

# Ion 550™ Kit – Chef

## USER GUIDE

Instructions for automated template preparation, chip loading,  
and sequencing

for use with:

Ion Chef™ System

Ion S5™ XL System

Ion GeneStudio™ S5 Plus System

Ion GeneStudio™ S5 Prime System

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Revision	Date	Description
D.0	31 July 2023	<ul style="list-style-type: none"> <li>Updated Ion S5™ Chef Supplies (Part No. A27755) kit to include the PCR Plate Frame.</li> <li>Updated instructions to include loading the PCR Plate Frame. See “Load the pipette tip racks and PCR Plate” on page 31.</li> <li>Updated instructions to include removing the PCR Plate Frame. See “Remove used consumables” on page 62.</li> </ul>
C.0	25 June 2019	<ul style="list-style-type: none"> <li>Updated for Torrent Suite™ Software 5.12.</li> <li>Added #unique_14/unique_14_Connect_42_GUID-440FE1AB-8075-4344-BAD8-69B208711F60 on page 8.</li> <li>In #unique_45/unique_45_Connect_42_GUID-0796DC72-2287-4303-9088-69174E8FD149, added clarification for setting up a 1X used Reagents cartridge for a single-chip run 2.</li> <li>Updated #unique_46/unique_46_Connect_42_GUID-9341033B-85D6-4411-A7D1-B0C72946C8EE to include the Run Options screen for step-by-step Ion Chef™ run setup.</li> <li>Updated the guidance for storing loaded chips before sequencing in #unique_28/unique_28_Connect_42_GUID-60C0B2D9-B171-4895-8AE0-D7204463C856.</li> <li>Added a note in #unique_46/unique_46_Connect_42_GUID-9341033B-85D6-4411-A7D1-B0C72946C8EE and #unique_28/unique_28_Connect_42_GUID-60C0B2D9-B171-4895-8AE0-D7204463C856 to NOT remove any residual liquid from chip wells after the Ion Chef™ run.</li> <li>Removed Ion Chef™ System maintenance procedures from #unique_97/unique_97_Connect_42_GUID-47C7590F-FFCD-472A-A61D-9161F491642E.</li> <li>Updated #unique_97/unique_97_Connect_42_GUID-47C7590F-FFCD-472A-A61D-9161F491642E to add references to the Ion Chef™ System maintenance procedures in <i>Ion Chef™ Instrument User Guide</i>.</li> <li>Updated #unique_120/unique_120_Connect_42_GUID-A2E734CE-DFB9-497D-8BFB-E2FAD0EDF618 to include the <i>Ion Chef™ Instrument User Guide</i>.</li> </ul>
B.0	6 December 2018	<ul style="list-style-type: none"> <li>Updated to include use with the Ion S5™ XL System Ion GeneStudio™ S5 Systems.</li> <li>Clarification made to dilute barcoded library pools, if used.</li> <li>Updated for Torrent Suite™ Software 5.10; Planned Run creation topics reorganized for ease of use.</li> <li>Ion S5™ Controls Kit replaced with Ion S5™ Controls Kit Plus.</li> <li>Removed reference to quality control of ISPs using the Attune™ NxT Acoustic Focusing Cytometer.</li> </ul>
A.0	8 January 2018	New user guide for use with the Ion 550™ Kit – Chef, the Ion Chef™ System, and the Ion GeneStudio™ S5 Plus or Ion GeneStudio™ S5 Prime System.

The information in this guide is subject to change without notice.

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**IMPORTANT!** Before using this product, read and understand the information in the “Safety” appendix in this document.

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## Product description

The Ion 550™ Kit – Chef (Cat. No. A34541) contains the reagents and materials that are required to perform the following steps in the sequencing workflow.

- Prepare enriched, template-positive Ion Sphere™ Particles (ISPs) and load them onto an Ion 550™ Chip using the Ion Chef™ Instrument.
- Sequence the loaded chips on the Ion GeneStudio™ S5 Plus Sequencer, Ion GeneStudio™ S5 Prime Sequencer, or Ion S5™ XL Sequencer.

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**IMPORTANT!** The Ion 550™ Kit – Chef and Ion 550™ Chips are **not** compatible with the Ion S5™ System or the base model Ion GeneStudio™ S5 System.

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Each kit contains all the supplies that are required to load and sequence 8 chips and is compatible for use with up to 200-base-read libraries. The kit supports 4 Ion Chef™ runs (2 chips/Ion Chef™ run) and 4 sequencer initializations (2 sequencer runs/sequencer initialization).

To provide more workflow flexibility, the Ion 550™ Chef Reagents and Ion 550™ Chef Solutions cartridges in the Ion 550™ Kit – Chef are now configured for both standard dual-chip templating runs, and single-chip templating runs. The Ion 550™ Chef Reagents and Ion 550™ Chef Solutions cartridges can be reused in a second single-chip templating run if the second run is performed within 8 days of the first run.

The Ion 550™ Single Chip Supplemental Kit must be ordered separately to obtain the additional single-use supplies that are required for 8 single-chip templating runs and 8 sequencer initializations. For ordering information for this kit, for the Ion 550™ Chip Kit, and for the Ion S5™ Controls Kit Plus, see “Related products” on page 10.

## Library compatibility

The Ion 550™ Kit – Chef can be used with up to 200-base-read libraries of any type prepared using any available Ion Torrent™-branded library kit.

## Sequencer compatibility

The Ion 550™ Chips are compatible with the Ion S5™ XL System, Ion GeneStudio™ S5 Plus System, and Ion GeneStudio™ S5 Prime System. The Ion 550™ Chips are NOT compatible with the Ion S5™ System or the Ion GeneStudio™ S5 System.

## Software compatibility

The Ion 550™ Kit – Chef is compatible with Torrent Suite™ Software 5.8 and later. Be sure to update your Torrent Suite™ Software to the latest available version before using this kit. For more information, see the *Torrent Suite™ Software User Guide* for your version of the software.

## Kit contents and storage

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**IMPORTANT!** Do not substitute components with components from other Ion sequencing kits. We have verified this protocol using these specific materials. Substitution can adversely affect system performance.

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**IMPORTANT!** Store all consumables and cartridges under the recommended conditions and in an upright position. Do NOT store the Ion S5™ Sequencing Reagents (Cat. No. A27768) on dry ice or in a closed environment where dry ice is present.

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Each Ion 550™ Kit – Chef contains all the supplies that are required to prepare and sequence 8 Ion 550™ Chips. Cat. No. A34541 supports 4 Ion Chef™ runs (2 chips/Ion Chef™ run) and 4 sequencer initializations (2 sequencing runs/sequencer initialization) for up to 200-base-read libraries.

To support 8 single-chip Ion Chef™ runs and 8 sequencer initializations with the Ion 550™ Kit – Chef, order the Ion 550™ Single Chip Supplemental Kit (see page 10).

On arrival, inspect all consumables. Contact Technical Support if any of the products have been damaged during shipping.



## Kit summary

Component	Part No.	Quantity per kit
Ion S5™ Chef Supplies	A27755	4 boxes
Ion 550™ Chef Solutions	A36410	1 box
Ion 550™ Chef Reagents	A34540	1 box
Ion S5™ Sequencing Solutions	A27767	1 box
Ion S5™ Sequencing Reagents	A27768	1 box

## Ion Chef™ reagents and materials

Contents	Amount / box	Storage
<b>Ion S5™ Chef Supplies (Part No. A27755)</b>		
Chip Adapter	2	15°C to 30°C
Enrichment Cartridge v2	1	
Tip Cartridge v2	1	
PCR Plate	1	
PCR Plate Frame	1	
Frame Seal v2	1	
Recovery Station Disposable Lid v2	2	
Recovery Tube v2	12	
<b>Ion 550™ Chef Solutions (Part No. A36410)</b>		
Ion 550™ Chef Solutions	4 cartridges	15°C to 30°C
<b>Ion 550™ Chef Reagents (Part No. A34540)</b>		
Ion 550™ Chef Reagents	4 cartridges	-30°C to -10°C

## Ion S5™ sequencing reagents and materials

Contents	Amount / box	Storage
<b>Ion S5™ Sequencing Solutions (Part No. A27767)</b>		
Ion S5™ Wash Solution	4 × 1.5 L	15°C to 30°C
Ion S5™ Cleaning Solution	250 mL	

Contents	Amount / box	Storage
<b>Ion S5™ Sequencing Reagents (Part No. A27768)</b>		
Ion S5™ Sequencing Reagents	4 cartridges	-30°C to -10°C <sup>[1]</sup>

<sup>[1]</sup> Cartridges ship at 2°C to 8°C. Store as indicated, do not store on dry ice.

**IMPORTANT!** Do not store the Ion S5™ Sequencing Reagents (Part No. A27768) on dry ice or in a closed environment containing dry ice.

## Related products

### Ion 550™ Single Chip Supplemental Kit

The Ion 550™ Single Chip Supplemental Kit (Cat. No. A36953), when used with the Ion 550™ Kit – Chef, provides reagents and materials for eight single-chip Ion Chef™ runs and eight sequencer initializations. The Ion 550™ Single Chip Supplemental Kit is ordered separately.

Contents	Part No.	Quantity per kit	Storage
Ion S5™ Chef Supplies	A27755	4 boxes	15°C to 30°C
Ion S5™ Sequencing Solutions	A27767	1 box	
Ion S5™ Sequencing Reagents	A27768	1 box	-30°C to -10°C

### Compatible Ion Chip™ kits

Description	Cat. No.	Quantity per kit	Storage
Ion 550™ Chip Kit (4-pack)	A34537	4 chips	15°C to 30°C
Ion 550™ Chip Kit (2 × 4-pack)	A34538	8 chips	

### Ion S5™ Controls Kit Plus

The Ion S5™ Controls Kit Plus (Cat. No. A30729) is ordered separately. See the *Ion S5™ Controls Kit Plus Product Information Sheet* (Pub. No. MAN0016206) for details.

Contents	Amount	Storage
Human CEPH Genomic DNA Control (red cap)	30 µL	-30°C to -10°C
Human CEPH Control 200 Library (yellow cap)	12 µL	
<i>E. coli</i> DH10B Control 400 Library (orange cap)	16 µL	

## Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier.

Item	Source
Ion Chef™ System	4484177
Microcentrifuge <sup>[1]</sup>	MLS
2-, 20-, 200-, and 1,000-μL pipettes and appropriate filtered tips	MLS
Microcentrifuge tubes, 1.5-mL or 1.7-mL	MLS
Vortex mixer with a rubber platform	MLS
Gloves, powder-free nitrile	MLS
Ice buckets and ice	—
Nuclease-free water, molecular biology grade	MLS
Isopropyl alcohol, 70% solution	MLS
Wipes, disposable lint-free	MLS
(Optional) Uninterruptible Power Supply (UPS) <sup>[2]</sup>	MLS

<sup>[1]</sup> Must fit standard 0.2- and 1.5-mL microcentrifuge tubes and generate 15,500 × g. To convert the RPMs of your centrifuge to RCF in units of gravity, see [tools.thermofisher.com/content/sfs/brochures/TR0040-Centrifuge-speed.pdf](https://tools.thermofisher.com/content/sfs/brochures/TR0040-Centrifuge-speed.pdf).

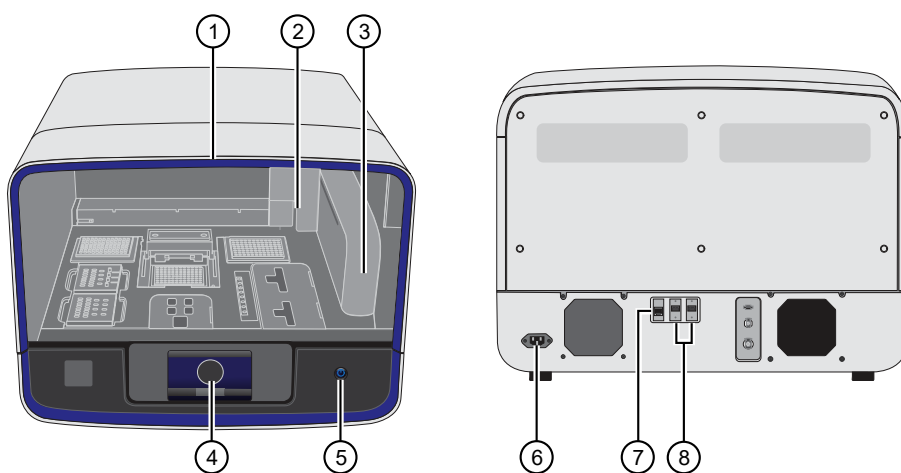
<sup>[2]</sup> For laboratories that experience frequent power outages or line voltage fluctuations, we recommend that you use an uninterruptible power supply that is compatible with 2500 W output or higher.

## About the Ion Chef™ System

The Ion Chef™ System (Cat. No. 4484177) provides automated, high-throughput template preparation and chip loading for use with the Ion S5™ XL Sequencer, Ion GeneStudio™ S5 Plus Sequencer, or Ion GeneStudio™ S5 Prime Sequencer. The system includes a set of cartridge-based consumables and reagents that enable a user to load two Ion 550™ Chips in approximately 16 hours with less than 15 minutes of hands-on time. The Ion Chef™ System features network integration with the Torrent Suite™ Software to enable sample and reagent traceability throughout the chip preparation workflow.

### Ion Chef™ Instrument components

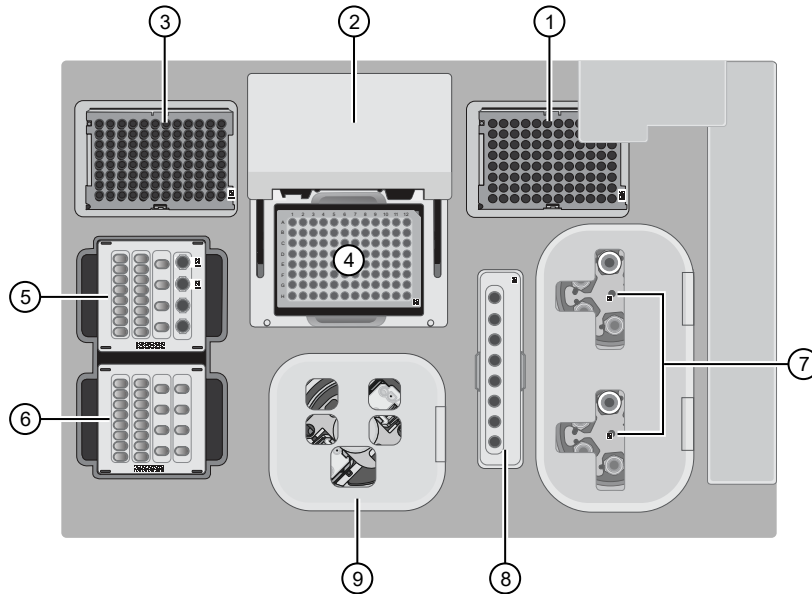
The following figure illustrates the major external and internal features of the Ion Chef™ Instrument.



- ① **Door** – Provides access to the interior of the instrument. The door is locked in the closed position during operation.
- ② **Micropipettor** – A mechanical positive-displacement pipettor that performs all fluid transfers during sample and chip preparation.
- ③ **Robotic arm** – Enables fluid transfer by the Micropipettor. The arm also contains an optical sensor that reads the barcodes of instrument reagents and consumables.
- ④ **Touchscreen** – Provides access to all instrument functions for operation, maintenance, and troubleshooting.
- ⑤ **Power button** – Power switch for the Ion Chef™ Instrument, where the states are on (illuminated) and off.
- ⑥ **Power port** – A 100–240 VAC port that provides power to the Ion Chef™ Instrument.
- ⑦ **Ethernet port** – An RJ45 port that provides Ethernet (100 Mbit) communication with the Ion Chef™ Instrument.
- ⑧ **USB port** – Provides USB communication with the Ion Chef™ Instrument. Used to update the instrument firmware and to transfer data during service or maintenance.

## Interior hardware and consumables

The following figure illustrates the interior of the Ion Chef™ Instrument and describes the stations involved in the preparation of chips for sequencing on an Ion S5™ XL System, Ion GeneStudio™ S5 Plus System, or Ion GeneStudio™ S5 Prime System.



- ① **Waste pipette tip rack** – The position of the rack containing waste (used) pipette tips.
- ② **Automated heated cover** – Transfers the plate cover to the PCR reaction plate within the sample block. During thermal cycling, the heated cover applies compression to seal the reaction plate and heats the cover to prevent condensation.
- ③ **New pipette tips** – The position of the rack containing unused pipette tips.
- ④ **Thermal cycler sample block** – Performs thermal cycling of the sequencing reactions on a 96-well PCR reaction plate.
- ⑤ **Reagents station** – The position on the instrument deck of the diluted libraries, NaOH, and the Ion 550™ Chef Reagents cartridge.
- ⑥ **Solutions station** – The position on the instrument deck of the Ion 550™ Chef Solutions cartridge, which is maintained at room temperature.
- ⑦ **Recovery centrifuges** – Twin stations that perform centrifugation of the ISPs during the recovery phase of template preparation.
- ⑧ **Enrichment station** – The position of the rack containing consumables for enrichment of the template-positive ISPs.
- ⑨ **Chip-loading centrifuge** – Performs centrifugation of sequencing chips that have been mounted to chip-loading adapters and loaded with template-positive ISPs.

## About the Ion Chef™ System touchscreen interface

The Ion Chef™ System features a simple interface for loading chips, cleaning the instrument, and performing system maintenance and configuration tasks.



