runs older than 30 days from the first time that you perform the task.

If you want to retain data older than 30 days or to protect data against potential disk failure, it is *your responsibility* to back up the database prior to running the Database Clean task (at least monthly).

The Database Clean task permanently deletes data, so it is highly recommended that you back up the database before performing this task, especially if retention of raw sample images is required.

Backup Strategy

Contact Customer Support to discuss best practices regarding database backup and to obtain sample scripts that automate the backup process.

Do not store database backups on the instrument computer, for two reasons:

- Data can be lost if the disk fails.
- Database backups are very large and consume disk space that is needed to operate the instrument.

If the backup strategy you select results in backup files being stored on the instrument hard drive, you must move them off the disk to an external hard drive, network drive, or similar destination.

You can perform a partial backup that contains all of the data in the database except for the raw IPL image files or a complete database backup that includes the raw IPL image files.

❖ To perform a database backup

See the *HD-X Analyzer Quanterix Customer Support Tool User Guide* for information on performing a full backup, or follow the instructions in *Simoa HD-X Analyzer IT Setup Guide* to make a partial backup.

❖ To perform the Database Clean task

Note! Be sure to back up the database before you perform this task to avoid permanently losing data.

In the Maintenance tab, touch the Database Clean checkbox > touch **Run Task**. After the Database Clean task completes, exit the HD-X software and reboot the PC.

Cleaning Exterior Instrument Surfaces

Clean exterior instrument surfaces whenever necessary (at least once per month) by wiping with a soft, clean cloth moistened with water or 10% ethanol.

Cleaning the Touchscreen

Use any glass cleaner that does not contain ammonia.

Wet a paper towel with glass cleaner and then gently wipe down the touchscreen. Do not spray glass cleaner directly onto the touchscreen.

Cleaning the System Resources Drawers

Once a month, clean the system resource drawers.

CAUTION! Do not attempt to clean the system resource drawers when an assay is running. If you open a drawer during a run, the instrument halts the run and you lose any accumulated data.

Completely pull out both drawers from the instrument and wipe the metal frame beneath the drawers with water and then with 70% ethanol.

Cleaning the Reagent and Sample Bays

Once a month, clean the inside surfaces of the reagent and sample bays.

WARNING! Turn off the instrument before you perform any interior surface cleaning. The robotic pipettors inside the instrument can injure your hands. Do not attempt to clean the incubator or the washer rings and do not insert your hands into the sample bay or the reagent bay if the instrument is turned on.

❖ To clean the reagent and sample bays

1. Turn off the instrument (see "Turning the Instrument On and Off" on page 24).
2. Remove all reagent racks, plate racks, tube racks, and RGP racks.
3. Wipe the walls, ceiling, and base of each bay first with water and then with 70% ethanol.
4. Replace the racks.

Cleaning the Liquid Waste Containers

Once a month, clean liquid waste containers and the platform on which they sit.

During this procedure, discard all fluids and rinsates according to your company's waste disposal policies.

❖ To clean the liquid waste containers

1. Power off the instrument and remove both liquid waste containers by disconnecting the container sensor. If the instrument is not powered off, it will halt when both liquid waste containers are removed.
2. Wipe the platform on which the containers sit first with water and then with 70% ethanol.
3. Rinse the containers by performing the following procedure three times on each container:
   a. Fill the container at least one-third full of deionized water.
   b. Shake to rinse inner surfaces.
   c. Discard the rinsate.
4. Replace the containers and reconnect the container sensors. Align the tab in the sensor with the cutout in the container. When done correctly, you will hear a click when you reinsert each sensor.

Cleaning the System Fluid Containers and Tube Rinsing

Once a month, the fluid resource containers must be cleaned. The tubing from the containers into the system must be primed with water for 30 minutes before they are primed with buffer for another 30 minutes.

During this procedure, discard all fluids and rinsates according to your company's waste disposal policies.

The DI water and wash buffer 1 each have two containers, which are defined as follows:

- **Secondary Container** – This is the removable container with a screw cap where new fluid is added. The wash buffer 1 and DI water secondary containers are identical in size.
- **Primary Container** – This is the pyramidal-shaped container behind the secondary containers. The liquid level sensor and tubing are connected through a screw cap on top.

Wash buffer 2 only has one container, which sits between the DI water and wash buffer 1 secondary containers. It has a screw cap for adding new fluid, a liquid level sensor, and a fluid port that connects directly to the instrument tubing.

The tubing leading to the sensor cap is color coded as follows:

- **Blue** – DI water
- **White** – Wash buffer 1
- **Yellow** – Wash buffer 2

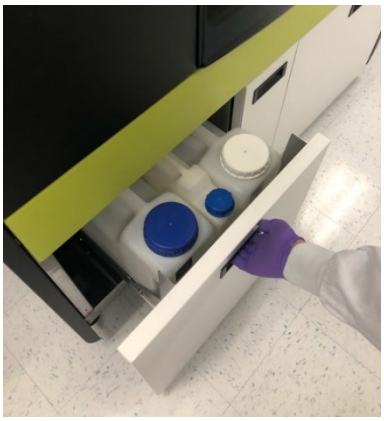
Note! Do not attempt to perform this procedure when an assay is running. The procedure involves disconnecting the fluid containers, which will prevent the instrument from obtaining the system fluids that are required to perform the assay.

