Ion PI™ Hi-Q™ Chef Kit

USER GUIDE

for use with:
Ion Chef™ System
Ion Proton™ System

Catalog Number  A27198
Publication Number  MAN0010967
Revision  C.0
The information in this guide is subject to change without notice.

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**Revision history:** MAN0010967

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
</table>
| C.0      | 2 July 2019| • Updated for Torrent Suite™ Software 5.12.  
• Guidance clarified for warming loaded chips that had been stored before sequencing.  
• "Create a Planned Run" moved from "Before you begin" to a new chapter for ease of use, and to align with the chapter structure of other user guides.  
• Corrected the number of Ion Proton™ Reagent Tubes and Reagent Tube Sippers provided in the Ion Proton™ Sequencing Supplies sub kit.  
• Clarification made in "Prepare the libraries and consumables" to dilute barcoded library pools, if used.  
• References to ISP quality control user guides updated.  
• "Install a firmware update" and "Change the instrument name" deleted from "Supplemental procedures". Users referred to the Ion Chef™ Instrument User Guide (Pub. No. MAN0018668) for this and other maintenance procedures, including deck calibration procedures (see page 87).  
| B.0      | 23 January 2017| • "Load the Ion Chef™ System™" and "Clean the Ion Chef™ Instrument" topics reorganized for ease of use  
• "Create a Planned Run" on page 23 updated for Torrent Suite™ Software v5.2  
• Designation of Chip-loading centrifuge positions changed from "A" and "B" to "1" and "2"  
• Instructions added to override warning for no Torrent Server or network connection  
• Recommended volume of 18 MΩ water and chlorite solution to add to C1 and C2 Reagent Tubes in the Ion Proton™ Sequencer cleaning procedures increased from 100 mL to 110 mL  
• ISP quality control sections updated and moved to new user guides:  
  - Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide (Pub. No. MAN0016387)  
  - Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide (Pub. No. MAN0016388)  
• Web links updated  
• Graphics enhanced |
| A.0      | 27 March 2015| New user guide, which includes instructions to set up and operate the Ion Chef™ System with the new Ion PI™ Hi-Q™ Chef Kit (Cat. No. A27198) |
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Purpose

This user guide describes how to use the Ion Chef™ System to prepare enriched, template-positive Ion Sphere™ Particles (ISPs) for up to 200 base-read sequencing of libraries on the Ion Proton™ Sequencer. The Ion PI™ Hi-Q™ Chef Kit (Cat. No. A27198) includes reagents and materials sufficient for performing 4 dual-sample, template-preparation runs on the Ion Chef™ System and subsequent sequencing on an Ion Proton™ System.

Prerequisites

This guide also assumes that you have:
- A general understanding of Ion Torrent™ sequencing chemistry and workflow
- Knowledge of techniques for handling and preparing DNA libraries
Product description

The Ion PI™ Hi-Q™ Chef Kit (Cat. No. A27198) includes the reagents and materials that are required for template preparation and chip loading on the Ion Chef™ System and sequencing of up to 200-base-read libraries on the Ion Proton™ System. The kit includes sufficient reagents and materials for 8 reactions of template preparation, chip loading, and sequencing using the Ion PI™ Chip v3 (ordered separately).

Library compatibility

The Ion PI™ Hi-Q™ Chef Kit can be used with up to 200-base-read libraries of any type prepared using any available Ion library kit.

Software compatibility

The Ion PI™ Hi-Q™ Chef Kit is compatible with Torrent Suite™ Software v4.6 or later. We recommend that you update to the latest available version of the software before using this kit.

Kit contents and storage

IMPORTANT! Do not substitute components from any other Ion sequencing kits. Thermo Fisher Scientific has verified this protocol using these specific materials. Substitution may adversely affect system performance.

IMPORTANT! Store all consumables and cartridges under the recommended conditions and in an upright position.

The Ion PI™ Hi-Q™ Chef Kit (Cat. No. A27198) contains all materials required for template preparation and sequencing. Upon arrival, inspect all consumables and contact Thermo Fisher Scientific if any of the products have been damaged during shipping.
## Ion PI™ Hi-Q™ Chef Kit summary

<table>
<thead>
<tr>
<th>Component</th>
<th>Part No.</th>
<th>Quantity per kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion PI™ Hi-Q™ Chef Supplies</td>
<td>A27283</td>
<td>4 boxes</td>
</tr>
<tr>
<td>Ion PI™ Hi-Q™ Chef Solutions</td>
<td>A27282</td>
<td>4 cartridges</td>
</tr>
<tr>
<td>Ion PI™ Hi-Q™ Chef Reagents</td>
<td>A27284</td>
<td>4 cartridges</td>
</tr>
<tr>
<td>Ion Proton™ Sequencing Supplies</td>
<td>4488651</td>
<td>1 box</td>
</tr>
<tr>
<td>Ion PI™ Hi-Q™ Sequencing 200 Solutions</td>
<td>A26430</td>
<td>1 box</td>
</tr>
<tr>
<td>Ion PI™ Sequencing Nucleotides</td>
<td>A26432</td>
<td>1 box</td>
</tr>
</tbody>
</table>