- ① **Set up run** button – Set up the Ion Chef™ template preparation and chip-loading routine. Choose **Step by Step** to have the instrument lead you stepwise through installation of reagents and consumables, or choose **Quick Start** to proceed if you have already installed the consumables.
- ② **Open Door** button.
- ③ **Notifications** button – View notifications about instrument status during and between runs.
- ④ **Quick Start** button – Proceed directly to the Quick Start instrument setup mode. User verifies the loading of a new pipette tip cartridge and an empty pipette tip rack to hold waste tips generated during the run, before proceeding to Deck Scan.
- ⑤ **Settings** button – Advance to a screen providing the following options:
  - **Notifications:** view notifications about instrument status during and between runs
  - **Instrument Settings:** view current settings and network configuration, set instrument name, adjust time zone
  - **Service tools:** access screens for service-related maintenance and instrument diagnostics
  - **Torrent Server:** add and manage Ion Torrent™ Server connections
  - **Clean Ion Chef:** proceed directly to the instrument cleaning routine
  - **Check for updates:** check availability of system software updates

## Workflow

The following workflow shows how to prepare and load samples using the Ion 550™ Kit – Chef and Ion Chef™ System for sequencing on an Ion S5™ XL Sequencer, Ion GeneStudio™ S5 Plus Sequencer, or Ion GeneStudio™ S5 Prime Sequencer.

### Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef Workflow

#### Create a Planned Run (page 19)

Create a planned run in Torrent Suite™ Software by entering run parameters for templating and sequencing your samples.



#### Dilute the library samples (page 26)

If needed, dilute sample and control libraries. Thaw the Reagents cartridge at room temperature for 45 minutes before use.



#### Load the Ion Chef™ Instrument (page 30)

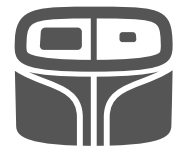
Load the Ion Chef™ Instrument with sequencing chips, reagents, and sample libraries.

#### Start the Ion Chef™ run (page 26)

Start the Ion Chef™ run by following the step-by-step instructions on the Ion Chef™ Instrument home touchscreen.

#### Unload the Ion Chef™ Instrument (page 50)

After the Ion Chef™ Run completes, unload the prepared sequencing chips from the Ion Chef™ Instrument.



#### Load the prepared sequencing chip into the sequencer and begin the sequencing run (page 56)

Load the prepared sequencing chips on a sequencer and follow the instructions on the touchscreen to start the sequencing run.

# 2

## Before you begin

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### Precautions

#### Avoid nucleic acid contamination

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**IMPORTANT!** A primary source of contamination is spurious DNA fragments from previous sample processing steps. Do not introduce amplified DNA into the library preparation laboratory or work area.

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#### Avoid chip damage

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**IMPORTANT!** To avoid possible damage to the chip due to electrostatic discharge, ground yourself before picking up a chip or placing a chip on a surface such as a lab bench. For example, touch the metal trim on the chip compartment before inserting or removing a chip from the chip clamp.

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#### Protection by equipment



**WARNING!** The protection that is provided by the equipment can be impaired if the instrument is operated outside the environment and use specifications, the user provides inadequate maintenance, or the equipment is used in a manner that is not specified by the manufacturer (Thermo Fisher Scientific).



## Types of Ion Chef™ runs possible with the Ion 550™ Kit – Chef

Three types of Ion Chef™ templating runs can be performed using the Ion 550™ Kit – Chef, depending on the number of chips that are loaded, and whether a single-chip run is the first or second run with a set of Reagents and Solutions cartridges. The Ion Chef™ Instrument tracks cartridge usage and uses the following names for the types of runs that are allowed with a given set of cartridges:

- Single-chip run 1—a run loading a single chip using a new set of cartridges
- Single-chip run 2—a run loading a single chip using a set of cartridges that have already been used in a single-chip run 1
- Dual-chip run—a run loading two chips using a new set of cartridges

## Guidelines for Ion 550™ Kit – Chef reagent use

To ensure the proper function of the Ion Chef™ System, handle the associated consumables and reagents according to the following guidelines:

- Store all Ion Chef™ System consumables and cartridges under the recommended conditions and in an upright position.
- Inspect all Ion Chef™ System consumables and cartridges for damage on arrival and again before use.
- Hold sequencing chips by gently gripping them by their edges.
- When the Ion Chef™ System is not in use, remove all consumables and reagents from the deck and close the instrument door.
- After performing a single-chip run 1, store the Ion 550™ Chef Reagents cartridge used in the run at 4°C. Store the Ion 550™ Chef Solutions cartridge used in the run at 15°C to 30°C. Use the cartridges in a single-chip run 2 within eight days of the first run.
- If you thaw a Ion 550™ Chef Reagents cartridge, and then decide not to perform an Ion Chef™ run, do not return the cartridge to the freezer for storage at –30°C to –10°C. Store a thawed Reagents cartridge at 4°C. Use the cartridge for a dual-chip run, or for both single-chip runs within eight days of thawing.
- It is good practice to label Reagents and Solutions cartridges that have been used in a single-chip run 1 with the date of the run.
- When performing a dual-chip run, always load two Ion 550™ Chips in the Ion Chef™ Instrument. You cannot load an Ion 550™ Chip with another chip type in an Ion Chef™ run.
- Use only Ion Torrent™ kits and supplies with the Ion Chef™ Instrument. The use of third-party reagents and supplies can adversely affect the performance of the Ion Chef™ Instrument and chips prepared.

- Remove and sequence chips as soon as possible after the Ion Chef™ System finishes loading them. If you cannot sequence a loaded chip immediately, store it in a chip storage container at 4°C until you are ready to run it (up to 24 hours maximum).

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**IMPORTANT!** If you choose to store a loaded chip, remove the chip from 4°C storage (but keep it in the chip storage container) at least 20 minutes before running it, allowing the chip to warm to room temperature.

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- If you sequence a second chip more than 24 hours after sequencer initialization was performed for the first chip, you must reinitialize the sequencer.

## Special guidelines for using the Ion 550™ Kit – Chef with the Ion Chef™ Instrument

- Ion Chef™ Instrument users must create a Planned Run for each chip in a templating run. Planned Runs are not optional for Ion Chef™ runs with the Ion 550™ Chip.
- The Ion Chef™ Instrument must be connected to a Ion Torrent™ Server to enable cartridge use tracking and proper use of the single-chip workflow with the Ion 550™ Kit – Chef.

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**Note:** If the instrument is not connected, see *Ion Chef™ Instrument Network Connection User Guide* (Pub. No. MAN0013444) for instructions on how to configure a direct or indirect network connection of the Ion Chef™ Instrument to a server.

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- To use the flexible workflow feature and enable cartridge use tracking between Ion Torrent™ Servers across multiple Ion Chef™ Instruments, servers must be linked in an Ion Mesh configuration. For further information on how to link servers in an Ion Mesh setup, go to **Software Administration** ▶ **Set up Ion Mesh** in *Torrent Suite™ Software Help*.

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**IMPORTANT!** If you have servers connected in an Ion Mesh configuration, and the connection to one server is disrupted or lost, cartridge tracking between servers is disabled. In this situation, an error message appears if you attempt to start a run. The run is not allowed to start because cartridge use status is not trackable. Ion Mesh communication must be restored to verify cartridge use status before the run can start.

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## Prepare the Ion Chef™ Instrument for use

- Ensure that the Ion Chef™ Instrument has been cleaned following the previous run. If not, clean the instrument *before* loading it with consumables.

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**Note:** For more information on the cleaning procedure, see Chapter 7, “Clean the Ion Chef™ System”.

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- Inspect the empty compartments of the Reagents and Solutions stations for condensation. Condensate can collect in these compartments, depending on temperature and humidity conditions. Before loading consumables into the instrument, wipe the compartments dry with a laboratory wipe or absorbent cloth, if needed.
- Thaw the Ion 550™ Chef Reagents cartridge at room temperature for 45 minutes before use.



# Create a Planned Run

- About Planned Runs ..... 19
- Create a Planned Run ..... 20
- Planned Run workflow key fields ..... 24

## About Planned Runs

Planned Runs are digital instructions that are created in Torrent Suite™ Software for controlling the template preparation and sequencing instruments. Planned Runs contain settings such as number of flows, kit types, barcodes, sample information, and reference files (if any). Planned Runs are also used to track samples, chips, and reagents throughout the workflow, from template preparation on the Ion Chef™ Instrument through sequencing on an Ion S5™ XL Sequencer, Ion GeneStudio™ S5 Plus Sequencer, or Ion GeneStudio™ S5 Prime Sequencer and subsequent data analysis. Each chip that is prepared in an Ion Chef™ run requires its own Planned Run.

---

**IMPORTANT!** For more information on creating a Planned Run in Torrent Suite™ Software, including a complete description of each field in the **Create Plan** workflow bar, see the *Torrent Suite™ Software Help*, available by clicking the **Help** button in the software.

---

## Create a Planned Run

**IMPORTANT!** If you are using the Ion 550™ Kit – Chef with libraries that were prepared using OncoPrint™ panels, see the corresponding OncoPrint™ Assay User Guide for assay-specific instructions on creating a Planned Run in Torrent Suite™ Software.

1. Sign in to the Torrent Suite™ Software.
2. In the **Plan** tab, in the **Templates** screen, select the application that you want to run (such as AmpliSeq DNA) from the left navigation menu, then click one of the following options:
  - **Plan New Run**—to plan a new run using the **Generic Sequencing** template for the selected application.
  - **⚙️ (Actions) ▶ Plan Run** in the row of the template—to plan a new run using a specific Planned Run template.

The screenshot shows the 'Plan' tab in the Torrent Suite™ Software. The left navigation menu is expanded to 'AmpliSeq DNA'. The main area displays a table of templates. The 'Plan New Run' button is highlighted in red. A context menu is open over the 'Oncomine Focus DNA for S5' template, with the 'Plan Run' option highlighted in blue.

Template Name	Instr	Sam... Prep	R... App	Barcodes	Reference	Project	Ion Reporter Account	Ion Reporter Workflow	Date	Source
Oncomine Focus DNA for S5				IonXpress	hg19				May 27 2018	ion torrent
Oncomine BRCA Research for S5				IonXpress	hg19					
Oncomine Comprehensive v3 DNA for 550				IonXpress	hg19					
Oncomine Comprehensive v3 DNA				IonXpress	hg19					
Oncomine Childhood Cancer Research DNA				IonXpress	hg19				Jan 20 2018	ion torrent

3. In the **Create Plan** workflow bar, review the **IonReporter** and **Research Application** steps, then make selections appropriate to your run. Click **Next**.
4. In the **Kits** step, make the following selections:
  - a. Select **Ion GeneStudio™ S5 System** from the **Instrument** dropdown list.
  - b. Select the Ion 550™ Chip from the **Chip Type** dropdown list.
  - c. Select the library kit used to prepare your libraries from the **Library Kit Type** dropdown list.
  - d. (Optional) For barcoded libraries, select the barcode set used during library preparation from the **Barcode Set** dropdown list.

- e. Select **IonChef** for **Template Kit**, then select **Ion 550 Kit-Chef** from the **Template Kit** dropdown list.
- f. Select **Ion S5 Sequencing Kit** from the **Sequencing Kit** dropdown list.
- g. Enter the appropriate number of flows in the **Flows** field.

Sequencing type	Number of flows
200-base-read	500
150-base-read	400
100-base-read	260

**IMPORTANT!** Do not exceed 1,200 total flows for one Ion S5™ Sequencing Reagents cartridge.

- h. Select or edit the remaining optional information fields appropriately for your run.
- i. Click **Next**.

Templates   Samples   Planned Runs   **Create Plan from Oncomine Focus DNA**

Create Plan from Onco   Ion Reporter   Research Application   **Kits**   Plugins   Projects   Plan

**Kits** Show Summary

**Instrument :**  
Ion GeneStudio™ S5 System

**Sample Preparation Kit (optional) :**  
[Dropdown]

**Library Kit Type :**  
Ion AmpliSeq 2.0 Library Kit

**Template Kit**  OneTouch  IonChef  IA :  
Ion 550 Kit-Chef

**Sequencing Kit :**  
Ion S5 Sequencing Kit

**Chip Type :**  
Ion 550™ Chip

**Control Sequence (optional) :**  
[Dropdown]

**Barcode Set (optional) :**  
IonCode

**Flows :**  
500

Mark as Duplicates Reads  :

Enable Realignment  :

**Advanced Settings** +

Use Recommended Defaults  Customize

← Previous Next →

5. Review the **Plugins** and **Projects** steps, then make selections appropriate to your run. Click **Next**.

6. In the **Plan** step, enter or make the following selections:

- a. Enter a Run Plan Name, then select Reference and BED files appropriate to your run.
- b. Enter the number of barcodes you are using in your combined library in the **Number of barcodes** field, then click  to the right of this field. Edit the auto-populated list of barcodes that appears, if needed.

---

**Note:** If you did not use barcode adapters in library preparation and did not select a **Barcode Set** in the **Kits** step, fields appear in the **Plan** step where you enter the number of chips that are used, then enter the sample name and chip barcode for each sample.

---

- c. Scan or enter the barcode of the Ion Chef™ Library Sample Tube into the **Sample Tube Label** field.
- d. Scan or enter the chip barcode into the **Chip Barcode** field.

e. Enter a sample name for each barcode in the appropriate **Sample Name (required)** fields.

Create Plan
Ion Reporter
Research Application
Kits
Plugins
Projects
Plan

**Template Name :**  
Oncomine Focus DNA Show Summary

**Run Plan Name (required) :**

**Analysis Parameters:**  Default (Recommended)  Custom Details +

**Default Reference & BED Files**

**Reference Library:**

**Target Regions:**

**Hotspot Regions:**

Use same reference & BED files for all barcodes

**Number of barcodes :**

**Sample Tube Label :**

**Chip Barcode :**

**Enter a sample name for each barcode used (require at least one sample)**   :

#	Barcode	Sample Name (required)	Control Type	Sample ID	Sample Description	Reference
1	IonCode_0101 (CTAAGGTAAC) ▼	Sample 1				
2	IonCode_0102 (TAAGGAGAAC) ▼	Sample 2				
3	IonCode_0103 (AAGAGGATTC) ▼	Sample 3				

7. After you have completed your selections, click **Plan Run** at the bottom right of the **Plan** step screen to save the run.

The run is listed in the **Planned Runs** screen under the name that you specified and is automatically used by the Ion Chef™ System when the associated sample is loaded.

If you are performing a dual-chip run, repeat step 2–step 7 to create a Planned Run for the second chip. You can copy the first Planned Run in the Planned Runs screen, then edit appropriately.

## Planned Run workflow key fields

Step/field name	Description
Ion Reporter	If Ion Reporter™ Software is installed and enabled, and you want to analyze the run data using the software, select the account and workflow.
Research Application	Select the sequencing application you are performing.
Instrument	Select <b>Ion GeneStudio™ S5 System</b> .
Chip Type	Select <b>Ion 550™ Chip</b> .
Library Kit Type	Select the kit used to prepare the library.
Template Kit	Select <b>Ion 550 Kit-Chef</b> .
Sequencing Kit	Select <b>Ion S5 Sequencing Kit</b> .
Barcode Set (optional)	<p>If you are using barcodes with:</p> <ul style="list-style-type: none"> <li>• <b>DNA libraries:</b> Select, for example: <ul style="list-style-type: none"> <li>– <b>IonXpress</b> for barcodes in the Ion Xpress™ Barcode Adapters Kits,</li> <li>– <b>IonCode</b> for barcodes in the IonCode™ Barcode Adapters 1–384 Kit, or</li> <li>– <b>Ion Dual Barcode Kit 1–96</b> for barcodes in the Ion Torrent™ Dual Barcode Kit 1–96.</li> </ul> </li> <li>• <b>RNA libraries prepared using the Ion Total RNA-Seq Kit v2:</b> Select the <b>IonXpressRNA</b> barcode set, which contains all 16 barcodes in the Ion Xpress™ RNA-Seq Barcode 1–16 Kit.</li> </ul> <p>If you are <i>not</i> using barcodes with:</p> <ul style="list-style-type: none"> <li>• <b>DNA libraries:</b> Leave the Barcode field blank.</li> <li>• <b>RNA libraries prepared using the Ion Total RNA-Seq Kit v2:</b> Select <b>RNA_Barcode_None</b> from the dropdown list. This will ensure that the proper trimming is performed on the resulting sequence when the RNA library does not have a barcode.</li> </ul>
Flows	Enter the appropriate number of flows for the read length (for example, 500 flows for 200-base-read sequencing).
Plugins	Select the appropriate plugins for your application.
Projects	Select or add a project to group your run data. You can include runs in multiple projects, and remove runs from a project at any time.
Planned Run Name	Enter a name for the run.
Reference Library	Select a reference library uploaded to the Ion Torrent™ Server, if any.
BED files	Select the <b>Target Regions</b> or <b>HotSpot Regions</b> BED file on the Ion Torrent™ Server, if any.



(continued)

Step/field name	Description
Sample Tube Label (Required)	<p>Enter or scan the barcode located on the Ion Chef™ Library Sample Tube that you will use to load the sample into the Ion Chef™ Instrument.</p> <p><b>IMPORTANT!</b> Each chip that is prepared in an Ion Chef™ run requires its own Planned Run. You must scan or enter the barcode of the appropriate Ion Chef™ Library Sample Tube for each Planned Run that is created for each individual chip.</p>
Chip Barcode	Enter or scan the chip barcode.
Sample Name (Required)	<p>Is Ion Reporter™ Uploader enabled?</p> <ul style="list-style-type: none"> <li>• <b>Yes</b> – Enter each sample name and select the appropriate values for workflow, relation, relation role, and set ID.</li> <li>• <b>No</b> – Scan the barcode of the Ion Chef™ Library Sample Tube into the "Sample Name" and "Sample Tube Label" fields for the specific sample.</li> </ul>
Monitoring Thresholds	Set thresholds for Bead Loading, Usable Sequence, and Key Signal. In the Torrent Browser <b>Monitor ▶ Runs in Progress</b> tab, an alert is displayed if the values for a run fall below the selected thresholds.