To clean the secondary and primary containers

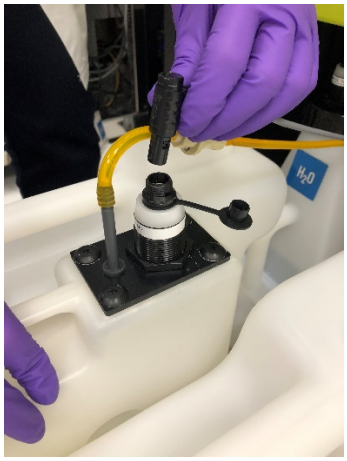
1. Close the Simoa HD-X Analyzer software and shut down the instrument.
2. Remove the secondary containers and wash buffer 2 container.

Note! Do not reuse the fluids after emptying the containers. Reuse of fluids has been associated with a reduction in data quality.

- **DI water and wash buffer 1:** Lift the container up and then pull out.



- **Wash buffer 2:** Disconnect the sensor and fluid port and then lift the container up.

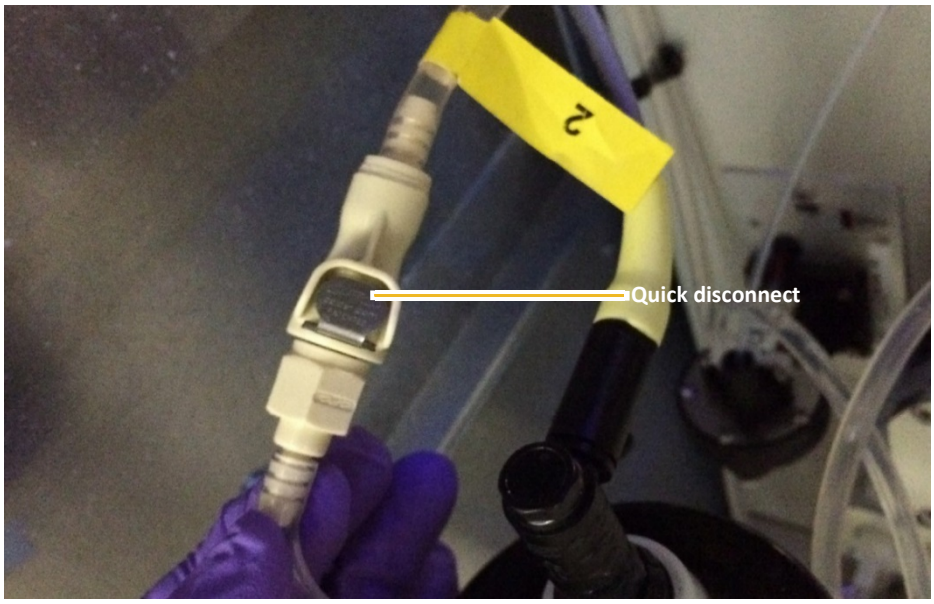


3 Empty and dispose of all secondary and wash buffer 2 container fluids.

Note! Cross-contamination of the wash buffers may result in poor assay performance. Take care during this procedure to prevent cross-contamination. Do not reuse the fluids after emptying the containers. Reuse of wash buffers can result in a reduction of data quality.

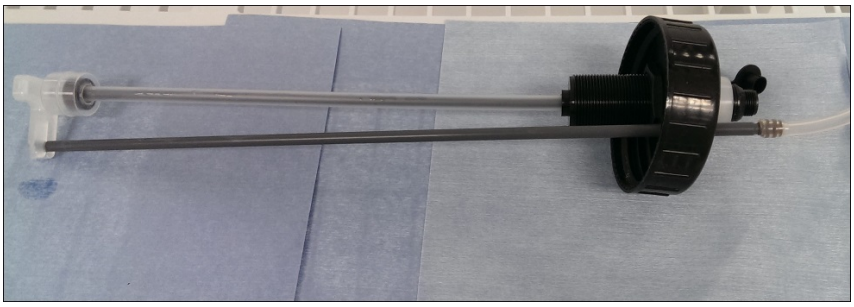
4 Remove all primary containers. The process is the same for each fluid type.

- Disconnect the sensor by pulling the black connector up.



- Press the metal button on the quick disconnect fitting to detach the two ends of the liquid line.
- Remove the primary container by pulling forward and then up.

- Unscrew the black cap and place it on a clean lint-free paper towel.



5 Empty and dispose of all primary container fluids.

6 Wash all fluid containers (primary, secondary, and wash buffer 2) by triple-rinsing them with DI water. Fill the containers to approximately 1/3 of their total capacity with DI water and shake to rinse all surfaces.

7 Clean the liquid level sensors by running under DI water.

❖ To prime the system with DI water

1 Fill the containers with DI water as follows. Do not fill the secondary wash buffer 1 container.

- **DI primary** – Full
- **Wash buffer 1 primary** – Full
- **Wash buffer 2** – Half full
- **DI secondary** – Half full

2 Replace the sensor caps for the DI water and wash buffer 1 primary containers. If they have been separated from the containers, identify the caps by the physical differences in the tubing. DI water uses blue tubing, wash buffer 1 uses white and wash buffer 2 uses yellow.

3 Replace the primary containers (DI water, wash buffer 1), wash buffer 2 container, and secondary DI container into the instrument. Do not replace the secondary container for wash buffer 1.

4 Reconnect the sensor cable and screw the fluid tubing together. Refer to the color code listed previously to ensure proper container connections.

- Connect the two ends of the quick disconnect fitting. You will hear an audible click sound.
- Connect the electrical connector into the top of the primary container lid, making sure that the tab on the cable is well-aligned with the slot in the lid. You will hear an audible click sound.

5 Turn on the instrument and open the Simoa HD-X software.

6 After initialization completes, run the Monthly Fluid Prime task. This step takes approximately 30 minutes to complete.

a Touch the Maintenance Tab > touch the Monthly Fluid Prime task checkbox.

b Touch **Run Task**. The task takes approximately 30 minutes to complete. The task window has a timer that indicates when the task will complete.