## Ion Chef™ reagents and materials

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Component</th>
<th>Amount per kit</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A27283</td>
<td>Ion PI™ Hi-Q™ Chef Supplies</td>
<td>4 boxes</td>
<td>15° to 30°C</td>
</tr>
<tr>
<td></td>
<td>• Chip Adapter (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Enrichment Cartridge v2 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Tip Cartridge v2 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• PCR Plate and Frame Seal v2 (1 each)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Recovery Station Disposable Lid v2 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Recovery Tube v2 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A27282</td>
<td>Ion PI™ Hi-Q™ Chef Solutions</td>
<td>4 cartridges</td>
<td>15° to 30°C</td>
</tr>
<tr>
<td>A27284</td>
<td>Ion PI™ Hi-Q™ Chef Reagents</td>
<td>4 cartridges</td>
<td>−30° to −10°C</td>
</tr>
</tbody>
</table>

## Ion Proton™ Sequencer reagents and materials

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion Proton™ Sequencing Supplies (Part No. 4488651)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion Proton™ Reagent Tube Caps</td>
<td>16 caps</td>
<td>15°C to 30°C</td>
</tr>
<tr>
<td>Ion Proton™ Wash Bottle Sippers</td>
<td>4 sippers</td>
<td></td>
</tr>
<tr>
<td>Ion Proton™ Reagent Tube Sippers</td>
<td>32 sippers</td>
<td></td>
</tr>
<tr>
<td>Ion Proton™ Reagent Tubes with labels (140 mL)</td>
<td>8 packs of 4 tubes each</td>
<td></td>
</tr>
</tbody>
</table>

## Ion PI™ Hi-Q™ Sequencing 200 Solutions (Part No. A26430) [1]

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion PI™ Hi-Q™ W2 Solution</td>
<td>4 × 125 mL</td>
<td>Shipped at 15°C to 30°C</td>
</tr>
<tr>
<td>Ion PI™ 1X W3 Solution</td>
<td>2 × 100 mL</td>
<td>Store at 2°C to 8°C (store W2 Solution protected from light)</td>
</tr>
<tr>
<td>Ion Cleaning Tablet</td>
<td>4 tablets</td>
<td></td>
</tr>
<tr>
<td>Component</td>
<td>Amount</td>
<td>Storage</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Ion PI™ dGTP (black cap)</td>
<td>280 µL</td>
<td>−30°C to −10°C</td>
</tr>
<tr>
<td>Ion PI™ dCTP (blue cap)</td>
<td>280 µL</td>
<td></td>
</tr>
<tr>
<td>Ion PI™ dATP (green cap)</td>
<td>280 µL</td>
<td></td>
</tr>
<tr>
<td>Ion PI™ dTTP (red cap)</td>
<td>280 µL</td>
<td></td>
</tr>
</tbody>
</table>

[1] Includes 1 bottle of Ion PI™ Annealing Buffer, and 1 tube each of Ion PI™ Foaming Solution and Ion PI™ Loading Buffer, which are not used in the Ion PI™ Hi-Q™ Chef Kit protocol.

**Ion Proton™ Wash 2 Bottle**

The Ion Proton™ Wash 2 Bottle (Cat. No. A24893), ordered separately, can be used for up to 20 Ion Proton™ Sequencer initializations (40 runs).

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion Proton™ Wash 2 Bottle with label</td>
<td>1 bottle</td>
<td>15°C to 30°C</td>
</tr>
</tbody>
</table>

**Ion PI™ Chip Kit v3**

The Ion PI™ Chip Kit v3 (Cat. No. A26771), ordered separately, is compatible with the Ion PI™ Hi-Q™ Chef Kit.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion PI™ Chip Kit v3 8-pack [2 boxes[1] of 4 chips each]</td>
<td>8 chips</td>
<td>15°C to 30°C</td>
</tr>
</tbody>
</table>

[1] Part No. A26770
## Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. MLS: Fisher Scientific (fisherscientific.com) or other major laboratory supplier.