# 4

## Start the Ion Chef™ run

■ Materials required .....	26
■ Dilute the libraries .....	26
■ Prepare the consumables .....	28
■ Add the diluted library to the Library Sample Tube .....	29
■ Load the Ion Chef™ System .....	30
■ Start the Ion Chef™ run .....	44
■ Unload the chips for sequencing .....	50

This chapter describes how to perform the following procedures:

- Set up the Ion Chef™ Instrument for use by diluting the libraries, and loading the instrument with all of the required reagents and consumables
- Start an Ion Chef™ run
- Unload the chips for sequencing

### Materials required

- From the Ion 550™ Kit – Chef (Cat. No. A34541):
  - Ion S5™ Chef Supplies
  - Ion 550™ Chef Reagents cartridge
  - Ion 550™ Chef Solutions cartridge
- Ion 550™ Chip (1 or 2)
- Ion Chef™ S5 Series Chip Balance (for a single-chip run)
- Library or combined library stock solution (1 or 2)
- Molecular-biology grade nuclease-free water
- P200 pipettor and filtered tips

### Dilute the libraries

Sample libraries are quantified at the end of the library preparation workflow and are typically diluted to a concentration of 100 pM. The Ion Library Equalizer™ Kit procedure also normalizes library concentration to 100 pM. Barcoded libraries are typically mixed in an equimolar ratio to create a combined library. Libraries or combined libraries can require further dilution before use in Ion Chef™ template runs.

## Guidelines for library dilution

- The optimal input concentration for a given library preparation depends on the library and chip type, and often needs to be empirically determined starting from the recommended concentration.
- The recommendations for diluting your sample libraries represent optimal input concentrations for control libraries.
- Recommendations are based on qPCR quantification. If libraries are quantified with an Agilent™ 2100 Bioanalyzer™ instrument, a higher calculated concentration may need to be used for equivalent input.
- Prepare a fresh dilution of each library or combined library before use with the Ion Chef™ System, and use the library dilutions within 48 hours.
- Barcoded libraries are typically combined (or pooled) before templating to allow multiple libraries to be sequenced on a single chip. We recommend that you combine equal volumes of individual barcoded libraries at the same concentration (for example, 100 pM), then dilute the combined library to the final concentration recommended in the following table.
- Follow the recommendations of your library preparation user guide if it recommends a specific final library concentration for an Ion Chef™ run.

## Dilute the libraries

---

**IMPORTANT!** Before proceeding, dilute the library or combined library (for a single-chip run), or libraries to the optimal input concentration. The quality of your sequencing data relies greatly upon achieving the correct concentration of starting library.

---

Dilute the individual or combined library with nuclease-free water according to the following table. If performing a dual-chip run, dilute both of the libraries or combined libraries.

Library	Recommended concentration
Ion Total RNA-Seq	50–100 pM
Ion TargetSeq™ Exome	50 pM
Ion AmpliSeq™ Exome RDY	50–100 pM
Ion AmpliSeq™ Transcriptome Human Gene Expression	50–100 pM
Ion AmpliSeq™ Transcriptome Mouse Gene Expression	70 pM
Ion AmpliSeq™ Comprehensive Cancer Panel	50 pM
Oncomine™ cfDNA Assays	50 pM
Human CEPH Control 200 Library <sup>[1]</sup>	Dilute 1 µL into 24 µL nuclease-free water

<sup>[1]</sup> Obtained from the Ion S5™ Controls Kit Plus (Cat. No. A30729).

## Prepare the consumables

1. Before use, unbox, then remove the Ion 550™ Chef Reagents cartridge from its plastic packaging. Allow the cartridge to warm to room temperature for 45 minutes.

---

**IMPORTANT!** The Reagents cartridge must sit at room temperature for at least 45 minutes before use.

---

2. Remove all cartridges and consumables from their packaging, then place them on the bench next to the Ion Chef™ Instrument.

Prepare the following:

- Chip Adapter (2)
- Enrichment Cartridge v2
- Tip Cartridge v2
- PCR Plate, PCR Plate Frame, and Frame Seal v2
- Recovery Station Disposable Lid v2 (2)
- Recovery Tube v2 (12)
- Thawed Ion 550™ Chef Reagents cartridge (from step 1)
- Ion 550™ Chef Solutions cartridge

---

**IMPORTANT!** Before use,

- Gently tap the Reagents and Solutions cartridges on the bench to force the reagents to the bottoms of the tubes. If bubbles are present below the surface of the liquid, continue tapping until the bubbles are dislodged.
  - Ensure that liquid in the tubes of the Reagents cartridge is thawed and no floating ice is present.
- 

**Note:** When stored under normal conditions, a precipitate can form in some tubes of the Ion 550™ Chef Reagents cartridge. If present, load the cartridge as directed. The precipitate dissolves when the reagents are mixed during instrument operation.

---

## Add the diluted library to the Library Sample Tube

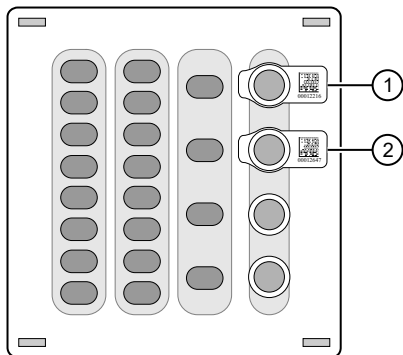
1. Pipet each diluted library or combined library (see “Dilute the libraries” on page 27) to the appropriate Library Sample Tube.
  - For a single-chip run 1 using a new Reagents cartridge, remove the Library Sample Tube from position A, then add 25  $\mu\text{L}$  of the diluted library or combined library. Leave the unused Library Sample Tube in Position B capped and in place during the run.
  - For a single-chip run 2 with a 1X used Reagents cartridge, remove the unused Library Sample Tube from Position B, then add 25  $\mu\text{L}$  of the diluted library or combined library. Load an empty capped 1.5-mL or 1.7-mL microcentrifuge tube into Position B and leave the tube in Position B during the run (see step 6 in “Load the Reagents cartridges—single-chip run” on page 33).

---

**Note:** For a single-chip run 2, the Library Sample Tube containing the library will be loaded into Position A, as described in step 5 in “Load the Reagents cartridges—single-chip run” on page 33.

---

- For a dual-chip run, remove both Library Sample Tubes (barcoded tubes) from Positions A and B, then pipet 25  $\mu\text{L}$  of each diluted library or combined library into the appropriate Library Sample Tube.



- ① Library Sample Tube in Position A
- ② Library Sample Tube in Position B

---

**Note:** If running the Human CEPH Control 200 Library from the Ion S5™ Controls Kit Plus (Cat. No. A30729), prepare the control library for use by diluting 1  $\mu\text{L}$  of stock library into 24  $\mu\text{L}$  nuclease-free water.

---

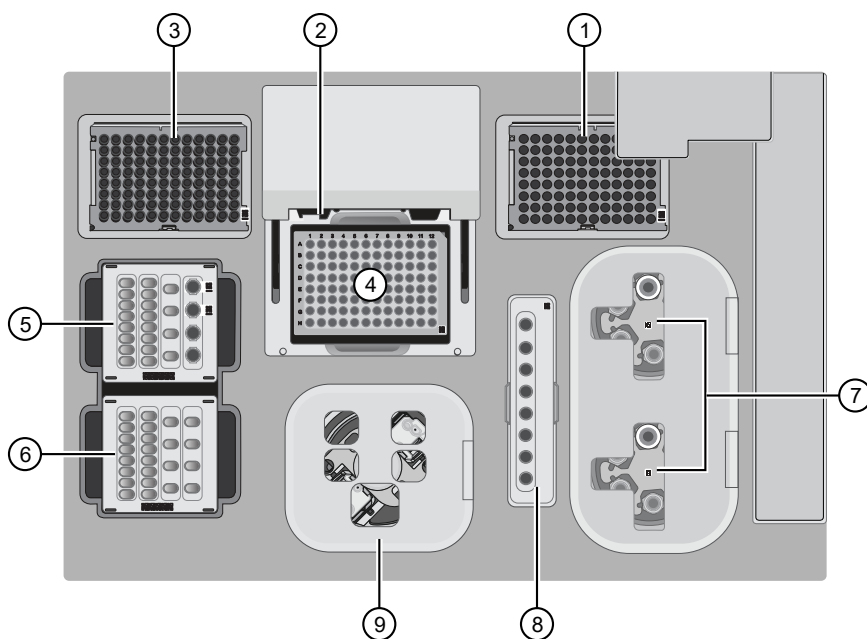
2. Cap and store the Library Sample Tubes on ice until you are ready to load onto the Ion Chef™ Instrument.

## Load the Ion Chef™ System

### IMPORTANT!

- Rated centrifuge speeds are intended only for operation with the provided buckets and approved consumable chips, tubes, and sample preparation reagents.
- The Chip-loading centrifuge is rated to operate at the listed rotational frequencies with the chip buckets, chips, and adapters. The centrifuge must be load-balanced. Proper care must be taken to load the buckets properly. If excessive vibrations arise, check that items are installed properly and rotors are load-balanced.
- Use only the materials supplied in the Ion 550™ Kit – Chef to run the centrifuges at the rated speeds. Do not remove or change the rotors. Inspect the buckets before each use to assure normal operation.
- Confirm that the instrument is powered on and was cleaned following the last use.
- Ensure that all components are clean and dry before loading them onto the Ion Chef™ Instrument.
- Ensure that the Reagents and Solutions station compartments are free of condensate before loading components.


Follow the procedures in this section to load the Ion Chef™ Instrument.



**Figure 1** A schematic of a loaded Ion Chef™ Instrument

- |   |   |
|---|---|
| ① Empty tip rack (move from new Tip Cartridge position) | ⑥ Ion 550™ Chef Solutions cartridge                     |
| ② Frame Seal v2   | ⑦ Recovery Tubes and Recovery Station Disposable Lid v2 |
| ③ New Tip Cartridge                                     | ⑧ Enrichment Cartridge v2                               |
| ④ PCR Plate and PCR Plate Frame                         | ⑨ Chip Adapter/Chip assemblies                          |
| ⑤ Ion 550™ Chef Reagents cartridge                      |   |

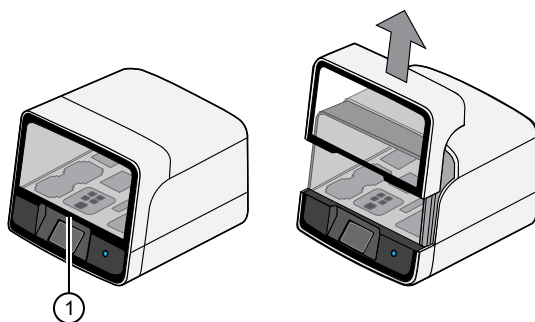
## Load the pipette tip racks and PCR Plate

1. Tap  (**Open Door**) in the instrument touchscreen to open the instrument door, then wait for the latch to open.
2. Lift the instrument door to the top of the travel until the latch mechanism engages.

---

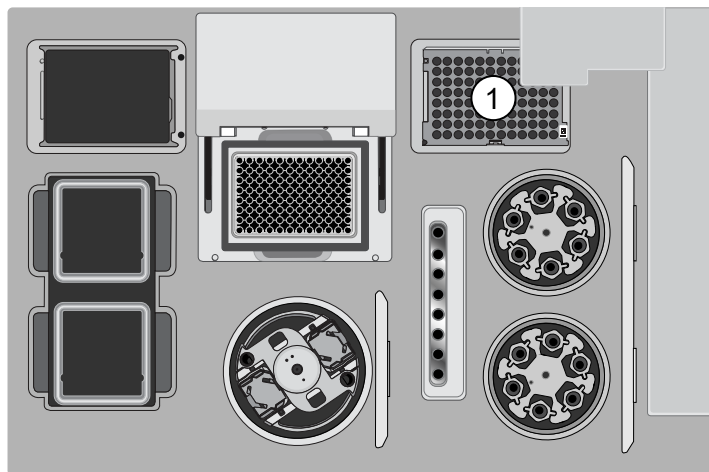
**IMPORTANT!** Lift the door from the center.

---



① Center of door

3. Load an empty pipette tip rack in the *Used (Waste)* Pipette Tip Position, then change gloves.



① Used Pipette Tip Position

---

### **IMPORTANT!**

- Ensure that the pipette tip rack in the *Used (Waste)* Pipette Tip Position does not contain any tips. The instrument aborts the run if tips are present in the *used* position.
- To prevent contamination, change gloves immediately after moving the empty pipette tip rack to the *Used (Waste)* Pipette Tip Position.

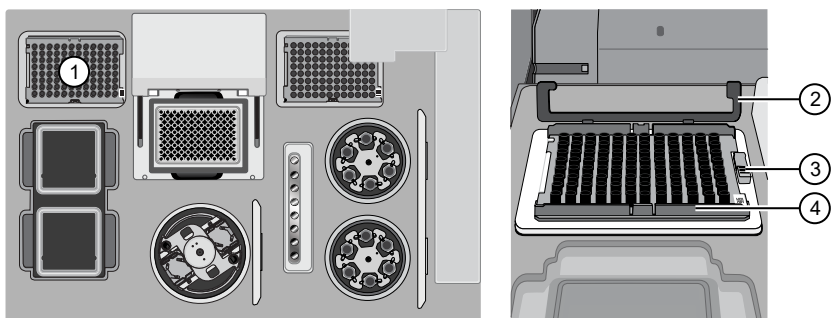
---

**Note:** A small amount of dried residue can be present in the tub of the empty pipette tip rack after a run. This residue does not affect the next run.

---

4. Unwrap a new Tip Cartridge v2 and remove the cover to expose the pipette tips, then load it in the *New Pipette Tip Position*. See the figure in step 5.

- Slide the catch forward to allow the locking bracket to pivot upward. Load the Tip Cartridge v2 into the New Pipette Tip Position, pull the bracket downward, then push the catch backward to lock the bracket and cartridge in place.

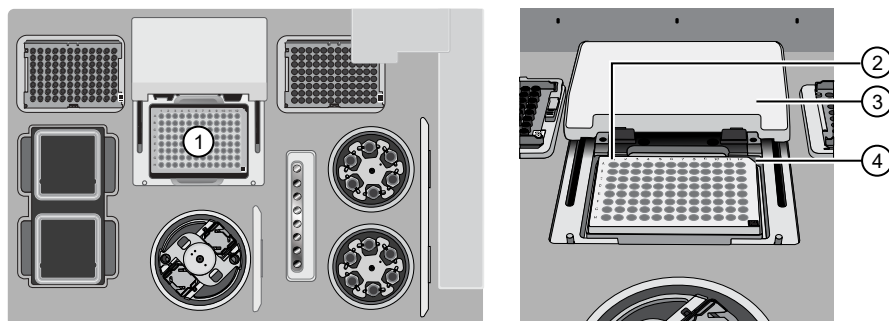


- New Pipette Tip Position
- Bracket

- Catch
- New Tip Cartridge v2

- Load a new PCR Plate into the thermal cycler sample block.
- With the white dot on the PCR Plate Frame facing upward, load the PCR Plate Frame into the thermo cycler sample block pressing down firmly on each corner, then slide a new Frame Seal v2 under the automated heated cover.

**IMPORTANT!** When the Frame Seal v2 is positioned correctly, its tabs project upward and contact the heated cover.



- Thermal cycler sample block
- Well A1

- Cover
- Keyed corner



## Load the Reagents cartridges—single-chip run

Use the following procedure if you are performing a single-chip run in an Ion Chef™ Instrument. If you are performing a dual-chip run, see “Load the Reagents cartridge—dual-chip run” on page 34.

---

**IMPORTANT!** Thaw the Reagents cartridge at room temperature for 45 minutes before use.

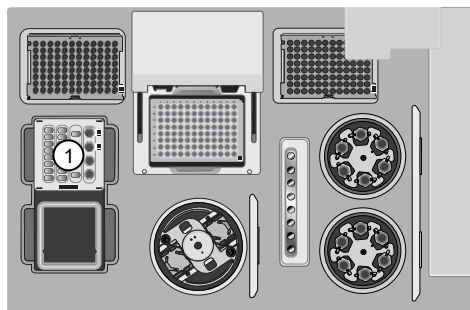
---

1. *(Single-chip run 1 using new Reagents cartridge only)* Label the Ion 550™ Chef Reagents cartridge with date of the run.
2. Gently tap the Reagents cartridge on the bench to force the reagents to the bottoms of the tubes.
3. If bubbles are present below the surface of the liquid, repeat step 2 until the bubbles are dislodged.
4. Load the cartridge into the Reagents station so that it snaps into place and is level on the deck.

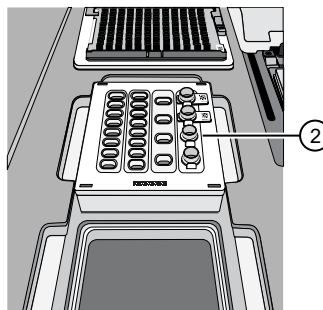
---

**IMPORTANT!** Do not force the Ion Chef™ cartridges into place. Each cartridge fits only one location on the deck and in one orientation. If a cartridge does not fit, ensure that you are loading the correct cartridge in the correct orientation.

---

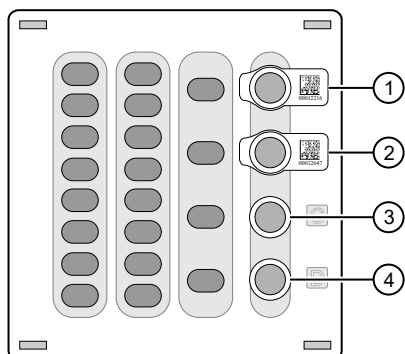


① Reagents station (4°C)



② Ion 550™ Chef Reagents cartridge

- Uncap, then load the prepared Library Sample Tube (from “Add the diluted library to the Library Sample Tube” on page 29) containing 25 µL of diluted library into Position A on the Reagents cartridge.