7 Close the Simoa HD-X software and shut down the instrument.

❖ To prime the system with wash buffer

1. Disconnect and remove the wash buffer 1 primary container and the wash buffer 2 container. Do not remove the DI Water primary container.
2. Unscrew the black cap for the wash buffer 1 primary container and place it and the liquid level sensors on a clean lint-free paper towel.
3. Empty the remaining water out of the containers.
4. Replace the wash buffer 1 primary container while it is still empty. Screw the sensor cap back on and connect the tubing and sensor again. Refer to the earlier instructions for detail.
5. Fill the containers with the following volumes:
   - **DI Water Secondary** – at least 5 L
   - **Wash buffer 1 Secondary** – at least 5 L
   - **Wash buffer 2** – at least 2 L
6. Replace the secondary containers and wash buffer 2 container into the instrument.
7. Turn on the instrument and open the Simoa HD-X software.
8. After initialization completes, run the Monthly Fluid Prime task again. This will take another 30 minutes to complete.

Maintaining Idle Instruments

When an instrument has been idle for a period of time, air can be introduced into the fluid lines, potentially impacting data quality and precision. To ensure proper instrument operation, perform maintenance tasks on idle systems as described in the following table.

Idle Period	Procedure
Less than 4 weeks	Perform the following maintenance tasks twice per week for each week of inactivity. 1 Power on computer and instrument. 2 Run Start of Day maintenance. 3 Run End of Day maintenance. 4 Power off computer and instrument. If you cannot perform these tasks on schedule, run the Monthly Maintenance task (which performs an extended fluid prime) followed by a Simoa Qualification Test.

Idle Period	Procedure
Greater than 4 weeks	Schedule a visit with Quanterix Service to perform maintenance before and after the scheduled idle period.

12 Configuring Software Settings

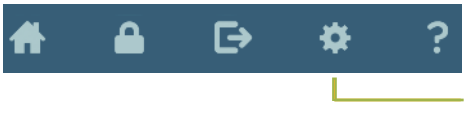
Software Settings

The Software Settings screen has the following tabs:

- System Settings
- User Settings
- Account Management (for users with administrator privileges only)
- Reporting Settings

❖ To view the software settings

Touch the Settings icon at the top right of the software to open the Software Settings screen.



Settings icon

Changing System Settings

In the System Settings screen, you can change the following settings:

- Sample loading mode
- Automatic lock out time
- Password policies
- Synchronization of loading screen and bay doors
- Default number of decimal places
- Customer-defined system name

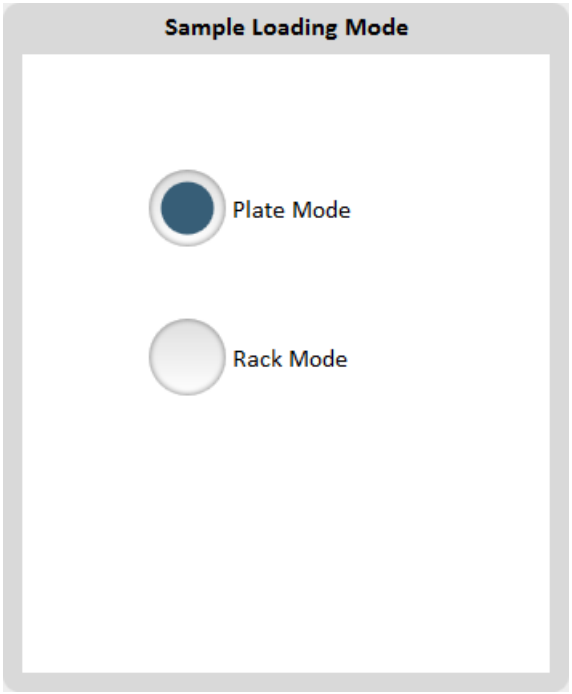
It is not necessary to restart the software after changes to the system settings.

Selecting the Sample Loading Mode

Plate mode is selected when you first open the software. To process samples in tubes, select Rack Mode.

❖ To change the sample loading mode

In the System Settings tab, touch **Plate Mode** or **Rack Mode** > touch **Done** in the upper right corner of the tab.

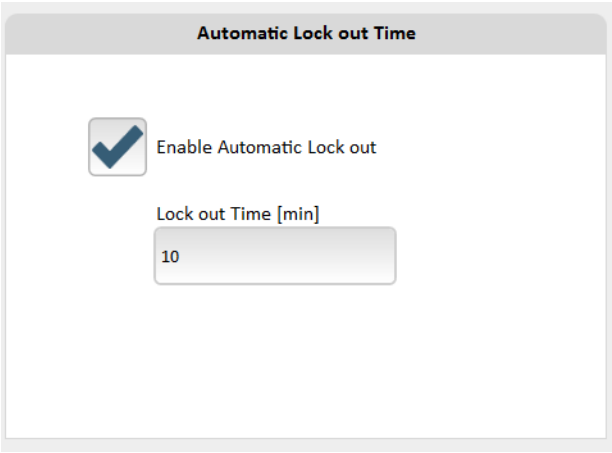


Setting an Automatic Lock Out Time

You can set the software to require users to log in again after a specified time period.

To set a lock out time

In the System Settings tab, touch the **Enable Automatic Lock out** checkbox > type a Lock Out Time > touch **Done** in the upper right corner of the tab.