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion Chef™ System</td>
<td>4484177</td>
</tr>
<tr>
<td>Ion Proton™ System (instrument and server) and included accessories</td>
<td>4476610</td>
</tr>
<tr>
<td>Ion PI™ Controls 200 Kit[1]</td>
<td>4488985</td>
</tr>
<tr>
<td>Tank of compressed nitrogen (grade 4.5, 99.995% pure or better)</td>
<td>MLS</td>
</tr>
<tr>
<td>Multistage (dual-stage) gas regulator (0-50 psi, 2-3 Bar output)</td>
<td>Fisher Scientific NC0393866 or MLS</td>
</tr>
<tr>
<td>(Optional) 1/8” x 1/4” stem reducing coupler (only required if using a separate tank for the wash station)</td>
<td>McMaster 5779K699</td>
</tr>
<tr>
<td>Uninterruptible Power Supply (UPS) [2]</td>
<td>MLS</td>
</tr>
<tr>
<td>ELGA™ PURELAB™ Flex 2 Water Purification System [or]</td>
<td>ELGA or MLS</td>
</tr>
<tr>
<td>ELGA™ PURELAB™ Flex 3 Water Purification System [or]</td>
<td>MLS</td>
</tr>
<tr>
<td>Equivalent 18 MΩ water system</td>
<td></td>
</tr>
<tr>
<td>NaOH (10 M) molecular biology grade</td>
<td>MLS</td>
</tr>
<tr>
<td>Isopropanol (100%)</td>
<td>MLS</td>
</tr>
<tr>
<td>Nuclease-free water molecular biology grade</td>
<td>MLS</td>
</tr>
<tr>
<td>0.22-µm or 0.45-µm vacuum filtration system and filters</td>
<td>MLS</td>
</tr>
<tr>
<td>Microcentrifuge[3]</td>
<td>MLS</td>
</tr>
<tr>
<td>P2, P10, P20, P200, P1000 µL pipette set and filtered tips</td>
<td>MLS</td>
</tr>
<tr>
<td>25-mL or 50-mL serological pipettes [or]</td>
<td>MLS</td>
</tr>
<tr>
<td>100-mL graduated cylinder</td>
<td></td>
</tr>
<tr>
<td>(If using serological pipettes) Pipet-Aid™ pipetter</td>
<td>Fisher Scientific 13-681-15</td>
</tr>
<tr>
<td>Rainin™ Pipet-Lite™ XLS with RFID LTS 2 µL to 20 µL</td>
<td>Rainin L-20XLS</td>
</tr>
<tr>
<td>Rainin™ Pipet-Lite™ XLS with RFID LTS 10 µL to 100 µL</td>
<td>Rainin L-100XLS</td>
</tr>
<tr>
<td>Rainin™ StableRak™ LTS tips 2-20 µL</td>
<td>Rainin SR-L20F</td>
</tr>
<tr>
<td>Rainin™ StableRak™ LTS tips 200 µL</td>
<td>Rainin SR-L200F</td>
</tr>
<tr>
<td>1.5-mL or 1.7-mL microcentrifuge tubes</td>
<td>MLS</td>
</tr>
<tr>
<td>Glass bottles (1 L)</td>
<td>MLS</td>
</tr>
</tbody>
</table>
Item Source

| Ice buckets and ice | — |
| Vortex mixer with a rubber platform | MLS |

[1] For installation and troubleshooting.
[2] 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.
[3] Must fit standard 1.5- and 0.2-mL microcentrifuge tubes and generate 15,500 × g.

**Note:** The procedures in this guide have been verified using these specific materials. Substitution may adversely affect system performance.

### Optional materials and equipment

The following optional materials can be used to verify and adjust the W2 Solution pH during initialization. Unless otherwise indicated, all materials are available through thermofisher.com. MLS: Fisher Scientific (fisherscientific.com) or other major laboratory supplier.

<table>
<thead>
<tr>
<th>Item</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermo Scientific™ Orion Star™ A111 pH Benchtop Meter Kit with electrode, electrode stand, and calibration buffers (or equivalent)</td>
<td>Fisher Scientific 13-645-503</td>
</tr>
<tr>
<td>1 N HCl</td>
<td>MLS</td>
</tr>
<tr>
<td>Magnetic stirrer (must hold 2-L bottle)</td>
<td>MLS</td>
</tr>
<tr>
<td>Magnetic stir bar (4 cm)</td>
<td>MLS</td>
</tr>
<tr>
<td>Squirt bottle</td>
<td>MLS</td>
</tr>
</tbody>
</table>
About the Ion Chef™ System

The Ion Chef™ System (Cat. No. 4484177) provides automated, high-throughput template preparation and chip loading for use with an Ion Proton™ Sequencer. The system includes a complete set of cartridge-based consumables and reagents that enable a user to load two chips in approximately 12.75 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.

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.
The following figure illustrates the interior of the Ion Chef™ Instrument and describes the stations involved in the preparation of Ion sequencing chips.

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 PI™ Hi-Q™ Chef Reagents.
6. **Solutions station** – The position on the instrument deck of the Ion PI™ Hi-Q™ Chef Solutions, 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 the 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.
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
About the Ion Proton™ Sequencer

The Ion Proton™ Sequencer performs automated, high-throughput sequencing of libraries loaded onto Ion PI™ Chip v3s using the Ion Chef™ Instrument.

**Ion Proton™ Sequencer components**

1. **Reagent compartment** – Contains all Ion Proton™ Sequencer reagents necessary for sequencing.
2. **Chip clamp** – Secures the Ion PI™ Chip v3 throughout the sequencing run.
3. **Touchscreen** – Provides access to all functions including operation, maintenance, and troubleshooting.
4. **Power button** – Power switch for the sequencer, where the states are on (illuminated) and off.
5. **Gas port** – A low-pressure port that provides compressed nitrogen gas to the sequencer.
6. **Ethernet port** – An RJ45 port that provides Ethernet (100 Mbit) communication with the sequencer.
7. **USB port** – A USB port that provides serial communication with the sequencer for use during service and maintenance.
8. **Power port** – A 100–240 VAC port that provides power to the sequencer.
9. **Power switch** – Power switch for the sequencer, where the states are on (O) and off (–).

**Ion Proton™ Sequencer reagent positions**

1. Wash 1 Solution (W1)
2. Wash 2 Solution (W2)
3. Wash 3 Solution (W3)
4. Clean 1 Solution (C1)
5. Clean 2 Solution (C2)
6. dGTP Solution (R1)
7. dCTP Solution (R2)
8. dATP Solution (R3)
9. dTTP Solution (R4)
10. Waste Container
The Ion Proton™ Sequencer features a simple interface for loading Ion chips, cleaning the instrument, and performing system maintenance and configuration tasks.