- Position A (Library)
- Position B (Empty Library Sample Tube for a single-chip run 1, or capped 1.5-mL microcentrifuge tube for a single-chip run 2)
- Position C (Empty tube)
- Position D (Empty tube)

---

**IMPORTANT!**

- Ensure that the Library Sample Tube containing the diluted library is loaded in Position A, and is uncapped before proceeding.
  - Ensure that the Library Sample Tube barcode is visible and oriented to the right.
  - Press down on the Library Sample Tube to ensure that it is firmly seated in the cartridge.
- 

The empty tubes in Positions B–D should remain capped during a run.

- (Single-chip run 2 using 1X used Reagents cartridge only) Load an empty capped 1.5-mL or 1.7-mL microcentrifuge tube into Position B.

---

**IMPORTANT!** Position B should be filled during the run. The presence of the empty microcentrifuge tube prevents the loss of chilled air from the Reagents station during the run.

---

Proceed to “Load the Solutions cartridge” on page 36.

## Load the Reagents cartridge—dual-chip run

Use the following procedure if you are performing a dual-chip run in an Ion Chef™ Instrument. See “Load the Reagents cartridges—single-chip run” on page 33 if you are performing a single-chip run.

---

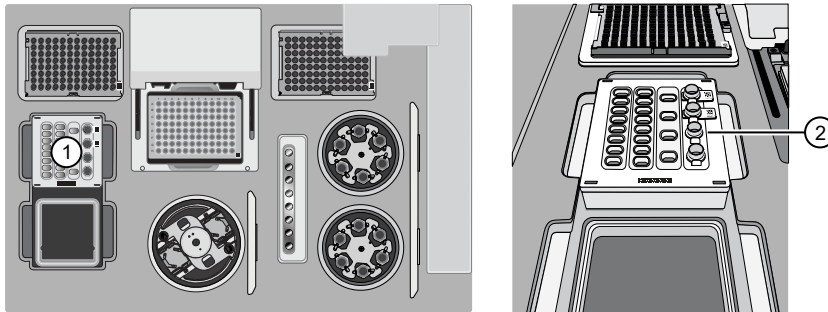
**IMPORTANT!** Thaw the Reagents cartridge at room temperature for 45 minutes before use.

---

- Gently tap the Ion 550™ Chef Reagents cartridge on the bench to force the reagents to the bottoms of the tubes.
- If bubbles are present below the surface of the liquid, repeat step 1 until the bubbles are dislodged.

3. Load the cartridge into the Reagents station so that it snaps into place and is level on the deck.

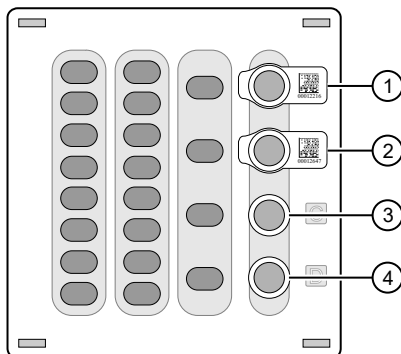
**IMPORTANT!** Do not force the Ion Chef™ cartridges into place. Each cartridge fits only one location on the deck and in one orientation. If a cartridge does not fit, ensure that you are loading the correct cartridge in the correct orientation.



① Reagents station (4°C)

② Ion 550™ Chef Reagents cartridge

4. Uncap, then load the two prepared Library Sample Tubes (from “Add the diluted library to the Library Sample Tube” on page 29), each containing 25 µL of diluted library, into Positions A and B on the Reagents cartridge.



① Position A (Library)

② Position B (Library)

③ Position C (Empty tube)

④ Position D (Empty tube)

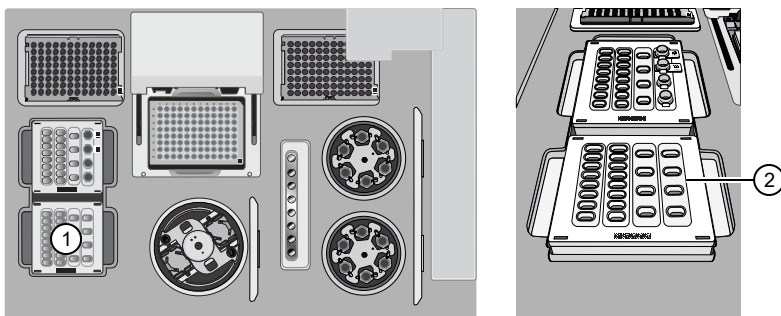
**IMPORTANT!**

- Ensure that the Library Sample Tube barcodes are visible and oriented to the right.
- Ensure that the caps of the Library Sample Tubes are removed before proceeding.
- Press down on the Library Sample Tubes to ensure that they are firmly seated in the cartridge.

The empty tubes in Positions C and D can remain capped during a run.

## Load the Solutions cartridge

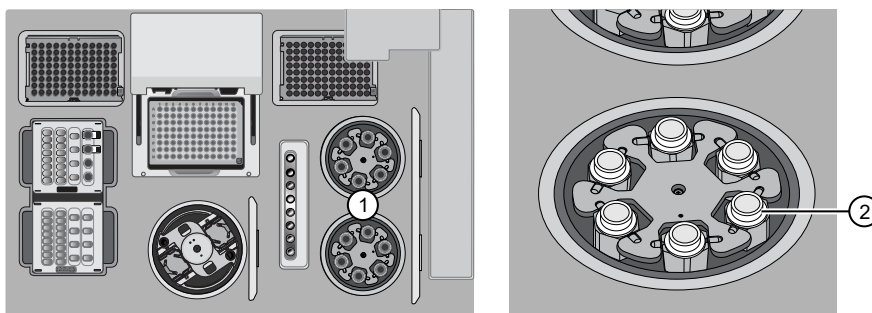
1. If the run is a single-chip run 1, label the Ion 550™ Chef Solutions cartridge with date of the run.
2. Gently tap the Solutions cartridge on the bench to force the reagents to the bottoms of the tubes.
3. Load the Solutions cartridge into the Solutions station until it snaps into place and is level on the deck.



- ① Solutions station (room temperature)  
② Ion 550™ Chef Solutions cartridge

## Load the Recovery Tubes and Enrichment Cartridge v2

1. Load six Recovery Tubes into each Recovery centrifuge.



- ① Recovery centrifuges  
② Recovery Tube v2

Before sealing each centrifuge, confirm that:

- The centrifuge is load-balanced with all required consumables.

---

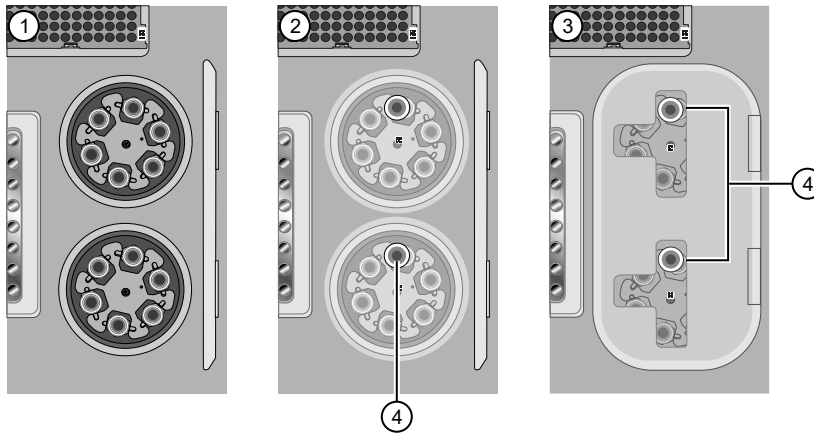
**IMPORTANT!** The centrifuge must be load-balanced.

---

- The buckets are securely seated in the centrifuge rotors.
- The buckets are oriented correctly in the centrifuge so that they pivot outwards.

2. Place a Recovery Station Disposable Lid v2 over each centrifuge by lining up the tab with the depression on the deck, then snap into place. Ensure that the lids snap completely into place by applying firm downward pressure along the lid perimeter.

- Close the hinged cover of the Recovery centrifuges. Confirm that the port of each disposable lid is positioned toward the rear of the instrument.



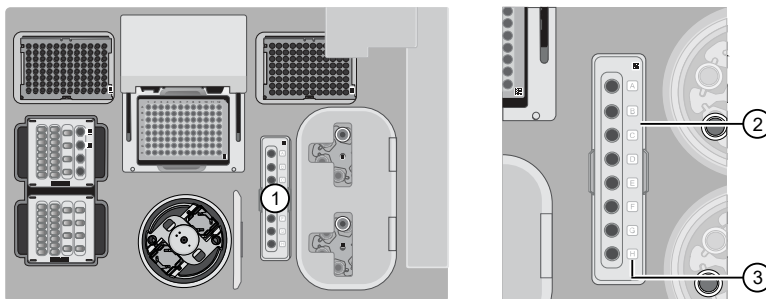
- ① Recovery Tubes installed
- ② Recovery Station Disposable Lid v2 installed
- ③ Recovery centrifuge cover closed
- ④ Port

**IMPORTANT!**

- Do not obstruct or place any object on top of the Recovery centrifuge cover.
- Use only the supplied materials, including buckets and disposables, to run the centrifuges at the rated speeds. Do not remove or change the rotors. To ensure normal operation, inspect the buckets before each use.

- Load the Enrichment Cartridge v2, then press down on the cartridge to ensure that it is level with the instrument deck.

**IMPORTANT!** Confirm that the Enrichment Cartridge v2 is loaded so that the lettering on the cartridge is right-side-up.



- ① Enrichment station
- ② Enrichment Cartridge v2
- ③ Lettering

## Load the Chip-loading centrifuge—single-chip run

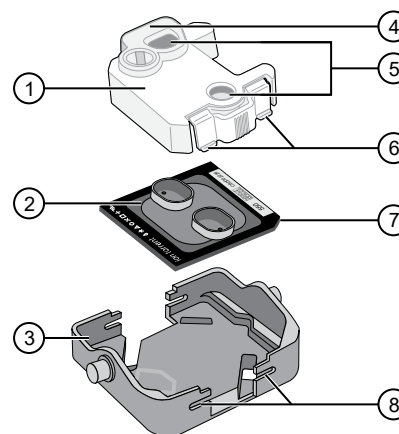
Load the Ion Chef™ S5 Series Chip Balance opposite to the Ion 550™ Chip in the Chip-loading centrifuge for a single-chip Ion Chef™ run. The Ion Chef™ S5 Series Chip Balance is provided in the Ion S5™ Installation Kit.



Ion Chef™ S5 Series Chip Balance

1. Load the sequencing chip into a centrifuge bucket, then attach a Chip Adapter to the assembly.
  - a. Place the chip in the chip-loading bucket with the keyed corners of the chip and bucket aligned, then align the wells of the Chip Adapter to the wells of the chip, orienting the adapter onto the chip so that the chip barcode is visible.
  - b. Place the adapter onto the chip, then insert the stationary tabs at the reservoir end of the adapter into the slots of the bucket.
  - c. Gently squeeze the flexible tabs at the other end of the adapter into the bucket slots until the adapter locks into place.
  - d. Confirm that the tabs at all four corners of the adapter are fitted into the slots in the centrifuge bucket.

**Note:** Loading can fail if the adapter is not attached securely.

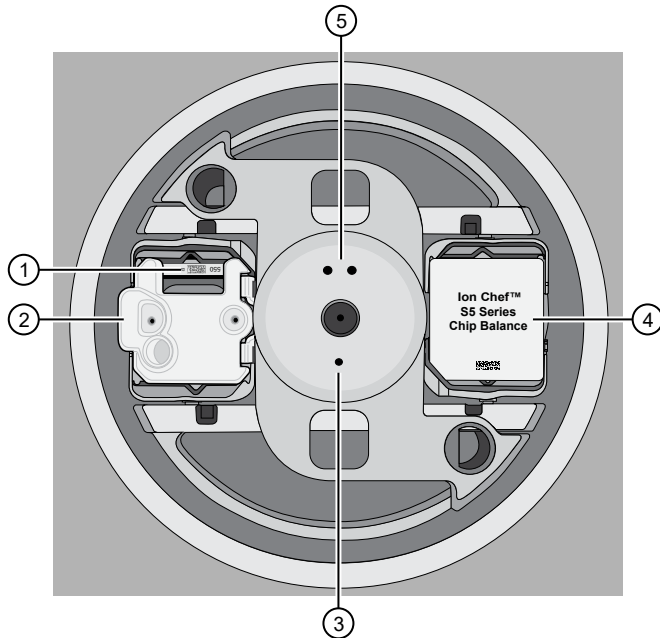


- ① Chip Adapter
- ② Ion 550™ Chip
- ③ Bucket
- ④ Reservoir end of Chip Adapter
- ⑤ Ports (align with chip)
- ⑥ Flexible tabs
- ⑦ Keyed corner (align with bucket)
- ⑧ Slots

2. Load the chip in Position 1 and the Ion Chef™ S5 Series Chip Balance in Position 2 of the Chip-loading centrifuge.

**IMPORTANT!** Do not use Ion Chef™ 314, 316/318, or P-Series versions of the chip balance with the Ion 550™ Chip. Each chip balance is weight-matched to the chip (and corresponding Chip Adapter) specified on the chip balance label.

**Note:** Position 1 of the Chip-loading centrifuge is the position 90° clockwise from the single hole in the rotor bucket cover at rest.



- ① Chip barcode
- ② Chip position 1 (Ion 550™ Chip/Chip Adapter)
- ③ Position 1 marker hole
- ④ Chip position 2 (Chip Balance)
- ⑤ Position 2 marker holes

The Ion Chef™ Instrument detects the presence of the single chip during Deck Scan before the run starts.

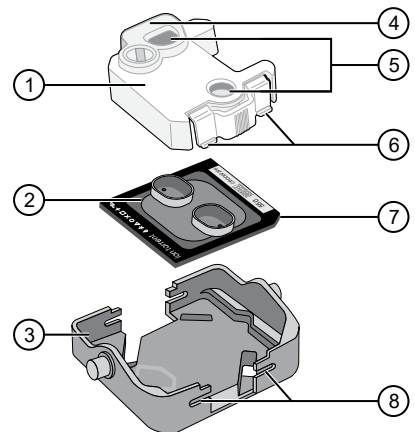
3. Ensure that the centrifuge is load-balanced, and the chip buckets are securely seated and oriented correctly in the centrifuge so that they pivot 90° outwards when touched. Then close the lid of the Chip-loading centrifuge.

**IMPORTANT!** Do not obstruct or place any object on top of the lid.

Proceed to “Confirm that consumables are correctly installed” on page 42.

## Load the Chip-loading centrifuge—dual-chip run

1. Load an Ion 550™ Chip into each centrifuge bucket, then attach a Chip Adapter to each assembly.
  - a. Place the chip in the chip-loading bucket with the keyed corners of the chip and bucket aligned, then align the wells of the Chip Adapter to the wells of the chip, orienting the adapter onto the chip so that the chip barcode is visible.
  - b. Place the adapter onto the chip, then insert the stationary tabs at the reservoir end of the adapter into the slots of the bucket.



① Chip Adapter

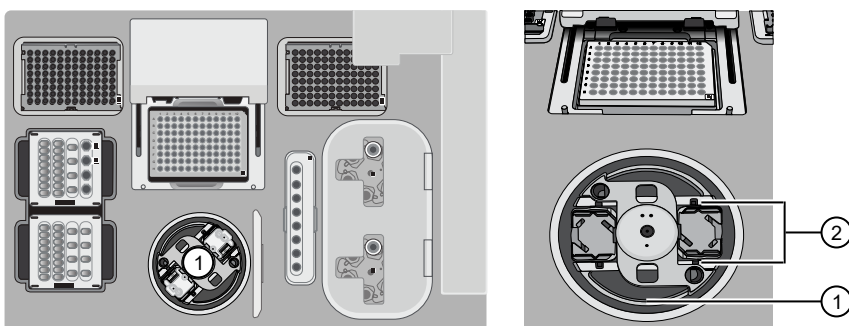
- c. Gently squeeze the flexible tabs at the other end of the adapter into the bucket slots until the adapter locks into place.
- d. Confirm that the tabs at all four corners of the adapter are fitted into the slots in the centrifuge bucket. Loading can fail if the adapter is not attached securely.
- ② Ion 550™ Chip
  - ③ Bucket
  - ④ Reservoir end of Chip Adapter
  - ⑤ Ports (align with chip)
  - ⑥ Flexible tabs
  - ⑦ Keyed corner (align with bucket)
  - ⑧ Slots

---

**Note:** If desired, you can label the tops of chips to distinguish them. Do not obstruct or overwrite the chip barcode with your label.

---

2. Load the adapter/chip/bucket assemblies into the Chip-loading centrifuge.



① Chip-loading centrifuge

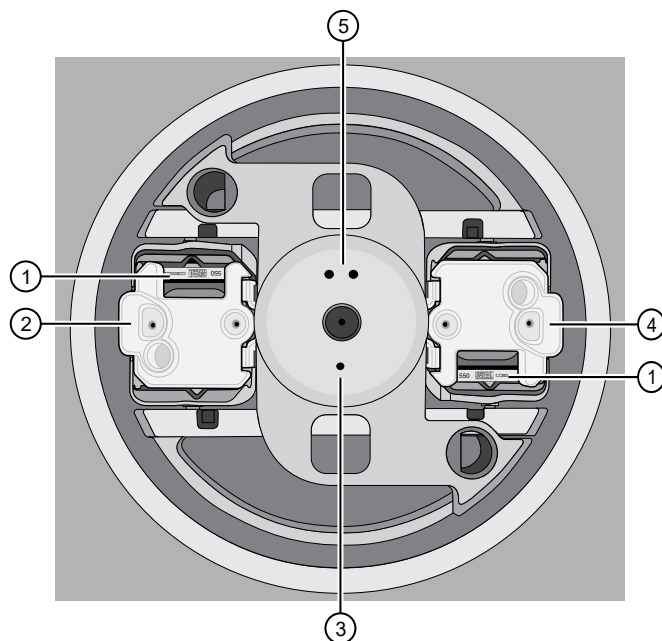
② Mounting grooves

---

**IMPORTANT!** When the Chip-loading centrifuge is loaded, ensure that each Chip Adapter is firmly attached to a bucket, and that the buckets are securely seated in the centrifuge rotors.