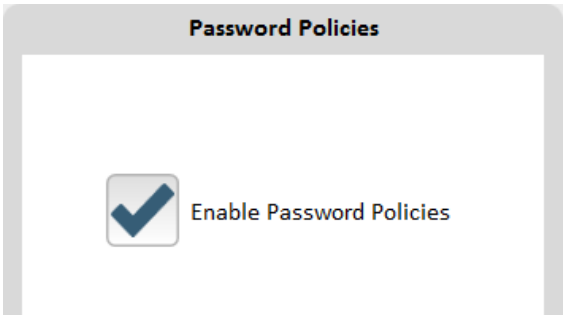
Enabling Password Policies

You can enable or disable the use of password policies. Enabling this setting places additional security requirements on new password values. The security requirements for new passwords are as follows:

- Usage of at least seven characters
- Usage of at least one upper case character
- Usage of at least one lower case character
- Usage of at least one non-alphanumeric character
- Usage of at least one numeric character

❖ To enable password policies

In the System Settings screen, scroll down to the General section > touch the **Enable Password Policies** checkbox > touch **Done** in the upper right corner of the tab.



Controlling Tab Switching when Bay Doors are Opened

By default, the software switches to the Load Reagents tab when you open the reagent bay door, and it switches to the Setup Run tab when you open the sample bay door. You can turn on/off tab switching with the **Synchronize Loading Screen and Bay Doors setting**.

❖ To set tab switching behavior

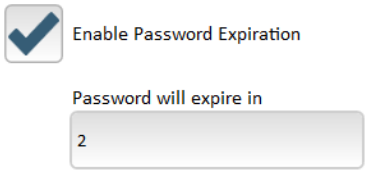
1. In the System Settings screen, scroll down to the General section.
2. Enable the **Synchronize Loading Screen and Bay Doors** checkbox to switch tabs when you open a bay door, or disable it to keep the current tab open.
3. Touch **Done** in the upper right corner of the tab.

Setting a Password Expiration Time

You can enable password expiration and set the amount of time in which you want user passwords to expire.

❖ To set a password expiration time

In the Systems Settings tab, touch the **Enable Password Expiration** checkbox > type the number of days in which you want user passwords to expire > touch Done in the upper right corner of the tab.

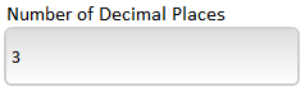


Setting Default Decimal Places

You can specify the number of decimal places that appear for all numbers (except concentrations, which are controlled at the Plex level). This setting controls the display of numbers throughout the application (in result tables, reports, etc.).

❖ To set the default digits to the right of the decimal place

In the System Settings screen, scroll down to the General section > enter an integer from 0 to 15 > touch **Done** in the upper right corner of the tab.



Specifying the Customer Defined System Name

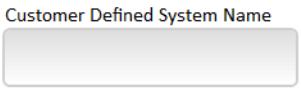
To assist with troubleshooting, you can specify a name that describes the system configuration. For example:

- Version 1.2.3.4
- Simoa 1234
- Lab Machine 12

This name is captured in the Event Log screen and the shellDebug log file when the software is started.

❖ To set the customer defined system name

In the System Settings screen, scroll down to the General section > type a string that describes the system configuration (up to 20 characters) > touch **Done** in the upper right corner of the tab.

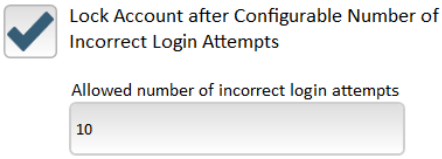


Locking an Account due to Incorrect Login Attempts

You can set the software to lock an account after a specified number of incorrect login attempts.

❖ To lock an account after a configurable number of incorrect login attempts

In the System Settings tab, scroll down to **General** > touch the **Lock Account after Configurable Number of Incorrect Login Attempts** checkbox > type the allowed number of incorrect login attempts > touch **Done** in the upper right corner of the tab.



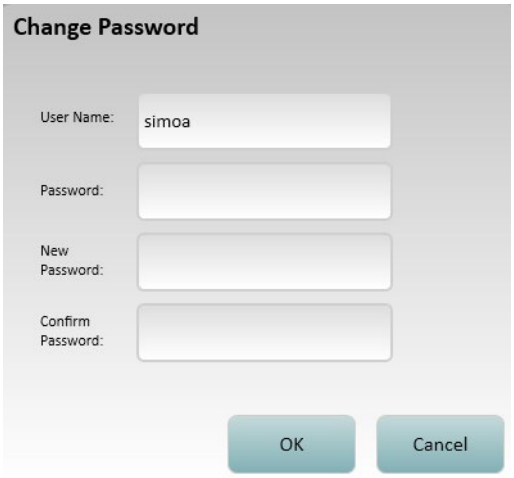
Specifying User Settings

All users can change their own system passwords. If you are a user with administrative privileges, you can:

- change another user's password
- show assays belonging to all users, even if the **Visible only to me** setting (in the Overview screen of the Custom Assay tab) is enabled for the account.

To change a password

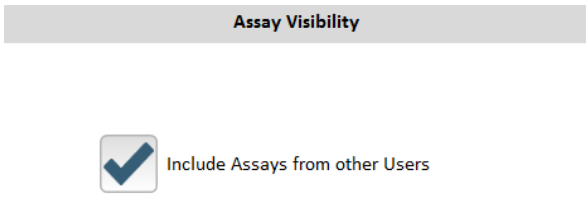
In the User Settings tab, type the user name, existing password, and new password > touch **OK**.