1. **Clean button** – Touch to clean the instrument before each run.
2. **Initialize button** – Touch to initialize the instrument before each run.
3. **Run button** – Touch to start the sequencing routine (loading, calibration, and sequencing).
4. **Options button** – Touch to open the Instrument Settings menu used to perform maintenance and configuration tasks.
5. **Tools button** – Touch to perform service-related actions:
   - **Auto pH** – Adjusts and checks the pH of the Wash 2 Bottle solution.
   - **Chip Cal** – Runs the chip calibration portion of the Run program.
   - **Data Mgt** – Manually delete run data or transfer data.
   - **Noise Screen** – Provides real-time measurement of electrical noise readings on the chip.
   - **Pressure Cal** – Calibrates the gas pressure.
   - **Reagent Check** – Measures the pH of all reagents on the instrument.
   - **Screen Cal** – Calibrates the touchscreen.
   - **Shut Down** – Shuts down or reboots the instrument.
6. **Chip Status icon** – Alerts you to the chip status where states include: Ready, Online, Imaging, Sleeping, and No chip detected.
7. **Instrument status** – Touch to view a list of alarms and events for the instrument. The number of alarms and events are indicated next to the associated labels on the button.
8. **Gauges button** – Toggles the presence of the instrument temperature data (items 9-12).
9. **Instrument gas pressure** – Indicates the instrument gas pressure (10.50 psi during cleaning, initialization, and sequencing).
10. **Chip compartment temperature** – Indicates the chip compartment temperature (35°C when the lid is closed).
11. **Disk space usage** – Indicates the percent of space used on the internal drives.
12. **On-instrument analysis** – Indicates that on-instrument analysis is in progress (indicates no analysis).
Workflow

Use the following workflow to prepare loaded chips using the Ion PI™ Hi-Q™ Chef Kit and Ion Chef™ System, and to sequence the chips on the Ion Proton™ Sequencer.

Chapter 3, “Create a Planned Run”

Chapter 4, “Start the Ion Chef™ run”

Chapter 5, “Clean and initialize the Ion Proton™ Sequencer”

Chapter 6, “Start the sequencing run”
Before you begin

Precautions

- Precautions .......................................................... 19
- Precautions - Read before using the Ion Proton™ System .................. 19
- Guidelines for using Ion Chef™ reagents and consumables ................ 20
- Prepare the Ion Chef™ Instrument for use ............................... 21

Precautions

Avoid nucleic acid contamination

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.

IMPORTANT! Handle nucleotides carefully to avoid cross-contamination. Always discard gloves after removing used Sippers from the sequencer in order to avoid cross-contamination of the nucleotides. Always discard gloves after handling concentrated dNTP stocks. Barrier tips are required for all dNTP pipetting steps.

Avoid chip damage

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.

Precautions - Read before using the Ion Proton™ System

Gas safety

WARNING! Ion instrumentation should be installed and operated in a well-ventilated environment as defined as having a minimum airflow of 6–10 air changes per hour. Assess the need for ventilation or atmospheric monitoring to avoid asphyxiation accidents from inert gases and/or oxygen depletion, and take measures to clearly identify potentially hazardous areas through training or signage. Please contact your Environmental Health and Safety Coordinator to confirm that the Ion instruments will be installed and operated in an environment with sufficient ventilation.
### Avoid nucleic acid contamination

**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.

### Avoid CO₂ contamination

**IMPORTANT!** Handle nucleotides carefully to avoid cross-contamination. Always discard gloves after removing used Sippers from the sequencer in order to avoid cross-contamination of the nucleotides. Always discard gloves after handling concentrated dNTP stocks. Barrier tips are required for all dNTP pipetting steps.

### Avoid introducing noise to instrument measurements

**IMPORTANT!** Dry ice (solid CO₂) should be kept away from areas where buffers, wash solutions or sources of molecular biology grade water for the Ion Proton™ System are used. High air concentrations of subliming CO₂ may influence the pH of such buffers during or after their preparation. The stability of the pH of these buffers is a critical factor in the performance of the Ion Proton™ System.

### Avoid chip damage

**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.

### Guidelines for using Ion Chef™ reagents and consumables

- Store all consumables and cartridges under the recommended conditions and in an upright position.
- Inspect all consumables and cartridges for damage on arrival and again before use.
- Hold sequencing chips by gently gripping them by their edges.
- When the instrument is not in use, remove all consumables and reagents from the deck and close the instrument door.
- Except for the new Tip Cartridge v2, do not reuse any of the consumables or reagents. After each run, the empty Tip Cartridge v2 is transferred to the waste tip station.

**IMPORTANT!** All components are single-use only.

- 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 instrument and chips.
• Remove and sequence chips within 1 hour after the instrument 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 6 hours maximum).

**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.

---

**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.

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

• 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 PI™ Hi-Q™ Chef Reagents cartridge at room temperature for 45 minutes before use.

• Ensure that the Ion Chef™ Instrument has a connection to the Ion Torrent™ Server. On the instrument home touchscreen, tap **Settings > Ion Torrent™ Server** to view the connection status.