---





- ① Chip barcode
- ② Chip position 1
- ③ Position 1 marker hole

- ④ Chip position 2
- ⑤ Position 2 marker holes

---

**Note:** Position 1 of the Chip-loading centrifuge is the position 90° clockwise from the single hole in the rotor bucket cover at rest. The chip that is loaded in Position 1 is loaded with ISPs prepared from the DNA library in the Library Sample Tube loaded in Position A of the Reagents cartridge. The chip that is loaded in Position 2 of the centrifuge is loaded with ISPs prepared from the DNA library in the Library Sample Tube loaded in Position B of the Reagents cartridge.

---

3. Ensure that the centrifuge is load-balanced, and the chip buckets are securely seated and oriented correctly in the centrifuge so that they pivot 90° outwards when touched. Then close the lid of the Chip-loading centrifuge.
- 

**IMPORTANT!** Do not obstruct or place any object on top of the lid.

---

## Confirm that consumables are correctly installed

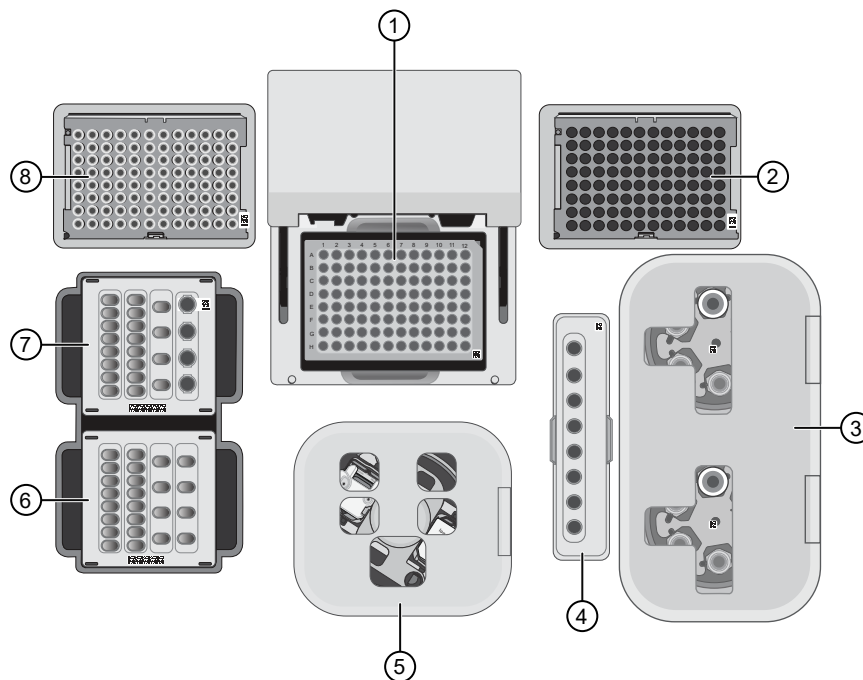
Before continuing:

- Confirm that each cartridge is at the correct location and in the correct orientation.
- Press down on all cartridges to confirm that they are firmly pressed into place.
- Confirm that all tubes in the Ion 550™ Chef Reagents cartridge are firmly pressed into place, and each Library Sample Tube that contains a library is uncapped.
- Confirm that the centrifuge lids are installed correctly so that the port is oriented toward the rear of the instrument.
- Confirm that the tube and chip buckets are seated securely in the rotor arms of the Chip-loading and Recovery centrifuges, and that the consumables they contain are correctly installed.



**CAUTION!** To ensure correct and safe instrument operation, you must confirm that all consumables are installed correctly to the deck before you start a run. The Ion Chef™ Instrument does not verify all aspects of the consumable setup prior to beginning each run.

## Ion Chef™ pre-run checklist



✓	Checklist item
<b>1 Thermal cycler sample block</b>	
<input type="checkbox"/>	A new PCR Plate is loaded in the thermal cycler sample block.
<input type="checkbox"/>	A PCR Plate Frame is loaded in the thermal cycler sample block.
<input type="checkbox"/>	A Frame Seal v2 is in place and oriented under the heated cover.
<b>2 Used Pipette Tip position</b>	
<input type="checkbox"/>	The tip rack from previous run is transferred from the New Pipette Tip position to the Used Pipette Tip position.
<b>3 Recovery centrifuge</b>	
<input type="checkbox"/>	Recovery Tubes (v2) are securely seated in the Recovery centrifuge buckets.
<input type="checkbox"/>	Recovery centrifuges are load-balanced.
<input type="checkbox"/>	Each Recovery Station Disposable Lid v2 is positioned so that the port is oriented toward the rear of the instrument.
<b>4 Enrichment station</b>	
<input type="checkbox"/>	Enrichment Cartridge v2 is pressed into place at the Enrichment station so that the cartridge is <b>firmly seated</b> and <b>level</b> with the deck.
<input type="checkbox"/>	Lettering on the cartridge is right-side up and positioned to the right of the enrichment tubes.

(continued)

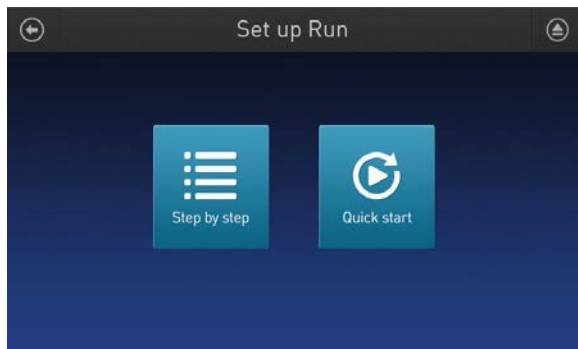
✓	Checklist item
<b>5 Chip-loading centrifuge</b>	
<input type="checkbox"/>	Adapter/chip assembly is correctly seated in the Chip-loading centrifuge bucket loaded in Position 1, with the adaptor clips inserted into the bucket slots.
<input type="checkbox"/>	Centrifuge buckets containing the adapter/chip assembly and Chip Balance, loaded in Position 2, are securely seated in the centrifuge rotor, and freely pivot 90° outwards.
<input type="checkbox"/>	Chip-loading centrifuge is load-balanced.
<b>6 Solutions station</b>	
<input type="checkbox"/>	Solutions cartridge is pressed into place at the Solutions station so that the cartridge is firmly seated and level with the deck.
<b>7 Reagents station</b>	
<input type="checkbox"/>	Reagents cartridge contents have been thawed at room temperature for 45 minutes.
<input type="checkbox"/>	Reagents cartridge tubes are uncapped and pressed into place at the Reagents station so that the tubes are <b>firmly seated</b> and <b>level</b> with the deck.
<input type="checkbox"/>	Library Sample tube is <b>firmly seated</b> and in the correct orientation with barcode facing the PCR Plate and cap removed.
<input type="checkbox"/>	For a single-chip run 2, ensure that a 1.5 mL centrifuge tube is inserted into the open position B.
<b>8 New Pipette Tip position</b>	
<input type="checkbox"/>	A new Tip Cartridge v2 is loaded in the New Pipette Tip position.
<input type="checkbox"/>	Bracket is pulled downward to lock the tip cartridge in place.

## Start the Ion Chef™ run

1. Ensure that you have loaded the instrument with all kits and consumables.
2. On the instrument home touchscreen, tap **Set up run**.

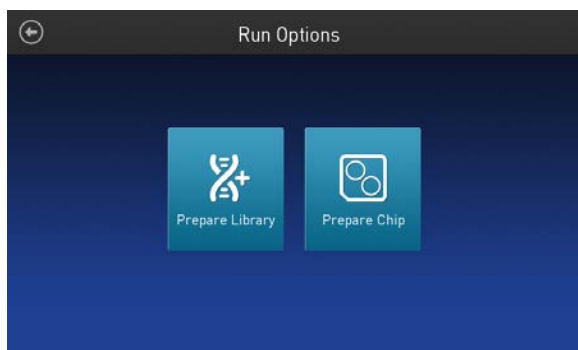


3. Tap **Step by Step** to have the instrument lead you through the instrument setup, or tap **Quick Start** to skip the instrument setup screens.



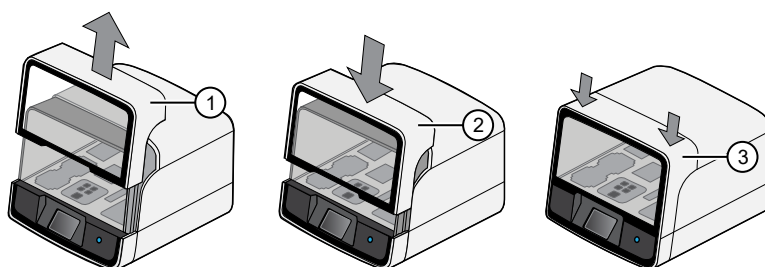
If you selected **Quick Start**, proceed to step 5, otherwise proceed to step 4.

4. (*Step by step setup only*) In the **Run Options** screen, tap **Prepare Chip** to select the templating run option.



5. Follow the on-screen instructions. When prompted, close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage.

**IMPORTANT!** Do not close the door by pulling it straight down from the open position. You must lift the door slightly before you can close it. Ensure that both sides of the door are locked after closing it.



- ① Lift door first
- ② Lower

- ③ Press down to lock

After the door closes, the instrument vision system activates.

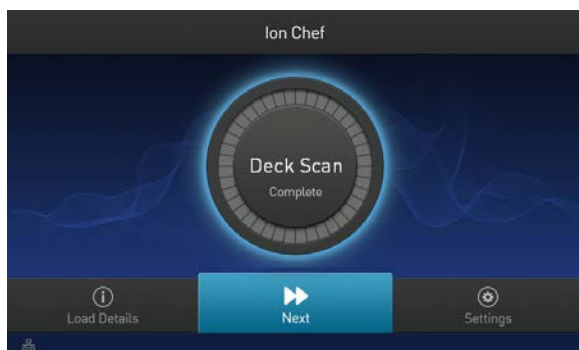
- When prompted, tap **Start check** to start Deck Scan. Wait while the instrument scans the barcodes of all consumables and reagents to confirm their presence and compatibility. During Deck Scan, the touchscreen displays warnings if the Ion Chef™ Instrument detects missing or incompatible consumables. You must address all warnings before the run can start. After you address each condition, tap **Yes** to continue.

---

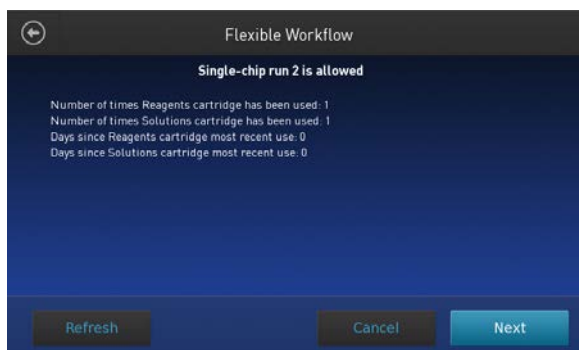
**IMPORTANT!** The Deck Scan function is not a substitute for manual inspection of the reagents and consumables on the Ion Chef™ Instrument before starting a run. To ensure proper and safe instrument operation, confirm that all consumables are installed correctly before you continue.

---

- After Deck Scan completes, tap **Next**.




- In the **Flexible Workflow** screen, confirm the run configuration that the instrument detects based on the consumables that are loaded and the usage status of the scanned Reagents and Solutions cartridges, then tap **Next** to proceed to the **Data Destination** screen.

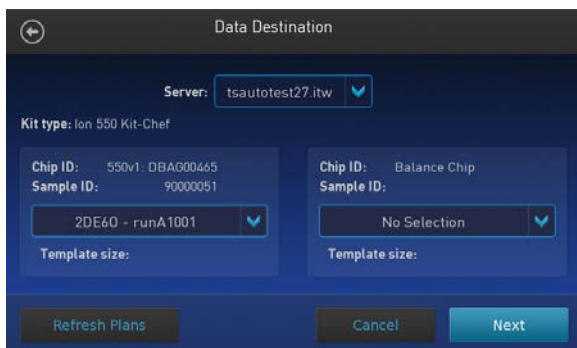


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**Note:** The instrument tracks the usage status of Ion 550™ Chef Reagents and Ion 550™ Chef Solutions cartridges and alerts you if it detects an incompatible configuration, or a problem with your Ion Mesh configuration. In the example shown, the instrument has detected a single-chip run 2 using Reagents and Solutions cartridges that have been used in a previous single-chip run 1. If you load these cartridges intending to start a dual-chip run, the instrument alerts you to an incompatible configuration and prevents the start of a run. The instrument also alerts you if the time between a single-chip run 1 and single-chip run 2 exceeds eight days.

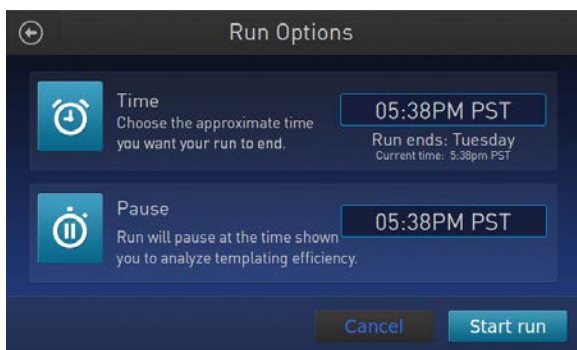
---

9. Ensure that the instrument displays the correct kit type, chip type, chip barcodes, and Planned Run. If the correct Planned Run does not display, tap the dropdown list  to select the correct Planned Run, then tap **Next**.



**IMPORTANT!** If the kit and chip type are not correct, ensure that you are using the correct kit and chip. If you are using the correct kit and chip, and an incorrect kit or chip type is displayed on the screen, contact Technical Support.

10. On the **Run Options** screen, tap the appropriate option to complete the run, then enter the desired time of run completion, if needed.



The Ion Chef™ Instrument provides two options for obtaining quality control (QC) samples that can be used to evaluate templating efficiency. Depending on your selection, the QC samples are made available either during (before chip loading) or after the run. In either case, you can obtain unenriched samples from the corresponding Library Sample Tubes at Positions A and B on the Reagents cartridge, or enriched samples from Positions A and E on the Enrichment Cartridge v2.

By selecting	You can obtain the QC samples	Approximate time after run start
Time	immediately after the run ends, at the time you specify:	<ul style="list-style-type: none"> <li>• 16 hours for a dual-chip run</li> <li>• 14 hours, 30 minutes for a single-chip run</li> </ul>
Pause	when the instrument pauses operation before the chip loading step:	<ul style="list-style-type: none"> <li>• 14 hours, 30 minutes for a dual-chip run</li> <li>• 13 hours for a single-chip run</li> </ul>

---

**Note:**

- The DNA library in the Library Sample Tube loaded in Position A of the Reagents cartridge is templated onto ISPs that can be sampled in Position E of the Enrichment Cartridge v2 after a run. The DNA library in the Library Sample Tube loaded in Position B of the Reagents cartridge is templated onto ISPs that can be sampled in Position A of the Enrichment Cartridge v2.
  - Select **Pause** if you are uncertain of library quality and want to evaluate templating efficiency before chips are loaded. If you do not pause the run, you can collect QC samples after the run. Save the samples until sequence analysis is complete to have them available for troubleshooting.
- 

11. On the **Run Options** screen, tap **Start run** to start the run.
- 

**Note:** If you must stop the run for any reason, tap **Cancel**, then tap **Yes** to confirm the cancellation.

---

If the Ion Chef™ Instrument encounters a problem during the run, it aborts the run and displays the error on the instrument touchscreen. If a run fails, perform the following tasks.

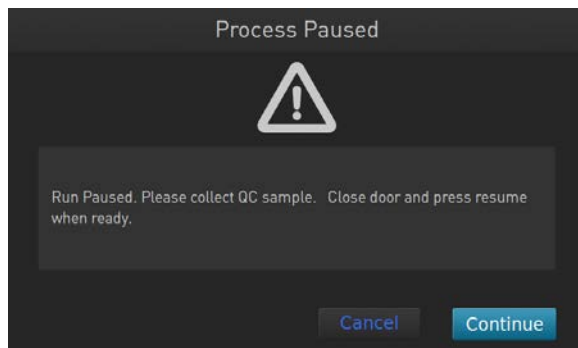
- a. Remove the consumables from the deck, then clean the instrument. If possible, retain the consumables for troubleshooting.
  - b. Reset, then reattempt the run. If the run fails again, contact Technical Support to troubleshoot the problem. Record the error message for reference.
12. Initialize the Ion S5™ XL Sequencer, Ion GeneStudio™ S5 Plus Sequencer, or Ion GeneStudio™ S5 Prime Sequencer at least 50 minutes before the Ion Chef™ Instrument finishes chip loading. See “Initialize the sequencer” on page 54.
- 

**Note:** Initialization of the instrument can be performed up to 24 hours before starting a sequencing run. If the intent is to perform two sequencing runs per initialization, the first run must be completed and the second run started within the 24-hour period. By initializing the sequencer before completion of chip loading, you ensure that the chips can be sequenced as soon as possible after loading is complete.

---



13. If you chose to pause the run to analyze the templating efficiency, remove the samples for testing when prompted to do so by the instrument (approximately 14.5 hours after the start of a dual-chip run).
  - a. When prompted to remove the QC sample, open the instrument door.