To display/hide assays from other users

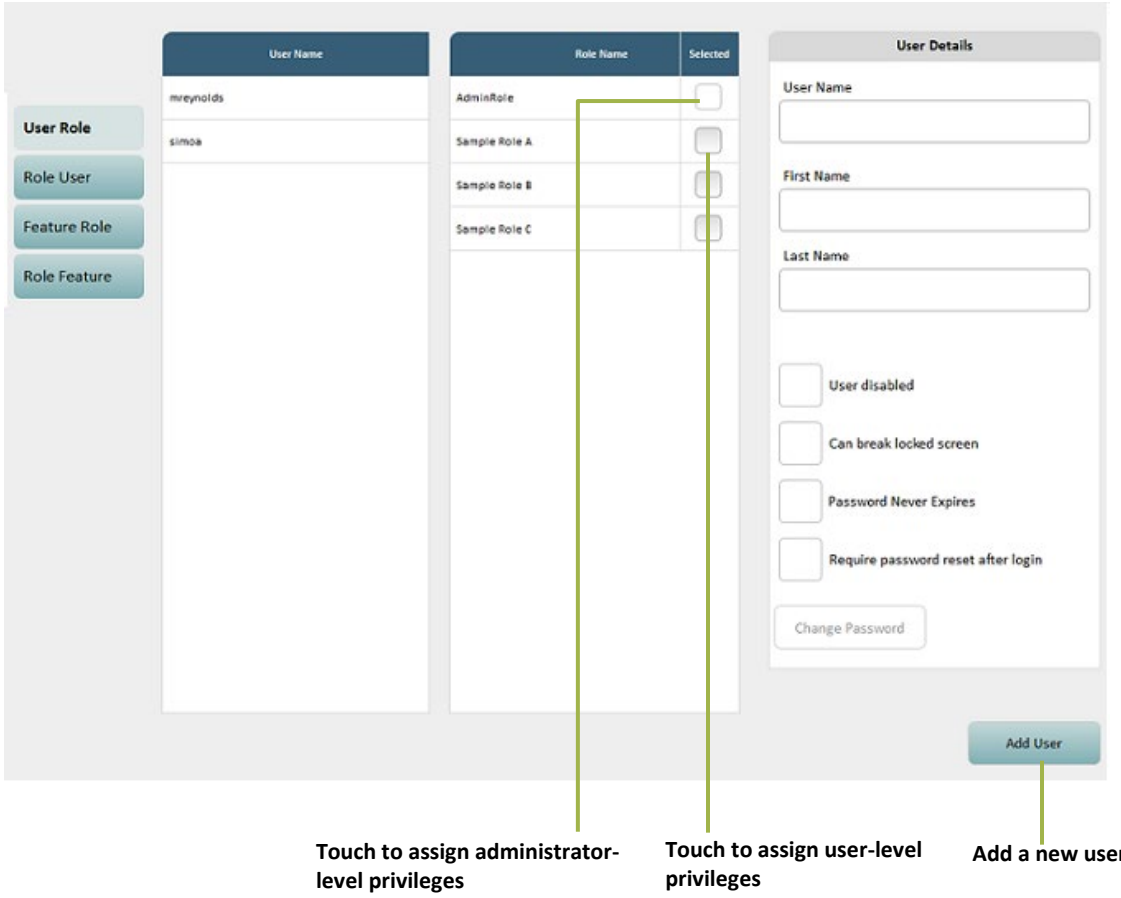
Note! You must be a user with administrative privileges to override the **Visible only to me setting** (in the Overview screen of the Custom Assay tab).

1. In the User Settings tab, enable **Include Assays from other Users** to display all assays, or disable it to show only your assays.
2. Touch **Done** in the upper right corner of the screen.



Specifying Account Management Settings

Use the Account Management screen to create and manage users and customize roles. The user that you define when you sign into the instrument software for the first time is assigned the AdminRole and appears as a user on this screen.

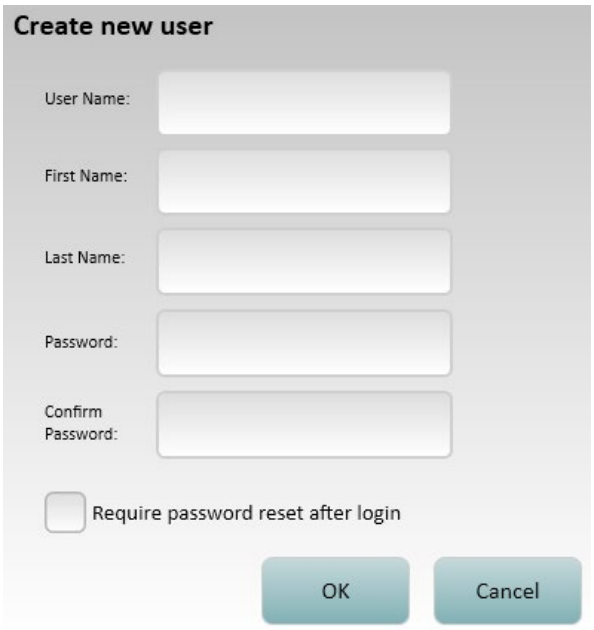


Managing Users

In the Account Management screen, you can add new users and define their access privileges.

To add a new user

1. Touch **Add User**.
2. Specify the User Name, First Name, Last Name, and Password.
3. To require the new user to reset their password after logging in, touch the **Require password reset after login** checkbox.
4. Touch **OK**.
5. Touch the **Selected** checkbox for the role you want to assign in the Role Name list.
6. If you have completed Account Management tasks, touch **Done**.