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

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 Proton™ 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

The following provides a summary of steps for creating a Planned Run in Torrent Suite™ Software.

1. Sign in to Torrent Suite™ Software on the Ion Torrent™ Server.

2. Select the Plan tab, click Templates, select the application that you want to run (such as AmpliSeq DNA), then click either:
   - **Plan New Run** to plan a new run using the generic template for the selected application.
   - **Plan Run** in the dropdown menu under the Settings tab to the right of the existing template you select from the template list.

3. In the Planned Run wizard, review the Ion Reporter and Research Application tabs, then make selections appropriate to your run. In the Kits step, make the following selections:
   a. Select Ion Proton™ System from the Instrument dropdown list.
   b. Select the appropriate chip type in the Chip Type dropdown list.
   c. Select the appropriate library kit from the Library Kit Type dropdown list.
   d. Select Ion Chef for Template Kit, then select Ion PI Hi-Q Chef Kit from the Template Kit dropdown list.
   e. Enter the appropriate Library Read Length if the correct read length is not shown.
f. Select Ion PI Hi-Q Sequencing 200 Kit from the Sequencing Kit dropdown list.

- Expand the Advanced Settings field, then select or edit optional information fields appropriately for your run, if needed.

- Click Next.

4. Review the Plugins and Projects steps, then make selections appropriate to your run.

5. In the Plan step, enter or make the following selections:
   a. Enter a Planned Run name, then select Reference and BED files appropriate to your run.

- If you are using barcoded sample libraries in the run, select the set that you used in library preparation from the Barcode Set dropdown list. If you are not using barcodes, leave this field empty.

- Enter the appropriate number of Flows.
b. Enter the number of barcodes you are using in your combined library, then click ☰ to the right of this field. Edit the auto-populated list of barcodes that appears, if needed.

c. Enter or scan the barcode of the Ion Chef™ Library Sample Tube that contains the library pool into the **Sample Tube Label** field.

d. Enter or scan the chip barcode into the **Chip Barcode** field.

e. Enter a sample name for each plan in the appropriate **Sample (required)** fields.
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 in the run, and enter the Sample Tube Label and chip barcode for each sample.
- For a complete description of the run planning fields of Torrent Suite™ Software, see Torrent Suite™ Software Help.

6. When you have completed your selections, click Plan Run at the bottom right of the Plan step screen to save the run. A Planned Run is created for each chip and appears on the Planned Runs page with the name that you specified, appended with sample name. The plan is automatically used by the Ion Chef™ System when the associated samples are loaded.

Planned Run workflow key fields

<table>
<thead>
<tr>
<th>Step or field name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion Reporter</td>
<td>If the Ion Reporter™ Software is installed and enabled, and you want to analyze the run data using the software, select the Ion Reporter Account, then select the Ion Reporter™ workflow from the Existing Workflow dropdown list.</td>
</tr>
<tr>
<td>Research Application</td>
<td>Select the Research Application and Target Technique that represent your sequencing experiment.</td>
</tr>
<tr>
<td>Instrument</td>
<td>Select Ion Proton™ System.</td>
</tr>
<tr>
<td>Chip Type</td>
<td>Select Ion PI™ Chip v3.</td>
</tr>
<tr>
<td>Library Kit Type</td>
<td>Select the kit used to prepare the library.</td>
</tr>
<tr>
<td>Template Kit</td>
<td>Select Ion PI Hi-Q Chef Kit.</td>
</tr>
<tr>
<td>Sequencing Kit</td>
<td>Select Ion PI Hi-Q Sequencing 200 Kit.</td>
</tr>
</tbody>
</table>
| Barcode Set (optional) | If you are using barcodes with:
- DNA libraries – Select the appropriate barcode set used.
- RNA libraries prepared using the Ion Total RNA-Seq Kit v2 – Select the IonXpressRNA barcode set, which contains all 16 barcodes in the Ion Xpress™ RNA-Seq Barcode 1–16 Kit.
If you are not using barcodes with:
- DNA libraries – Leave the Barcode Set field blank.
- RNA libraries prepared using the Ion Total RNA-Seq Kit v2 – Select RNA_Barcode_None 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. |
<p>| 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. |</p>
<table>
<thead>
<tr>
<th>Step or field name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Projects</td>
<td>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.</td>
</tr>
<tr>
<td>Run Plan Name</td>
<td>Enter a name for the run.</td>
</tr>
<tr>
<td>Reference Library</td>
<td>Select a reference library uploaded to the Torrent Suite™ Software, if any.</td>
</tr>
<tr>
<td>BED files</td>
<td>Select the <strong>Target Regions</strong> or <strong>HotSpot Regions</strong> BED file in the Torrent Suite™ Software, if any.</td>
</tr>
<tr>
<td>Sample Tube Label (Required)</td>
<td>Enter or scan the barcode of the Ion Chef™ Library Sample Tube that you will use to load your sample into the Ion Chef™ Instrument.</td>
</tr>
<tr>
<td></td>
<td><strong>IMPORTANT!</strong> 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.</td>
</tr>
<tr>
<td>Chip Barcode</td>
<td>Enter or scan the chip barcode.</td>
</tr>
<tr>
<td>Sample Name (Required)</td>
<td>Did you select a <strong>Barcode Set</strong> in the <strong>Kits</strong> step?</td>
</tr>
<tr>
<td></td>
<td>• <strong>No</strong> – Enter the sample name for each sample loaded onto each chip in the <strong>Sample Name (required)</strong> column, then enter or scan the corresponding Ion Chef™ Library Sample Tube barcode and the chip barcode in the <strong>Sample Tube Label</strong> and <strong>Chip Barcode</strong> columns respectively.</td>
</tr>
<tr>
<td></td>
<td>• <strong>Yes</strong> – Enter the sample name for each sample in the <strong>Sample Name (required)</strong> column, then select the barcode that is associated with each sample from the dropdown list in the <strong>Barcode</strong> column.</td>
</tr>
<tr>
<td></td>
<td>Did you select the <strong>Ion Reporter Account</strong> and the associated Ion Reporter™ workflow?</td>
</tr>
<tr>
<td></td>
<td>• <strong>No</strong> – For each sample name listed, make the appropriate selections in each column in the table.</td>
</tr>
<tr>
<td></td>
<td>• <strong>Yes</strong> – For each sample name listed, also make the appropriate selections in the <strong>Ion Reporter Workflow</strong>, <strong>Relation</strong>, <strong>Gender</strong>, and <strong>IR Set ID</strong> columns.</td>
</tr>
<tr>
<td>Monitoring Thresholds</td>
<td>Set thresholds for <strong>Bead Loading</strong>, <strong>Usable Sequence</strong>, and <strong>Key Signal</strong>. In the Torrent Suite™ Software <strong>Monitor ▶ Runs in Progress</strong> screen, an alert is displayed if the values for a run fall below the selected thresholds.</td>
</tr>
</tbody>
</table>
Start the Ion Chef™ run