---

**IMPORTANT!** If you unintentionally close the instrument door before you obtain the QC samples, you must wait until the end of the run before you can collect them. You cannot pause the run or open the door after it has been closed.

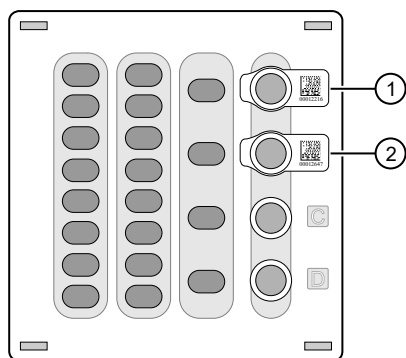
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- b. Transfer the entire volume of each QC sample from Positions A and B of the Ion 550™ Chef Reagents cartridge on the instrument deck to two new labeled microcentrifuge tubes.

---

**IMPORTANT!** Do not remove the Library Sample Tubes from the Ion 550™ Chef Reagents cartridge.

---



① Position A (QC Sample)

② Position B (QC Sample, if performing a dual-chip run)

- c. If you are performing quality assessment of enriched samples, transfer QC samples from positions A and E of the Enrichment Cartridge v2 to two new labeled microcentrifuge tubes.
    - d. Analyze the QC samples. For more information, see “Quality control of Ion 550™ ISPs” on page 75.
    - e. Close the instrument door, then tap **Continue** to complete the run.

14. When the run is complete, unload the Ion Chef™ Instrument and sequence the chips as soon as possible. You can collect QC samples from the Reagents and/or Enrichment cartridges if you have not done so already.

---

**IMPORTANT!** Liquid may be present in the chip wells after the Ion Chef™ run. Do NOT remove any residual liquid from the wells.

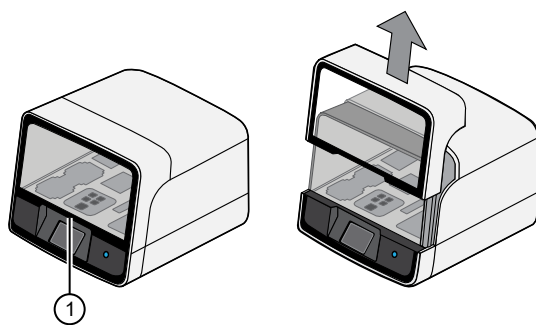
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**Note:** If you cannot sequence a loaded chip immediately or plan to sequence two chips per initialization, place the chip into a separate chip storage container and store at 4°C until you are ready to sequence it (up to 24 hours maximum).

---

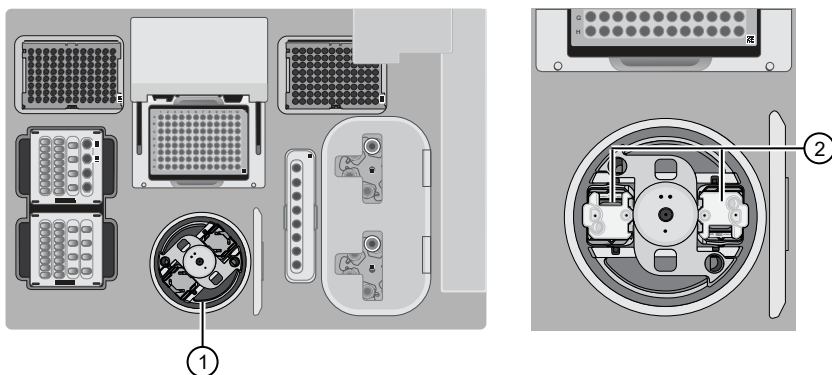
## Unload the chips for sequencing

1. Open the instrument door.
  - a. In the instrument touchscreen, tap  (**Open Door**) then wait for the latch to open.
  - b. Lift the instrument door to the top of the travel until the latch mechanism engages.



① Hold here, then lift

2. Open the lid of the Chip-loading centrifuge, then unload both adapter/chip/bucket assemblies from the instrument.

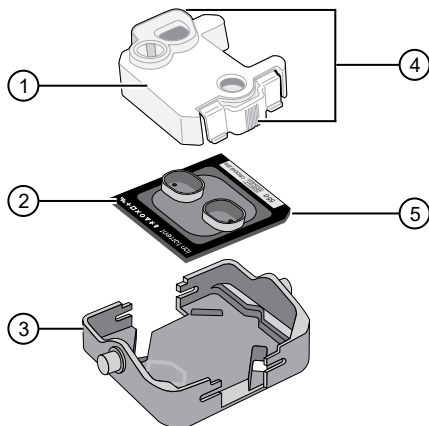


① Chip-loading centrifuge

② Remove

3. Unload each chip from the adapter/chip/bucket assembly.
  - a. Apply pressure to both ends of the Chip Adapter, then remove and discard the Chip Adapter.

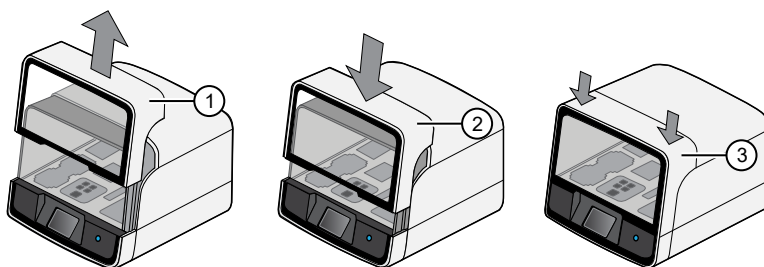
- b. Grasp the chip by its edges, carefully lift the chip out of the bucket, then set it aside on a clean, static-free surface. Return the bucket to the Chip-loading centrifuge.



- |                |                                |
|----------------|--------------------------------|
| ① Chip Adapter | ④ Press and remove adapter     |
| ② Ion Chip™    | ⑤ Remove from bucket carefully |
| ③ Bucket       |                                |

4. Close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage.

**IMPORTANT!** Do not close the door by pulling it straight down from the open position. You must lift the door slightly before you can close it. Ensure that both sides of the door are locked after closing it.



- |                   |                      |
|-------------------|----------------------|
| ① Lift door first | ③ Press down to lock |
| ② Lower           |                      |

5. Load one or both chips into a sequencer, then promptly start the sequencing run.

If you cannot sequence a loaded chip immediately, or plan to sequence two chips per initialization, place the chip into a separate chip storage container and store at 4°C until you are ready to sequence it (up to 24 hours maximum).

**IMPORTANT!**

- Liquid may be present in chip wells after the Ion Chef™ run. Do NOT remove any residual liquid from the wells.
- If you choose to store a loaded chip, remove the chip from 4°C storage (but keep it in the storage container) at least 20 minutes before running it, allowing the chip to warm to room temperature.

# 5

## Initialize the sequencer

- Ion GeneStudio™ S5 System component positions ..... 52
- Before you begin ..... 53
- When a manual cleaning of the sequencer is required ..... 53
- Initialize the sequencer ..... 54

Initialization takes ~50 minutes.

---

**Note:** The instructions in this chapter also apply to both Ion S5™ Systems and Ion S5™ XL Systems.

---

### Ion GeneStudio™ S5 System component positions

---

**Note:** The positions also apply to Ion S5™ Systems and Ion S5™ XL Systems.

---



- ① Touchscreen
- ② Power button
- ③ Ion S5™ Sequencing Reagents cartridge
- ④ Chip clamp
- ⑤ Ion S5™ Wash Solution bottle. Waste reservoir located behind the Ion S5™ Wash Solution bottle (shown on the right).
- ⑥ Ion S5™ Cleaning Solution bottle
- ⑦ Waste reservoir

---

**Note:**

- The system uses RFID technology to verify that the proper reagents are loaded in positions 3, 5, and 6. Reagents that exceed their expiration date or usage count generate an error message prompting the user to replace the reagent before performing the run.
  - RFID regulatory information is on the main screen under **Options ▶ Regulatory info**.
- 

## Before you begin

The Ion S5™ XL Sequencer, Ion GeneStudio™ S5 Plus Sequencer, and Ion GeneStudio™ S5 Prime Sequencer are equipped to verify the compatibility of each chip and consumable that is loaded during initialization and sequencing, and that these components do not exceed their expiration date. To avoid exceptions during initialization, inspect this information for each consumable before installing onto the instrument.

- Unbox the Ion S5™ Sequencing Reagents cartridge 45 minutes before use, then allow it to equilibrate to room temperature.  
Do not remove the Ion S5™ Sequencing Reagents cartridge from its packaging until immediately before loading, so that you can return the unused cartridge to storage if your sequencing run is delayed.
- Unbox the Ion S5™ Wash Solution bottle. Invert the bottle 5 times within its vacuum-sealed bag, then swirl at an angle to mix thoroughly.
- Remove the Ion S5™ Wash Solution bottle from its vacuum-sealed bag, then remove the red cap from the Ion S5™ Wash Solution and Ion S5™ Cleaning Solution bottles immediately before installing on the instrument.

## When a manual cleaning of the sequencer is required

The Ion S5™ XL Sequencer, Ion GeneStudio™ S5 Plus Sequencer, and Ion GeneStudio™ S5 Prime Sequencer require that a cleaning be performed before initialization. Cleaning is normally performed automatically at the completion of the previous sequencing run. When two sequencing runs are performed on a single initialization, the post-run cleaning is performed after the second sequencing run. However, if the "Enable post-run clean" checkbox (see "Start the sequencing run") is deselected to allow a second run, and a second run is not performed, the instrument will not allow the subsequent initialization to proceed until a manual cleaning has been performed. For more information on how to perform a manual cleaning, see "Perform a manual cleaning of the sequencer" on page 72.

If the sequencer is initialized and a sequencing run is not started within 24 hours, or a run is not started or completed due to a power failure or an abort, do not perform a manual cleaning. An instrument reset run is required before reinitialization. For more information, see "Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge" on page 73.

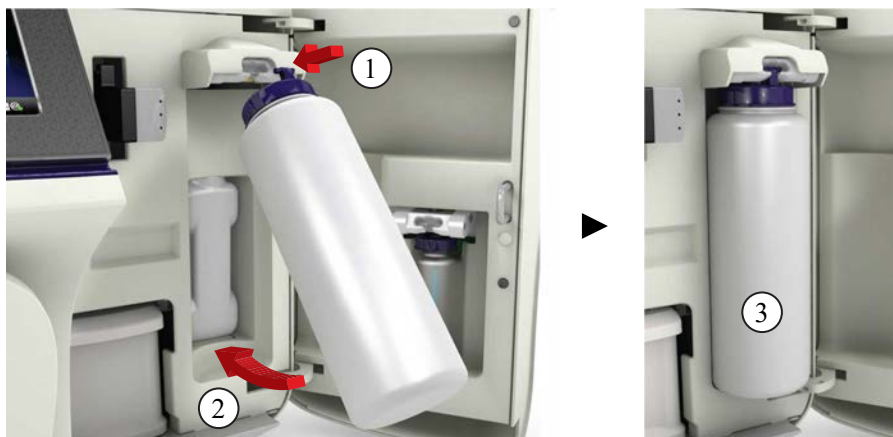
## Initialize the sequencer

1. In the instrument touchscreen main menu, tap **Initialize**.



The door, chip, and Reagent cartridge clamps unlock.

2. When prompted, remove the Ion S5™ Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.
3. Reinstall the empty waste reservoir.
4. Replace the expended Ion S5™ Sequencing Reagents cartridge with a new cartridge equilibrated to room temperature.
5. Ensure the new Ion S5™ Wash Solution bottle is thoroughly mixed. Then remove the red cap and install.



6. Ensure that the used sequencing chip from the previous run is properly seated in the chip clamp and the chip clamp is pushed in all the way.

7. If necessary, install a new Ion S5™ Cleaning Solution bottle.

---

**Note:** The Ion S5™ Cleaning Solution bottle contains sufficient reagent to complete 4 cleanings.

---

8. Close the door, then tap **Next**.

The instrument confirms that the consumables and chip are properly installed and that the Ion S5™ Cleaning Solution contains sufficient reagent to perform the post-run clean. Follow all on-screen recommendations to ensure proper installation of required consumables.

---

**IMPORTANT!** If the allowed number of post-run cleans has been met, the instrument prompts the user to replace the Ion S5™ Cleaning Solution bottle.

---

**Note:** If a **Checking Reagent: Failed** warning appears, see “Bottle leak check fails” on page 70.

---

9. When initialization is complete (~50 minutes), tap **Home**.

The instrument is now ready for a sequencing run.

For information on recycling and disposal of used components, see “Reagent consumables disposal” on page 59.

# 6

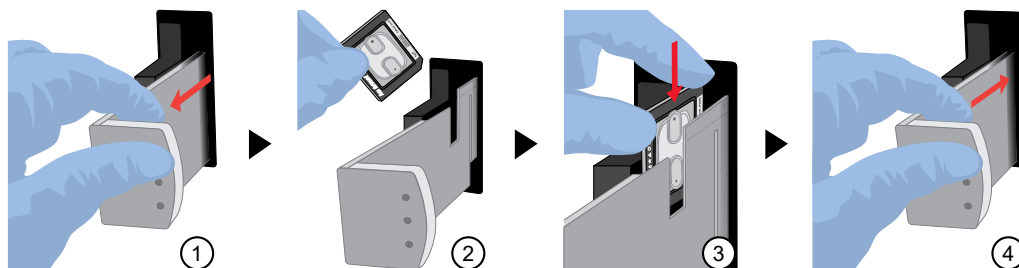
## Start the sequencing run

■ Chip handling guidelines .....	56
■ Start the sequencing run .....	57
■ Maintain the sequencer .....	59
■ Reagent consumables disposal .....	59

**Note:** The instructions in this chapter also apply to both Ion S5™ Systems and Ion S5™ XL Systems.

### Chip handling guidelines

To place a chip in the chip clamp, perform the steps that are described in the following figure:



- ① Slide the chip clamp out.
- ② Remove the chip currently in the clamp.
- ③ Place the appropriate loaded chip in the chip clamp with the chip notch in the bottom-front corner.

**Note:** Do not force the chip into the clamp. If the chip does not fit easily in the clamp, confirm that the notch is oriented as shown in the drawing.

- ④ Slide the metal tab in fully to engage the clamp, then close the instrument door.



## Start the sequencing run

We recommend that you start a sequencing run as soon as possible after chip loading and instrument initialization are complete. However, successful sequencing runs can be started up to 24 hours after instrument initialization.

---

### IMPORTANT!

- Do *not* press the power button during a run. Interrupting power to the instrument during a run can result in sequencing run failure and loss of sample.
- We recommend that you verify the status of the Planned Run you will use as "Planned" before inserting the loaded chip. If your Planned Run does not advance to the Planned status in Torrent Suite™ Software after successful completion of an Ion Chef™ run, see "Planned Run status does not advance to "Planned"" on page 69.

- 
1. After completion of initialization, tap **Run** in the instrument touchscreen. The door and chip clamp unlock.
  2. Remove the used sequencing chip, then secure a chip loaded with template-positive Ion Sphere™ Particles in the chip clamp.
  3. Push the chip clamp all the way in to engage, close the instrument door, then tap **Next**.

---

**Note:** Do not remove the chip from the chip clamp until completion of the run. Removing and reinserting the chip risks introducing air bubbles in the chip.

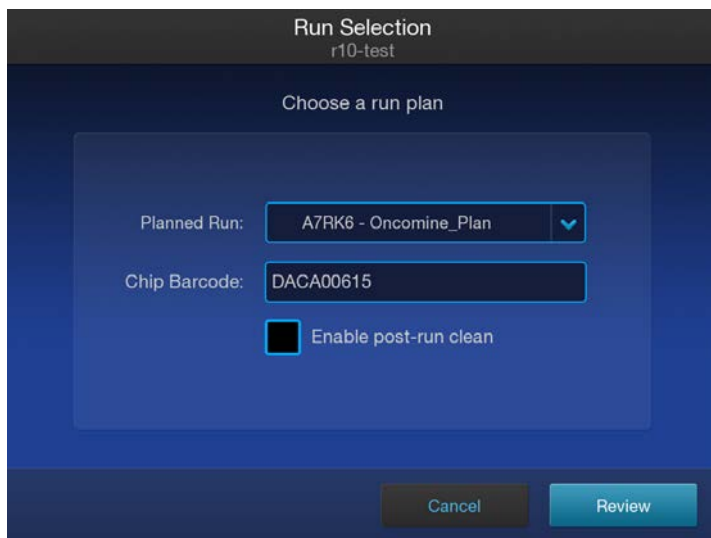
---

4. Confirm that the correct Planned Run has auto-populated. If this run is the first of two sequencing runs on this initialization, **deselect** the **Enable post-run clean** checkbox, then tap **Review**.

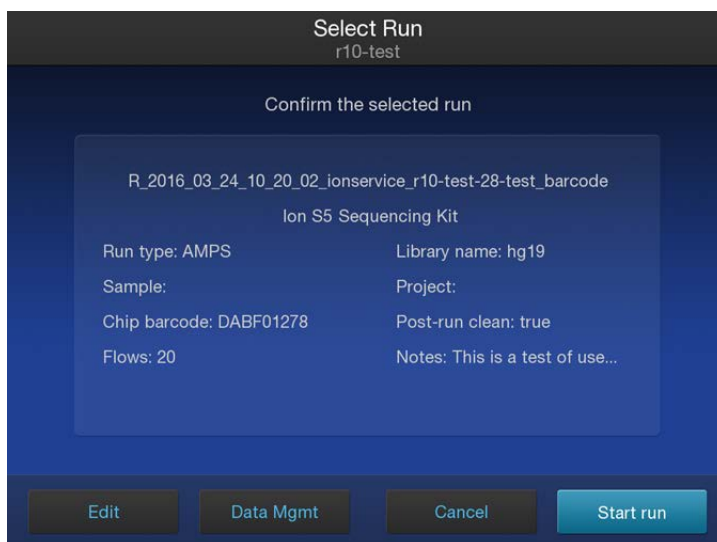
---

### IMPORTANT!

- Failure to deselect the checkbox results in a cleaning performed automatically after the first run. A second run is not available.
  - When starting the second sequencing run on a single initialization, ensure that the **Enable post-run clean** checkbox is selected so that the post-run cleaning is performed automatically.
-



5. Confirm that the remaining pre-populated settings are correct, or tap **Edit** to make changes if needed.



6. Confirm that the instrument door is closed, then tap **Start run** to begin the sequencing run.

---

**IMPORTANT!** During a run, do not open the instrument door, and avoid touching the instrument. Touching the instrument during the sequencing run can reduce the quality of the measurements.