To change a user's role

1. Touch **User Role**.
2. Select the user in the User Name list.
3. Touch the **Selected** checkbox for the role you want to assign in the Role Name list.
4. If you have completed Account Management tasks, touch **Done**.

To change a user's password

1. Touch **User Role**.
2. Select the user in the User Name list.
3. Touch **Change Password**.
4. Touch **Done** in the upper right corner of the screen.
5. Type the existing password and the new password.
6. Touch **OK**.
7. If you have completed Account Management tasks, touch **Done**.

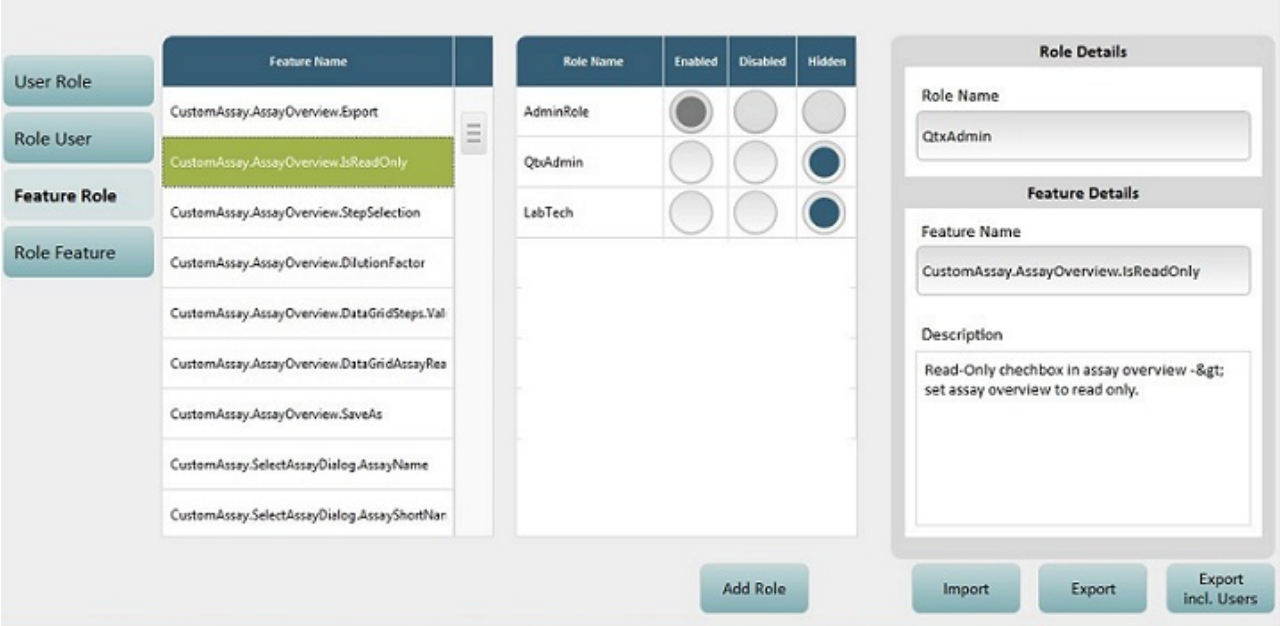
To assign multiple users to a single role

1. Touch **Role User.**
2. Select the role you are assigning in the Role Name list.
3. Touch the **Selected** checkbox for each user you want to assign.
4. If you have completed Account Management tasks, touch **Done**.

Managing Roles and Features

In the Account Management screen, you can create new roles and define the product features that each role can access. For example, you might want to limit the ability to modify imported assay definitions to administrators only. To do this, you can enable the Hidden option for the Read/Write button on the Custom Assay screen for nonadministrator roles.

The default role, AdminRole, can access all features. You cannot change the status of any feature to Disabled or Hidden for AdminRole.



To define features for a role

1. Touch **Feature Role**.
2. Select the feature you are assigning in the Feature Name list or select multiple features with the Shift key or Control key.
3. Touch **Enabled** (role can use the feature), **Disabled** (role cannot use the feature), or **Hidden** (feature does not appear in the software) for each role.
4. If you have completed Account Management tasks, touch **Done**.

Note! You can use the **Import**, **Export**, and **Export incl. Users** options to share settings among multiple instruments.

To define multiple features for a role

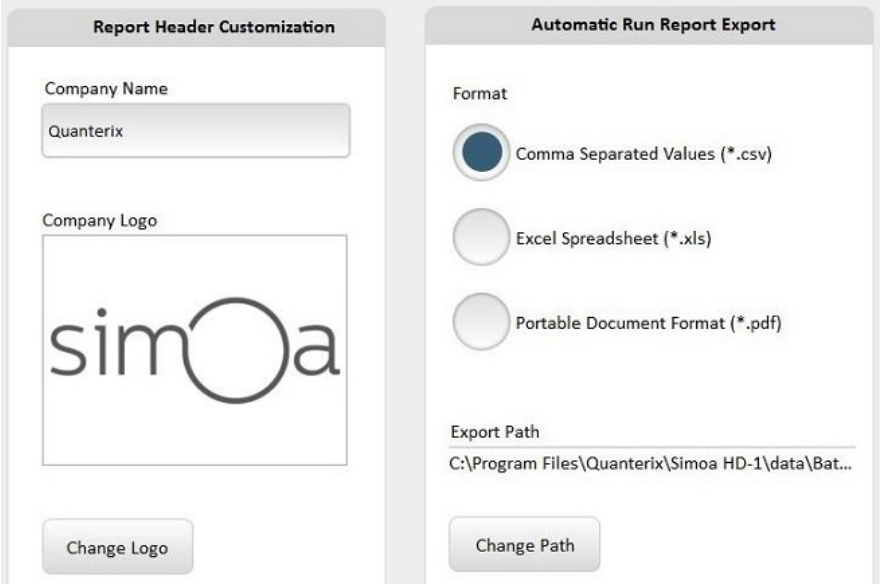
1. Touch **Role Feature**.
2. Select the role name you are configuring in the Role Name list or select multiple roles with the Shift key or Control key.
3. Touch **Enabled** (role can use the feature), **Disabled** (role cannot use the feature), or **Hidden** (feature does not appear in the software) for each feature.
4. If you have completed Account Management tasks, touch **Done**.