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Prepare the consumables ......................................................... 29
Add the diluted libraries to the Library Sample Tubes ..................... 30
Load the Ion Chef™ System ...................................................... 30
Start the Ion Chef™ run ......................................................... 43
Unload the chips for sequencing ............................................... 48

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

• Ion PI™ Hi-Q™ Chef Kit (Cat. No. A27198)
• Ion PI™ Chip v3 (2)
• Molecular-biology grade Nuclease-free Water
• P200 pipette and filtered tips
• Waste container
Dilute the sample libraries

**IMPORTANT!** Before proceeding, dilute the two libraries or combined library pools (if using barcoded libraries) to the optimal input concentration. The quality of your sequencing data relies on achieving the correct concentration of starting library.

Dilute the two libraries or combined library pools with nuclease-free water according to the following table. Prepare a fresh dilution of each library before use with the Ion Chef™ System, and use the library dilutions within 48 hours.

<table>
<thead>
<tr>
<th>Library</th>
<th>Recommended concentration [1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion Total RNA-Seq</td>
<td>50–100 pM</td>
</tr>
<tr>
<td>Ion AmpliSeq™ Exome RDY</td>
<td>50–100 pM</td>
</tr>
<tr>
<td>Ion AmpliSeq™ Transcriptome Human Gene Expression</td>
<td>50–100 pM</td>
</tr>
<tr>
<td>Ion AmpliSeq™ Transcriptome Mouse Gene Expression</td>
<td>70 pM</td>
</tr>
<tr>
<td>Ion AmpliSeq™ Comprehensive Cancer Panel</td>
<td>50 pM</td>
</tr>
<tr>
<td>Oncomine™ cfDNA Assays</td>
<td>50 pM</td>
</tr>
<tr>
<td>Human CEPH Control 200 library[2]</td>
<td>Dilute 1 µL into 24 µL nuclease-free water</td>
</tr>
</tbody>
</table>

[1] 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 the consumables

1. At least 45 minutes before use, unbox the Ion PI™ Hi-Q™ Chef Reagents cartridge and allow it to warm to room temperature.

   **IMPORTANT!** The Ion PI™ Hi-Q™ Chef 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 and Frame Seal v2
- Recovery Station Disposable Lid v2 (2)
- Recovery Tube v2 (12)
- Ion PI™ Hi-Q™ Chef Solutions
- Ion PI™ Hi-Q™ Chef Reagents (from step 1)
IMPORTANT! Before use, gently tap the Hi-Q™ Chef Reagents and the Hi-Q™ Chef Solutions cartridges on the bench to force the reagents to the bottoms of the tubes.

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

Add the diluted libraries to the Library Sample Tubes

1. Pipet 25 µL of each diluted library or combined library pool to the bottom of the appropriate Ion Chef™ Library Sample Tube (flagged tubes in the Ion PI™ Hi-Q™ Chef Reagents cartridge).

   Note: If running the Human CEPH Control 200 Library from the Ion PI™ Controls 200 Kit (Cat. No. 4488985), prepare the control library for use by diluting 1 µL of stock library into 24-µL Nuclease-free Water.

2. Cap, then store the two Library Sample Tubes on ice until you are ready to load them in 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 PI™ Hi-Q™ Chef Kit 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 procedure described in the following sections to load the Ion Chef™ Instrument.