---

When the sequencing run is complete, the instrument automatically performs the cleaning procedure unless the **Enable post-run clean** checkbox was deselected. After cleaning, the touchscreen returns to the main menu. Use Torrent Suite™ Software to review the results.

If you are sequencing a second chip on a single initialization, start the second run within 24 hours of start of initialization.

## Maintain the sequencer

### Materials required

- Lint-free wipes
- 70% isopropanol
- *(Optional)* 10% bleach solution

### Clean or decontaminate the sequencer

In the event of a spill or leak on or inside the instrument, perform the following steps.

---

**Note:** Dispose of all waste in appropriate liquid or solid waste containers.

---

1. Remove the Ion S5™ Wash Solution bottle, then remove and empty the waste reservoir.
2. Remove the Ion S5™ Sequencing Reagents cartridge.
3. Inspect the waste and nucleotide reagent bays for liquid.
4. Using absorbent paper soak up as much liquid as possible, then wash the affected area with 10% bleach solution.
5. Wipe the affected surfaces with 70% isopropanol, then allow to air-dry.

## Reagent consumables disposal

---

**IMPORTANT!** Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of Ion S5™ reagent consumables.

---

### CO<sub>2</sub> scrubber removal and disposal

To properly dispose of the CO<sub>2</sub> scrubber, you must first remove it from the Ion S5™ Sequencing Reagents cartridge.

1. Invert the Ion S5™ Sequencing Reagents cartridge over an appropriate receptacle to drain all residual liquid.
2. Wearing gloves, insert the Ion S5™ Cartridge Tool firmly into the CO<sub>2</sub> scrubber until the flange stops on the top of the cartridge.



Ion S5™ Cartridge Tool

3. Pull straight up on the tool while holding the nucleotide reagent cartridge down.



4. Remove the scrubber from the cartridge tool, then dispose of the scrubber according to applicable hazardous waste regulations.

The remaining nucleotide reagent cartridge should be disposed of appropriately.

## Recycle Ion S5™ Wash Solution and Ion S5™ Cleaning Solution bottles

The Ion S5™ Wash Solution and Ion S5™ Cleaning Solution bottles are made of recyclable plastic.

1. Open the expended bottle by unscrewing the cap.
2. Remove the cap, sipper, and filter, then pour any residual liquid into an appropriate receptacle.
3. Rinse the empty bottle with water. Pour out the rinse water into the same liquid waste receptacle.
4. Recycle or dispose of the clean bottle, cap, sipper, and filter according to applicable regulations.



# Clean the Ion Chef™ System

- About the cleaning protocol ..... 61
- Materials required ..... 61
- Clean the Ion Chef™ Instrument ..... 61

## About the cleaning protocol

The Ion Chef™ System includes an automated cleaning function that must be performed following every run. The cleaning routine is initiated from the Ion Chef™ Instrument touchscreen and is designed to minimize potential contamination. During the routine, the instrument irradiates the deck with ultraviolet light for 1 minute after all consumables have been removed from the instrument.

---

**IMPORTANT!** Although the Ion Chef™ Instrument cleaning routine provides some protection against contamination, it is not a substitute for good laboratory technique or precautions. When preparing DNA libraries for use or when preparing the Ion Chef™ Instrument, make certain to observe sterile laboratory procedures at all times to ensure minimal contamination.

---

## Materials required

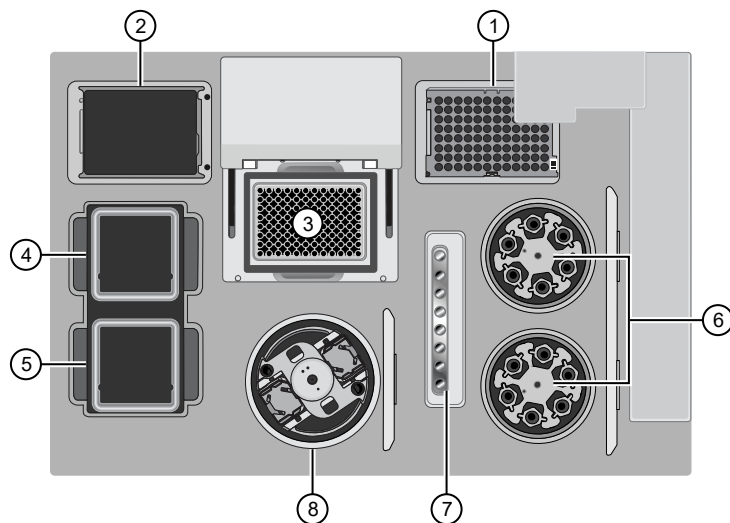
- Gloves, powder-free nitrile
- Isopropanol, 70% solution
- Wipes, lint-free

## Clean the Ion Chef™ Instrument

---

**IMPORTANT!** Clean the Ion Chef™ Instrument as described in the following pages after every run. To prevent contamination, do not operate the instrument unless it has been recently cleaned.

---




#### Ion Chef™ Instrument stations

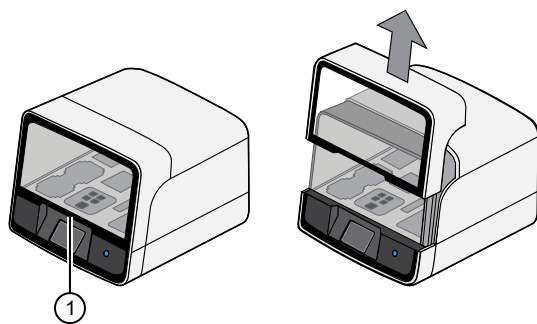
- |   |                           |
|---|---------------------------|
| ① Waste pipette tip position                                | ⑤ Solutions station       |
| ② Empty Tip Cartridge v2: move to waste pipette tip station | ⑥ Recovery centrifuges    |
| ③ Thermal cycler sample block                               | ⑦ Enrichment station      |
| ④ Reagents station  | ⑧ Chip-loading centrifuge |

## Remove used consumables

### IMPORTANT!

- Do not discard the empty Tip Cartridge.
- Do not discard the Reagents and Solutions cartridges if they were used for a single-chip run 1. If you have not done so already, write the date of use on the partially used cartridges and store them for a single-chip run 2.
- Ensure that you transfer the QC samples before you remove the Reagents cartridge.

1. Tap  (Open Door) in the instrument touchscreen, then wait for the latch to open.
2. Lift the instrument door to the top of the travel until the latch mechanism engages.



- ① Hold here, then lift

3. Remove, then discard the PCR Plate with the PCR Plate Frame and Frame Seal v2 from the thermal cycler sample block in unison.

---

**IMPORTANT!** Do not attempt to separate the PCR Plate Frame from the PCR Plate and Frame Seal v2, as this may cause PCR product to splash and contaminate the instrument deck.

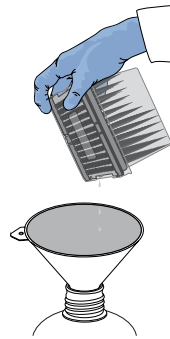
---

4. Remove, then discard the box of used pipette tips from the waste tip position.

---

**IMPORTANT!** Handle the disposable reservoir in the waste tip position with care. During the run, liquid waste collects in the reservoir. Dispose of the liquid waste by tipping the reservoir on one corner and pouring the waste into an appropriate waste container:

---



---

**IMPORTANT!** Do not reuse the waste pipette tip rack. Always move the empty Tip Cartridge from the new tip position to the waste tip position.

---

5. Move the empty Tip Cartridge to the waste tip position.
6. Remove and discard, or remove and store the cartridges for a second run:
  - If the Ion Chef™ run was a single-chip run 1, remove and **store** the
    - Ion 550™ Chef Reagents cartridge at 4°C for up to eight days
    - Ion 550™ Chef Solutions cartridge at room temperature for up to eight daysRemove and discard the Enrichment Cartridge v2.
  - If the Ion Chef™ run was a single-chip run 2, or a dual-chip run, remove, then **discard** the
    - Ion 550™ Chef Reagents cartridge
    - Ion 550™ Chef Solutions cartridge
    - Enrichment Cartridge v2
7. Remove, then discard the consumables from the Recovery centrifuges, including the:
  - Recovery Station Disposable Lid v2
  - Recovery Tube v2
8. Close the Chip-loading centrifuge cover.

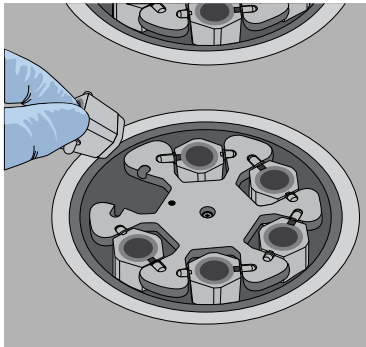
## Inspect and clean the Recovery centrifuges and buckets

1. Inspect the Recovery centrifuges, then clean the components if excess liquid is present.

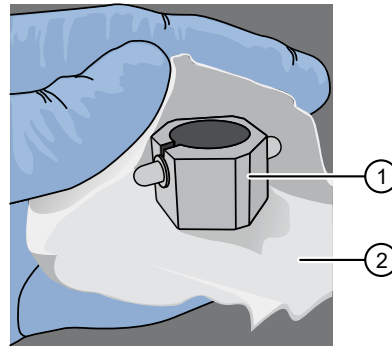
Is liquid present?	Action
No	Proceed to “Start the cleaning” on page 65.
Yes	Clean the centrifuge bowl and buckets as described below. <b>IMPORTANT!</b> Clean the Recovery centrifuges occasionally, only when excess liquid is noticeable in the bowl and/or buckets. You do <i>not</i> need to clean the centrifuges after every run.

**IMPORTANT!** Wear powder-free, nitrile gloves when cleaning the Recovery centrifuge.

2. Remove the buckets from the Recovery centrifuges. Clean the inside and outside of each bucket using a lint-free wipe, then place the buckets on a clean, dry surface while you clean the centrifuge.

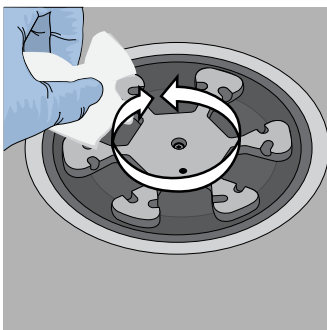


① Bucket

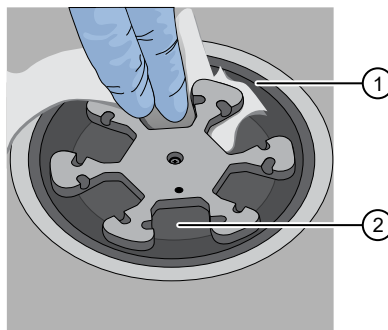


② Lint-free wipe

3. Use lint-free wipes to remove all fluid from inside the centrifuge bowl.



① Inside rim of the centrifuge

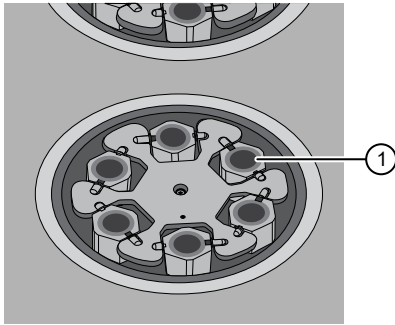


② Bottom of the centrifuge bowls

4. Use lint-free wipes treated with 70% isopropanol to clean the following parts.
  - Inside rim of the centrifuge.
  - Bottom of the centrifuge bowl.
  - Outside and inside of the centrifuge buckets.



5. Dry the centrifuge and buckets with lint-free wipes.
6. Install the centrifuge buckets, then close the Recovery centrifuge cover.



① Buckets (cleaned and installed)

## Start the cleaning

1. Close the instrument door by first lifting it up slightly to disengage the locking mechanism, then pushing down on the door until the locks engage.

---

**IMPORTANT!** Before closing the door, ensure the covers of the Chip-loading and Recovery centrifuges are closed.

---

2. To start the cleaning, tap **Next** on the Ion Chef™ Instrument touchscreen that appears after run completion.

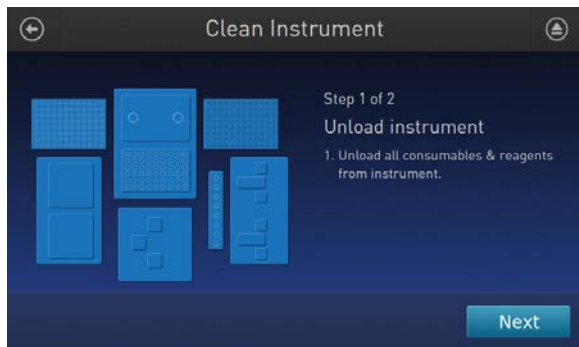


---

**Note:** You can also clean the instrument at any time starting from the home touchscreen. Tap **Settings** ▶ **Clean Ion Chef**.

---

3. Confirm that you have removed all consumables from the Ion Chef™ Instrument, except the empty pipette tip rack in the waste tip position, then tap **Next**.



4. With the door closed, tap **Start**. The instrument performs a Deck Scan before starting the cleaning routine. The Ion Chef™ Instrument stops ventilation, then illuminates the ultraviolet (UV) light in the instrument for ~1 minute.



**CAUTION!** The Ion Chef™ Instrument emits UV light at 254 nm. Wear appropriate eye wear, protective clothing, and gloves when working near the instrument. Do not look directly at the UV light while it is illuminated during the cleaning routine.



# Troubleshooting

- View troubleshooting and FAQs online ..... 67
- Ion Chef™ System setup and operation ..... 67
- Ion GeneStudio™ S5 System setup and operation ..... 70
- Ion Sphere™ Assay troubleshooting table ..... 71

## View troubleshooting and FAQs online


Visit our online Support Centers and FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated to ensure accurate and thorough content.

- For the Next-Generation Sequencing Support Center: <http://thermofisher.com/ngssupport>
- For FAQs for this product: [https://www.thermofisher.com/search/results?query=ion%20550&persona=DocSupport&refinementAction=true&personaClicked=true&filter=document.result\\_type\\_s%3AProduct%20FAQs](https://www.thermofisher.com/search/results?query=ion%20550&persona=DocSupport&refinementAction=true&personaClicked=true&filter=document.result_type_s%3AProduct%20FAQs)
- To browse the FAQ database and search using keywords: [thermofisher.com/faqs](http://thermofisher.com/faqs)

## Ion Chef™ System setup and operation


Observation	Possible cause	Recommended action
Instrument does not display the home screen when powered on	Multiple causes are possible.	<ol style="list-style-type: none"> <li>1. Power off the instrument, wait 30 seconds, then power on the instrument.</li> <li>2. If the instrument fails again, contact Technical Support.</li> </ol>
The instrument door cannot be opened	An obstruction was present on or around the door mechanism.	Remove the obstruction blocking the door, then operate the instrument normally.
	A hardware or software error occurred.	Contact Technical Support to report the problem and for further assistance.
The instrument stops during a run	The instrument encountered an internal error.	<ol style="list-style-type: none"> <li>1. Record the error displayed on the instrument display, then tap <b>OK</b>.</li> <li>2. Contact Technical Support to report the problem and for further assistance.</li> </ol>
	A consumable was improperly loaded.	Ensure that all consumables are loaded according to the instructions provided.