To add a new role

1. Touch **Feature Role**.
2. Touch **Add Role**.
3. Enter a **Role Name**.
4. Optionally, select a role that is similar to the one you are creating from the dropdown list.
5. Touch **OK**.

Managing Report Settings

In the Report Settings tab, you can customize the company name and logo (.jpg, .jpeg, .jpe, .png, or .bmp format) that appear on reports. You can also specify the default output format and path for automatic batch reports.



To customize report headers

1. In the Report Settings screen, enter the Company Name.
2. Touch **Change Logo**.
3. Browse to the logo file > touch **Open.**
4. Touch **Done** in the upper right corner of the tab.

To customize report export defaults

1. In the Report Settings screen, select the Format.
2. Touch **Change Path**.
3. Browse to the folder you want to use for report output, or touch **New Folder**, specify a new folder name for reports.
4. Touch **Select.**
5. Touch **Done** in the upper right corner of the tab.

Appendix A – Configure Columns Definitions

The following table describes each column in the Configure Table Header screen.

Column Name	Description
Analog AEB	AEB value calculated using the analog mode. See **Analysis Mode** in this table to find out whether digital or analog mode was used to calculate the result AEB value. If the sample was run in replicate, the majority rule is used to determine the mode (see *Simoa HD-X Data Analysis Guide*).
Analysis Mode	Indicates whether the AEB calculation was performed using the digital or analog mode.
Assay	Name of the assay scheduled for the sample.
Assay Revision	The revision of the assay definition used.
Batch ID	Unique system identifier of the sample batch.
Batch Name	Name of the batch specified on the Setup Run screen. If you do not specify a batch name, it defaults to batch creation date and time.
Bead Concentration	Value that specifies bead concentration of Homebrew beads during a bead aggregation run. See *Simoa Bead-Based Homebrew Assay Development Guide* for instructions on calculating bead quality value.
Calibration Curve ID	Unique system identifier of the calibration curve used in sample concentration calculations. This value is blank for calibrators as more than one calibration curve can be generated against calibrator AEB values.
Carrier Barcode	Barcode value of a microtiter plate or tube rack specified in the Setup Run screen.

Column Name	Description
Completion Date	Date and time when processing of the sample result completed.
Curve Name	Name of the calibration curve used in sample concentration calculations. This value defaults to Measured. The calibration curve name can be edited in the Data Analysis tab.
CV AEB	Coefficient of variation of sample replicate AEB values for a given plex.
CV Conc.	Coefficient of variation of sample replicate concentration values for a given plex.
Date Curve Created	Date and time when the calibration curve used in the sample concentration calculations was created. This value can be used to distinguish between curves if they all have the default name of Measured.
Digital AEB	AEB value calculated using the digital mode. See **Analysis Mode** in this table to determine if digital or analog mode was used to calculate the result AEB value. If the sample was run in replicate, the majority rule is used to determine the mode (see *Simoa HD-X Data Analysis Guide*).
Dilution Description	Name of the dilution applied to the sample test. The dilution description normally appears as a ratio of sample to diluent, for example, 4x, but it can carry any name, for example, One to Four, etc.
Dilution Factor	Multiplication factor applied to sample results when the concentration is reported. For example, for 4x dilution, the Dilution Factor will be 4.
Errors	IDs of error flags associated with a sample test. To view additional information in the Event Log, click the error hyperlink.

Column Name	Description
Estimated Time to Result	Date and time when the system expects to deliver the result value.
Extended Properties	A collection of image analysis diagnostic information. For system diagnostic use only.
Flags	The number of warning or error flags associated with processing of a sample test.
Fraction Monomeric Beads	Fraction of bead content that is monomeric – used to calculate actual bead concentration during bead aggregation assays. See *Simoa Bead-Based Homebrew Assay Development Guide* for instructions on calculating bead quality value.
Fraction On	Fraction of beaded wells with enzyme activity for a given plex. Both the digital and analog AEB calculation methods use this value.
Ibead	Average signal growth of beaded wells with enzyme activity. This value is used to calculate the result AEB value in analog mode. In digital mode, this value is not used. Isingle is calculated with Ibead values of jobs meeting the appropriate constraints.
Image Quality Score	Metric that helps the software determine whether a result should be included or excluded from analysis, as determined by image-processing algorithms. Currently, the value defaults to 1 for all sample results. For system diagnostic use only.
Instrument SN	Unique instrument identifier.
Isingle	Estimated average signal growth of beaded wells that contain one enzyme. Certain conditions are required to calculate this value. (see *Simoa HD-X Data Analysis Guide*).