A schematic of a loaded Ion Chef™ Instrument:
1. Empty tip rack (move from new Tip Cartridge v2 position)
2. Frame Seal v2
3. New Tip Cartridge v2
4. PCR Plate
5. Ion PI™ Hi-Q™ Chef Reagents cartridge
6. Ion PI™ Hi-Q™ Chef Solutions cartridge
7. Recovery Tubes and Recovery Station Disposable Lid v2
8. Enrichment Cartridge v2
9. Chip Adapter/Chip assemblies
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.

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

   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.

   Note: Two Ion Chef™ Piercing Tips are pre-loaded into tip positions G7 and H7 on the Tip Cartridge v2.
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.

6. Load a new PCR plate into the thermal cycler sample block, 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.

**Load the Reagents and Solutions cartridges**

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

1. Gently tap the Ion PI™ Hi-Q™ Chef Reagents cartridge on the bench to force the reagents to the bottoms of the tubes.

2. If bubbles are present below the surface of the liquid, repeat step 1.
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, confirm that you are loading the correct cartridge in the correct orientation.

![Reagents station (4°C) and Ion PI™ Hi-Q™ Chef Reagents cartridge]

1. Reagents station (4°C)
2. Ion PI™ Hi-Q™ Chef Reagents cartridge

4. Uncap, then load the two Library Sample Tubes, each containing 25 µL of diluted library or combined library pool, into Positions A and B on the Reagents cartridge.

![Library Sample Tubes in Positions A and B]

1. Position A (Library)
2. Position B (Library)
3. Position C (NaOH)
4. Position D (Empty tube)

**IMPORTANT!**
- Orient the sample tubes so that the barcodes are visible and oriented to the right.
- Remove the caps from each Library Sample Tube before proceeding.
- Because 200- and 400-base-read libraries require different run parameters, do not load a 200-base-read library and 400-base-read library in a single Ion Chef™ run. Both libraries that are loaded in a run must have a similar read length.
5. Uncap both the tube of NaOH in Position C and the empty tube in Position D on the Reagents cartridge.

**IMPORTANT!** When the Reagents cartridge is loaded:

- Press down on each Library Sample Tube to ensure that they are firmly seated in the cartridge.
- Ensure that all tubes are uncapped, including the tube at Position D.

6. Gently tap the Ion PI™ Hi-Q™ Chef Solutions cartridge on the bench to force the reagents to the bottoms of the tubes.

7. Load the Solutions cartridge into the Solutions station until it snaps into place and is level on the deck.

---

**Load the Recovery Tubes and Enrichment Cartridge v2**

1. Load six Recovery Tubes into each Recovery centrifuge.

---

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.

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

**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.

4. 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. Load each chip into a centrifuge bucket, then attach a Chip Adapter to the assembly.

**IMPORTANT!** When attaching a Chip Adapter:

- On the underside of each Chip Adapter, confirm that the rubber fittings are in place. If a fitting has become detached but can be found, reattach it to the empty port before you use the adapter. Otherwise, discard the Chip Adapter and use a replacement.
- Place the chip in the chip-loading bucket, and align the wells of the Chip Adapter to the wells of the chip. Then place the adapter onto the chip, inserting the tabs of one end of the adapter into the slots of the bucket. Gently squeeze the other end of the adapter into the bucket slots until it locks into place.
- Listen for an audible 'snap', which indicates that the Chip Adapter is attached. Loading can fail if the adapter is not attached securely.
- Confirm that the tabs at all four corners of the Chip Adapter are fitted into the slots in the centrifuge bucket.

**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.

![Diagram of Chip-loading centrifuge]

1. **Chip-loading centrifuge**
2. **Mounting grooves**
3. **Chip-loading centrifuge**

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

![Diagram of centrifuge rotor]

1. **Position 1 chip**
2. **Chip barcode**
3. **Position 2 chip**
4. **Position 1 marker hole**
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. Close the lid of the Chip-loading centrifuge.

**IMPORTANT!** Do not obstruct or place any object on top of the lid.
Before closing the centrifuge, confirm that:

- The centrifuge is balanced.

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

- The pins on the sides of the chip buckets are securely seated in the centrifuge.

- The chip buckets are oriented correctly in the centrifuge so that they pivot 90° outwards when touched.

**IMPORTANT!** The chip buckets must be correctly seated in the centrifuge.

**IMPORTANT!** 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 bucket properly. If excessive vibrations arise, check to ensure that items are installed properly and rotors are equally balanced on each side.

**IMPORTANT!** Use only the materials that are supplied in the Ion PI™ Hi-Q™ Chef Kit, including buckets and disposables, 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 consumables are correctly installed**

- 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 PI™ Hi-Q™ Chef Reagents cartridge, including the tube of NaOH in Position C, are uncapped and firmly pressed into place.

- 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 before beginning each run.
Single chip loading workflow

You can set up an Ion Chef™ Hi-Q™ run to load a single chip instead of two, using the appropriate Ion Chef™ Chip Balance loaded opposite to the chip in the Chip-loading centrifuge. Contact Customer Service to obtain an Ion Chef™ Chip Balance Pack. The pack contains a set of barcoded Chip Balances for use with singly loaded Ion PGM™ chips, and P-Series chips.

Ion Chef™ Chip Balance Pack

Load the Ion Chef™ Instrument as you would normally load the system. For single chip loading, perform the following steps:

1. Add the single diluted DNA library to an Ion Chef™ Library Sample Tube, then load the tube into Position A of the Reagents cartridge.