Observation	Possible cause	Recommended action
Liquid residue is present in the Recovery centrifuges following a run	During normal instrument operation, a noticeable coating of liquid collected on the bowl and buckets of the Recovery centrifuges after repeated runs.	Remove the residue as instructed in “Inspect and clean the Recovery centrifuges and buckets” on page 64.
Instrument will not begin a run	The instrument has encountered a Deck Scan error (one or more consumables are absent or loaded improperly).	<ol style="list-style-type: none"> <li>1. Confirm that the touchscreen does not display any Deck Scan warnings. If alarms are present, note the error(s) displayed, replace the missing consumable as directed, tap <b>No</b> when prompted then tap <b>Next</b> to cancel the run. After returning to the home screen, restart the run.</li> <li>2. If the error persists, confirm that: <ul style="list-style-type: none"> <li>• All buckets are seated correctly in the rotors of the Recovery and Chip-Loading Centrifuges.</li> <li>• All cartridges are loaded correctly and are level on the instrument deck.</li> <li>• The barcodes of the Library Sample Tubes are visible and positioned correctly.</li> <li>• The tube(s) containing a library sample are both present <i>and</i> uncapped on the Ion 550™ Chef Reagents ( Library Sample Tubes, NaOH tube, and the empty tube).</li> </ul> </li> <li>3. If the error persists after you check the consumables on the instrument deck, do one of the following: <ul style="list-style-type: none"> <li>• If you are confident that the Ion Chef™ Instrument is set up correctly and you are comfortable disregarding the warnings, tap <b>YES</b> following Deck Scan to proceed with the run.</li> <li>• If the instrument cannot begin the run, contact Technical Support for further assistance.</li> </ul> </li> </ol>
	Multiple Torrent Servers are connected in an Ion Mesh configuration, and connection to one of the servers is lost.	<ol style="list-style-type: none"> <li>1. Restore the connection to all Ion Torrent™ Servers to reestablish the Ion Mesh configuration.</li> <li>2. Return to the Ion Chef™ home screen, then tap <b>Set up run</b>. Proceed with run set up.</li> </ol>

Observation	Possible cause	Recommended action
Instrument will not begin a run <i>(continued)</i>	The instrument has encountered an internal error.	<ol style="list-style-type: none"> <li>Record the error displayed on the instrument display, then tap <b>OK</b>.</li> <li>Contact Technical Support to report the problem and for further assistance.</li> </ol>
Instrument displays one or more alerts during a run	The instrument detected one or more problems during the run.	<p>After the instrument completes the run, contact Technical Support. If possible, capture an image of the alert or error message to help troubleshoot.</p> <p><b>IMPORTANT!</b> The detected problem might impact the performance of the sequencing run.</p>
	<ul style="list-style-type: none"> <li>Network connection to the server was interrupted.</li> <li>User name or password was incorrect.</li> </ul>	<ol style="list-style-type: none"> <li>Tap the Instrument status button to view the alert(s).</li> <li>In the Instrument status screen, confirm that the name of the Torrent Server connection is red.</li> <li>Contact your network administrator to confirm that:                             <ul style="list-style-type: none"> <li>The Torrent Server can be accessed from the network port used by the Ion Chef™ Instrument. If not, troubleshoot the network connection.</li> <li>The user name and password used by the Ion Chef™ Instrument are valid. If not, contact the server administrator to renew the credentials.</li> </ul> </li> <li>If the alert persists, capture an image of the alert or error message, if possible, to help troubleshoot, then contact Technical Support for further assistance.</li> </ol>
Planned Run status does not advance to "Planned"  <b>Details:</b> The status of a successfully completed run remains listed as "Reserved" in Torrent Suite™ Software.	Connectivity was temporarily lost or interrupted between the Ion Chef™ Instrument and Torrent Suite™ Software.	Manually change the status of the run to "Planned". <ol style="list-style-type: none"> <li>Sign in to the Torrent Suite™ Software.</li> <li>In the <b>Plan</b> tab, click <b>Planned Runs</b>.</li> <li>For the Planned Run of interest, click  <b>Completed on Chef</b>.</li> </ol> The status changes to "Planned".

# Ion GeneStudio™ S5 System setup and operation

## Instrument alarms and events

Observation	Possible cause	Recommended action
<p>Red "Alarms" and/or "Events" message in Main Menu</p> 	<ul style="list-style-type: none"> <li>Available software updates were detected.</li> <li>Connectivity issues were detected.</li> <li>Instrument did not detect required files or hardware.</li> </ul>	<p>Tap the red <b>Alarms</b> icon to see detailed messages.</p> <ul style="list-style-type: none"> <li>If a message states "Newer Software Available": <ul style="list-style-type: none"> <li><b>IMPORTANT!</b> After updates are installed, the instrument must be restarted. <ol style="list-style-type: none"> <li>In the main menu, tap <b>Settings</b> ▶ <b>Check for Updates</b>.</li> <li>Select the <b>Released Updates</b> checkbox, then tap <b>Update</b>.</li> <li>When installation is complete, follow the onscreen prompts to restart the instrument. <ul style="list-style-type: none"> <li><b>Note:</b> In some cases, the instrument restarts automatically after software installation.</li> </ul> </li> </ol> </li> <li>If a message states "No Connectivity to Torrent Server", "No Connectivity to ftp server", or "Network Manager not connected", disconnect and re-connect the ethernet cable, confirm that the router is operational, and verify that the network is up and running.</li> <li>For any other messages: <ol style="list-style-type: none"> <li>Power off the instrument: On the home screen, tap <b>Settings</b> ▶ <b>System Tools</b> ▶ <b>Shut Down</b> ▶ <b>Shut Down</b>.</li> <li>Wait 30 seconds, then press the button on the side of the instrument to power on the instrument.</li> </ol> </li> <li>If the red "Alarms" and/or "Events" message still appears in the main menu, contact Technical Support.</li> </ul> </li> </ul>

## Initialization—General errors

Observation	Possible cause	Recommended action
Bottle leak check fails	<ul style="list-style-type: none"> <li>Bottle seal was not tight.</li> <li>Bottle was damaged or defective.</li> </ul>	<ol style="list-style-type: none"> <li>Finger-tighten the bottles.</li> <li>If the bottle continues to leak, replace the bottle.</li> <li>If leak check continues to fail, contact Technical Support.</li> </ol>

## Ion Sphere™ Assay troubleshooting table

The following table contains troubleshooting information for unenriched ISPs tested with the Ion Sphere™ Assay on a Qubit™ Fluorometer.

Qubit™ Fluorometer <sup>[1]</sup> observation	Ion GeneStudio™ S5 System observation	Possible cause	Recommended action
<10% Templated ISPs	<ul style="list-style-type: none"> <li>• Lower loading</li> <li>• Lower % enriched</li> <li>• Lower key signal</li> <li>• Lower throughput</li> </ul>	Too little library input into template preparation.	<ul style="list-style-type: none"> <li>• Increase library input to target 10–25% templated ISPs.</li> </ul> <i>or</i> <ul style="list-style-type: none"> <li>• Continue with sequencing; expect lower throughput.</li> </ul>
>25% Templated ISPs, but <70%	Increased number of filtered out reads	Too much library input into template preparation.	<ul style="list-style-type: none"> <li>• Decrease library input to target 10–25% templated ISPs.</li> </ul> <i>or</i> <ul style="list-style-type: none"> <li>• Continue with sequencing; expect lower throughput.</li> </ul>
>70% Templated ISPs	<ul style="list-style-type: none"> <li>• Increased % primer dimer filtered out reads</li> <li>• Lower throughput</li> </ul>	Adapter dimer contaminating library, more likely in short amplicon libraries, Ion AmpliSeq™ libraries, or miRNA libraries.	<ul style="list-style-type: none"> <li>• Check Agilent™ 2100 Bioanalyzer™ traces for adapter dimer peak (Amplicon library or Ion AmpliSeq™ library peak around 70 bp; miRNA library peak around 60bp).</li> <li>• Re-purify library using Agencourt™ AMPure™ XP Kit clean-up steps as outlined in the appropriate user guides.</li> </ul>
	<ul style="list-style-type: none"> <li>• Low loading</li> <li>• Low % enriched</li> <li>• Lower throughput</li> <li>• High % filtered out reads</li> </ul>	Ion Chef™ System underperformance	Troubleshoot with Technical Support or a Field Application Scientist.

<sup>[1]</sup> Qubit™ 2.0 Fluorometer or later.

- Perform a manual cleaning of the sequencer ..... 72
- Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge ..... 73
- Quality control of Ion 550™ ISPs ..... 75
- Maintain the Ion Chef™ System ..... 77

## Perform a manual cleaning of the sequencer

A cleaning protocol is normally performed automatically at the completion of each sequencing run. If a cleaning is necessary, perform the following procedure.

1. On the home screen, select **Settings ▶ Clean Instrument**.  
The instrument door unlocks allowing access to the consumables.
2. Remove the Ion S5™ Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.



3. Reinstall the empty waste reservoir and a *used* Ion S5™ Wash Solution bottle.
4. Ensure that the Ion S5™ Sequencing Reagents cartridge and Ion S5™ Wash Solution bottle are properly installed.

---

**IMPORTANT!** Perform the cleaning with a used reagent cartridge and wash solution bottle installed. The cleaning procedure pumps cleaning solution into the wash solution bottle and reagent cartridge making them unsuitable for sequencing.

---



5. Place a used sequencing chip in the chip clamp, then push the chip clamp in all the way to engage.
6. Close the instrument door, then tap **Next**.  
Cleaning takes ~35 minutes to complete. On completion the instrument door automatically unlocks and the chip and cartridge clamps disengage.
7. Proceed to “Initialize the sequencer” on page 54.

## Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge

Cleaning is normally automatically performed at completion of a sequencing run. If an Ion S5™ XL Sequencer, Ion GeneStudio™ S5 Plus Sequencer, or Ion GeneStudio™ S5 Prime Sequencer is initialized, an instrument reset run is required to ensure proper cleaning before reinitialization in one of the following situations.

- A sequencing run is not started within 24 hours after initialization.
- A sequencing run is not completed due to a power failure or an abort, and <200 flows occurred before the stoppage.

Do NOT perform a manual cleaning with an unused, initialized Ion S5™ Sequencing Reagents cartridge.

---

**Note:**

- If a power failure or abort occurs during the second of two runs started after a single initialization, a manual cleaning is sufficient (see “Perform a manual cleaning of the sequencer” on page 72).
- If the number of flows that occurred before a power failure or abort is unknown, perform an instrument reset run.

---

To perform an instrument reset run, use the following procedure before reinitialization.

1. In the instrument touchscreen main menu, tap **Run**.  
The instrument door unlocks and the chip clamp disengages.
2. Ensure that a used sequencing chip is in the chip clamp, then push the chip clamp in all the way to engage.
3. Close the instrument door, then tap **Next**.
4. When prompted, select **Planned Run (none)**. Ensure that the **Enable post-run clean** checkbox is selected, then tap **Review**.
5. In the **Select Run** screen, tap **Edit**, then in the **Detail** screen set the number of flows to **200**. Ensure that the **Post-Run/Clean** checkbox is selected, then tap **Close**.
6. Tap **Start run**, then tap **Accept** to confirm that Post-Run Clean is enabled, and to start the run.



## Appendix B Supplemental procedures

*Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge*

When the instrument reset run completes, the instrument automatically performs the cleaning procedure. After cleaning, the touchscreen returns to the main menu.

## Quality control of Ion 550™ ISPs

### Quality control using the Ion Sphere™ Assay on the Qubit™ Fluorometer

The Qubit™ Fluorometer can be used with the Ion Sphere™ Quality Control Kit (Cat. No. 4468656) to perform a quality assessment of unenriched Ion Sphere™ Particles generated for up to 200-base-read sequencing on an Ion S5™ XL Sequencer, Ion GeneStudio™ S5 Plus Sequencer, or Ion GeneStudio™ S5 Prime Sequencer.

For detailed protocols, see the *Ion Sphere™ Quality Control Kit User Guide* (Pub. No. MAN0017531) available at [thermofisher.com](http://thermofisher.com).

---

**Note:** This protocol supports the Qubit™ 2.0 Fluorometer or later.

---

### Acceptance criteria for unenriched Ion 550™ ISPs

The optimal amount of library corresponds to the library dilution point that gives Percent Templated ISPs between 10–25%.

Samples that fall within the recommended range generally produce the most data; however, samples that fall outside of the recommended range can still meet the throughput specifications on the Ion Chips™.

The recommended optimal range is not intended to be a pass/fail criterion. The range provides guidance for the quality of the sample.

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**Note:** If the results are outside the desired Percent Templated ISPs range, then increase or decrease the library input appropriately. For more information, see the “Ion Sphere™ Assay troubleshooting table” on page 71.

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Percent Templated ISPs	Description
<10%	Sample contains an insufficient number of templated ISPs to achieve optimal loading density on the Ion Chip™.
10–25%	Optimal amount of library.
>25%	Sample will yield multi-templated ISPs (mixed reads).

### Quality control using the Guava™ easyCyte 5 Benchtop Flow Cytometer

The Guava™ easyCyte 5 Benchtop Flow Cytometer can be used for quality assessment of unenriched and enriched Ion Sphere™ Particles generated for up to 200-base-read sequencing on an Ion S5™ XL Sequencer, Ion GeneStudio™ S5 Plus Sequencer, or Ion GeneStudio™ S5 Prime Sequencer.

- **Unenriched samples** – Obtain the QC samples from the corresponding Ion Chef™ Library Sample Tubes on the Ion 550™ Chef Reagents cartridge (positions A and B).
- **Enriched samples** – Obtain sample 1 from position E and sample 2 from position A on the Enrichment Cartridge v2.



Appendix B Supplemental procedures  
*Quality control of Ion 550™ ISPs*

For details, see the *Ion Sphere™ Particles Quality Assessment for the Ion Proton™ and Ion S5™ Systems Using the Guava™ easyCyte 5 Benchtop Flow Cytometer User Bulletin* (Pub. No. MAN0007496), available at [thermofisher.com](http://thermofisher.com).

## Maintain the Ion Chef™ System

For further information on the following Ion Chef™ System maintenance procedures, see the *Ion Chef™ Instrument User Guide* (Pub. No. MAN0018668), available at [thermofisher.com](http://thermofisher.com).

- Install a firmware update
- Change the instrument name
- Replace the ultraviolet lamp
- Perform an XY $\theta$  calibration
- Perform a Z calibration

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**Note:** You must install the Ion Chef™ Instrument firmware update in Torrent Suite™ Software 5.12 or later to access the XY $\theta$  calibration and Z calibration routines.

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**WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit [thermofisher.com/support](https://www.thermofisher.com/support).

## Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

- **CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.



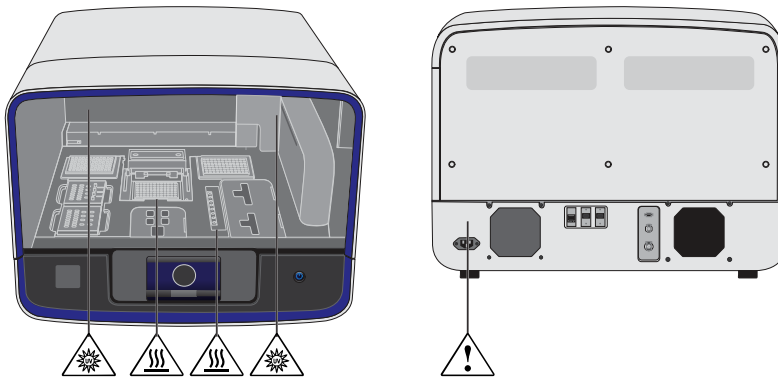
Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Moving parts	Parties mobiles
	Caution, hot surface	Attention, surface chaude
	Ultraviolet light	Rayonnement ultraviolet
	On/Off	On/Off (marche/arrêt)
	Standby	En attente
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
	Terminal that can receive or supply alternating current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant de type alternatif
	Do not dispose of this product in unsorted municipal waste  <b>CAUTION!</b> To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.  <b>MISE EN GARDE !</b> Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.



## Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union Low Voltage Directive 2006/95/EC, EMC Directive 2014/30/EU, and R&TTE Directive 1999/5/EC.
	Indicates conformity with Australian standards for electromagnetic compatibility.

## Location of safety labels on this instrument



## Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

## Instrument safety

### General



**CAUTION! Do not remove instrument protective covers.** If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.



## Physical injury



**CAUTION! Moving and Lifting Injury.** Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.



**CAUTION! Moving Parts.** Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

## Electrical safety



**WARNING! Ensure appropriate electrical supply.** For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.



**WARNING! Power Supply Line Cords.** Use properly configured and approved line cords for the power supply in your facility.



**WARNING! Disconnecting Power.** To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.



## Cleaning and decontamination



**CAUTION! Cleaning and Decontamination.** Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

## Ultraviolet (UV) Safety

The Ion Chef™ System uses a UV lamp which emits light at 254 nm. Under normal operating conditions, the UV lamp is powered on when performing the cleaning protocol. Safety interlocks are used to ensure that the UV lamp is not powered when the door is open.

## Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

### Safety compliance

Reference	Description
EU Directive 2006/95/EC	European Union "Low Voltage Directive"
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	<i>Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements</i>
IEC 61010-2-010 EN 61010-2-010	<i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials</i>
IEC/EN 61010-2-020	<i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-020: Particular requirements for laboratory centrifuges</i>
IEC 61010-2-081 EN 61010-2-081	<i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes</i>



## EMC

Reference	Description
Directive 2014/30/EU	European Union “EMC Directive”
FCC Part 15	U.S. Standard “Industrial, Scientific, and Medical Equipment”
AS/NZS 2064	Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment
ICES-001, Issue 3	Industrial, Scientific and Medical (ISM) Radio Frequency Generators
US FCC CFR Title 47 Part 15.225, Subpart C	Operation within the band 13.110–14.010 MHz.
Industry Canada RSS 210, Issue 8, Annex 2 EN 302 291-1/2 V1.1.1	Licence-Exempt Radio Apparatus: Category I Equipment

## Environmental design

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive” – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive” – Restriction of hazardous substances in electrical and electronic equipment

## Instrument safety – Ion GeneStudio™ S5 System

For detailed information on Ion GeneStudio™ S5 System instrument safety symbols and alerts, safety and electromagnetic compatibility standards, and general instrument safety, see the Safety appendix of the *Ion S5™ and Ion S5™ XL Instrument User Guide* (Pub. No. MAN0010811), or the *Ion GeneStudio™ S5 Instrument User Guide* (Pub. No. MAN0017528), available at [thermofisher.com](http://thermofisher.com).



## Chemical safety



**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



**WARNING! HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



## Biological hazard safety



**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:  
[www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf](http://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf)
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:  
[www.who.int/publications/i/item/9789240011311](http://www.who.int/publications/i/item/9789240011311)

# Documentation and support

## Related documentation

Document	Pub. No.
<i>Ion Chef™ Instrument User Guide</i>	MAN0018668
<i>Ion GeneStudio™ S5 Instrument User Guide</i>	MAN0017528
<i>Ion S5™ and Ion S5™ XL Instrument User Guide</i>	MAN0010811

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**Note:** For additional documentation, see “Customer and technical support”.

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## Customer and technical support

Visit [thermofisher.com/support](http://thermofisher.com/support) for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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**Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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## Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at [www.thermofisher.com/us/en/home/global/terms-and-conditions.html](http://www.thermofisher.com/us/en/home/global/terms-and-conditions.html). If you have any questions, please contact Life Technologies at [www.thermofisher.com/support](http://www.thermofisher.com/support).