Column Name	Description
Job ID	Unique system identifier for a set of activities that lead to one result. For example, if a sample is scheduled to run an assay with one specimen in Neat and 4x dilutions in triplicate, the system will generate results for 6 unique jobs.
Job Start Cycle	The internal system clock used to coordinate sample processing activities. For system diagnostic use only.
Job Status	Status of each job.
Location	Location where the sample was loaded in the instrument.
Mean AEB	Average of sample replicate AEB values for a given plex.
Mean Conc.	Concentration calculated by averaging available individual replicate concentrations.
Number of Beads	Number of beads identified and used to produce the result for a given plex.
Plex	Name of the assay plex.
Replicate AEB	Sample AEB value for a given plex of a given job.
Replicate Conc.	Sample concentration value for a given plex of a given job.
Replicate Result ID	Unique system identifier for a replicate summary result. For example, if a sample was processed in triplicate, the replicate summary result will contain the mean, standard deviation, and coefficient of variation of the replicates (see Mean AEB, Mean Conc., SD AEB, SD Conc., CV AEB, CV Conc. in this table). For system diagnostic use only.
Result ID	Unique system identifier for a single result generated by the system. For system diagnostic use only.
Result Status	Indicates whether the result was included or excluded from result calculations. The value can be one of: Automatically

Column Name	Description
	Included, Automatically Excluded, Manually Included, and Manually Excluded.
Sample Barcode	Sample identifier assigned by the user in the Sample Assignment screen. The value is entered manually or by scanning a barcode.
Sample Type	Specimen or calibrator sample type.
Selected	A column of check boxes for selecting records.
SD AEB	Standard deviation of sample replicate AEB values for a given plex.
SD Conc.	Standard deviation of sample replicate concentration values for a given plex.
SW Version	Software version. Provide to Quanterix Customer Support during troubleshooting.
Test Order ID	Unique system identifier for a grouping of sample jobs. For system diagnostic use only.
Unit	Unit of sample concentration.
Used Reagents	A list of reagent barcodes used to produce the result.
User Name	The user who initiated the sample test that produced this result.

Appendix B – Instrument Specifications

The instrument must be installed by qualified service personnel only. Please do not move the instrument once it is installed.

Physical Specifications

Weight (without fluids)	294 kg (648 lb)
Dimensions	Width: 141.4 cm (55.66 in) Depth: 78.7 cm (31 in) Depth with monitor: 89.7 cm (35.3 in) Height: 161.2 cm (63.5 in)

Installation & Service Dimension Requirements

Doorway width for installation	80.0 cm (31.5 in)
Service clearance at instrument rear	60.0 cm (23.6 in) If clearance at the rear is not sufficient, enough front clearance must be available to move the instrument away from the wall for service.
Service clearance at instrument sides	60.0 cm (23.6 in)
Operator clearance at instrument front	100.0 cm (39.38 in)
Ventilation clearance at instrument rear	Minimum of 20.5 cm (8 in)
Earthquake safety	For sites that require the system to be secured from tipping due to an earthquake, adhesive straps must be affixed to the rear panel of the instrument only.

Power Requirements

Instrument

Instrument	Electrical receptacles (2 each): • Europe: DIN49441 • North America: 5 – 15R
Voltage	100 V – 240 V ± 10%
Frequency	50 – 60 Hz
Input current	7.5 A @100 VAC, 3.2 A @240 VAC
Fuse	Thermal overload protection

Computer and UPS

Computer	Input: 100 – 240 VAC ~50 – 60 Hz, 300W Ethernet (RJ45)
Recommended UPS	APC Smart-UPS SRT 2200VA 120V recommended

Environmental Operating Conditions

Operating temperature	18°C to 25°C (64.4°F to 77°F)
Operating humidity	30% to 80%, noncondensing
BTU output during operating conditions (non-standby)	3288 BTU/Hr

Noise Emission

Noise emission	72 dB(A), distance 1 m (39.4 in)

Lasers

The Simoa HD-X Analyzer is a class 2 laser product. It incorporates the following laser sources.

Laser Barcode Scanner

Maximal output radiation	1.3 mW
Maximal pulse duration	110 µs
Wavelength	650 nm
Reading distance	3.0 – 30.0 cm

Laser Light Barrier

Maximal output radiation	1.0 mW
Wavelength	650 nm

Packaging

See delivery note enclosed with the instrument for the contents, weight, and dimensions.

Number of crates	3
Crate 1	
Contents	Instrument
Dimensions (WxDxH)	160.0 cm x 92.0 cm x 178.0 cm (63 in x 36.2 in x 70.1 in)
Weight	378 kg (833 lb)

Crate 2	
Contents	Instrument covers
Dimensions (WxDxH)	157.0 cm x 80.0 cm x 97.0 cm (61.8 in x 31.5 in x 38.2 in) (Box 3 on top of crate 2 bundled)
Weight	130 kg (287 lb)
Crate 3	
Contents	Accessories
Dimensions (WxDxH)	120.0 cm x 80.0 cm x 50.0 cm (47.2 in x 31.5 in x 19.7 in) (packaged in card board box and can be handled separately)
Weight	39 kg (86 lb)

Environmental Conditions

The following table shows the range of conditions needed to run the system safely.

Environmental conditions	The system is made for indoor use.
Temperature	Operating: 18°C to 25°C (64.4°F to 77°F) Storage: 1°C to 45°C (33.8°F to 113°F) Transport: –20°C to 60°C (–4°F to 140°F)
Humidity	Operating: 30% – 80% noncondensing Storage: 5% – 80% noncondensing Transport: 20% – 80% noncondensing
Pollution degree	Degree 2
Overvoltage category	Class 2

Limit class	Class A (For industrial use. Domestic use restricted.)
Sunlight	No direct sunlight. May mislead optical sensors and affect performance.
Altitude	Up to 2000 m (1.24 mi) above mean sea level.
Dust	No excessive dust

Instrument Installation Dimensions

The following diagram shows a top-down view of the instrument.