2. Load an empty Ion Chef™ Library Sample Tube into Position B of the Reagents cartridge. Uncap both tubes.

3. Load a chip in Position 1 and the appropriate Ion Chef™ Chip Balance in Position 2 of the Chip-loading centrifuge.

   **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. Position 1 (Chip)
2. Position 2 marker hole
3. Position 2 (Chip Balance)
4. Position 1 marker hole
IMPORTANT! Use the Chip Balance appropriate for the sequencing chip you have loaded. Each Chip Balance is weight-matched to the chip and chip adapter specified.

4. Resume the normal workflow in “Load the Chip-loading centrifuge” on page 37 at step 4. The Ion Chef™ Instrument detects the presence of the single chip during Deck Scan before the run starts.
ion Chef™ pre-run checklist

☐ 1) A new Hi-Q™ Chef Tip Cartridge v2 is loaded in the New Pipette Tip position.
☐ 2) Bracket is pulled downward to lock the Tip Cartridge in place.

☐ 1) A new PCR Plate is loaded in the thermal cycler sample block.
☐ 2) A Frame Seal v2 is in place and oriented under the heated cover.

☐ 1) The tip rack from previous run is transferred from the New Pipette Tip position to the Used Pipette Tip position.

☐ 1) Hi-Q™ Chef Reagents cartridge contents are thawed (leave at RT for 45 minutes).
☐ 2) Hi-Q™ Chef Reagents cartridge tubes are uncapped and pressed into place at the Reagents station so that the tubes are firmly seated and level with the deck.
☐ 3) Library Sample tubes are firmly seated and in the correct orientation with barcodes facing the PCR Plate and caps removed.

☐ 1) Hi-Q™ Chef Solutions cartridge is pressed into place at the Solutions station so that the cartridge is firmly seated and level with the deck.

☐ 1) Recovery Tubes (v2) are securely seated in the Recovery Centrifuge buckets.
☐ 2) Recovery centrifuges are load-balanced.
☐ 3) Recovery Station Disposable Lids (v2) are positioned so that the port is correctly oriented: port should not be located in the position facing the front of the instrument.

☐ 1) Enrichment Cartridge v2 is pressed into place at the Enrichment station so that the cartridge is firmly seated and level with the deck.
☐ 2) Lettering on the cartridge is right-side up and positioned to the right of the enrichment tubes.

☐ 1) Adapter/chip assemblies are correctly seated in the Chip-loading centrifuge buckets, with the adaptor clips inserted into the bucket slots.
☐ 2) Centrifuge buckets containing the adapter/chip assemblies are securely seated in the centrifuge rotor, and freely pivot 90° outwards.
☐ 3) Chip-loading centrifuge is load-balanced.
Start the Ion Chef™ run

1. Ensure that you have loaded the instrument with all kits and consumables.

2. On the Ion Chef™ 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.

4. If you selected Step by step, the Run Options screen opens. 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. After the door closes, the instrument vision system activates.

**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.

6. 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 can display 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, touch **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, ensure that all consumables are installed correctly before you continue.

7. When Deck Scan is complete, tap **Next** to display the **Data Destination** screen.
8. Confirm that the instrument displays the correct kit name, chip types, chip barcodes, and Planned Runs. If the correct Planned Runs do not display, tap the dropdown menu to select the Planned Run for each chip, then tap Next.

   ![Data Destination screen]

**IMPORTANT!** If the kit name 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.

**Note:** If the Ion Chef™ Instrument is not connected to an Ion Torrent™ Server, a warning screen appears after tapping Next.

![Network Cannot Be Found]

See Ion Chef™ and Torrent Server Network Setup User Guide (Pub. No. MAN0013444) to troubleshoot your network connection. Tap the close symbol in the upper right corner to return to the Data Destination screen. To bypass an Ion Torrent™ Server or network connection and proceed with the run, tap OK.

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

   ![Run Options screen]
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 will be made available either during 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 Ion PI™ Hi-Q™ Chef Reagents cartridge, or enriched samples from Positions A and E on the Enrichment Cartridge v2.

<table>
<thead>
<tr>
<th>By selecting</th>
<th>You can obtain the QC samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>immediately after the run ends, at the time you specify [12.75 hours after run start].</td>
</tr>
<tr>
<td>Pause</td>
<td>when the instrument pauses operation before the chip loading step [10.8 hours into the run].</td>
</tr>
</tbody>
</table>

**Note:** The DNA library in the Library Sample Tube loaded in Position A is templated onto ISPs that can be sampled in Position E of Enrichment Cartridge v2 after a run. The DNA library in the Library Sample Tube loaded in Position B is templated onto ISPs that can be sampled in Position A of Enrichment Cartridge v2.

**Note:** 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, collect QC samples after the run and save until sequence analysis is complete to have available for troubleshooting.

10. **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 display the error on the instrument touchscreen. If a run fails:

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.

11. **Clean and initialize the Ion Proton™ Sequencer approximately 1.5 hours before the Ion Chef™ Instrument finishes chip loading.**

By preparing the sequencer during the last stages of chip loading, you ensure that the chips can be sequenced as soon as possible after loading is complete.
12. If you chose to pause the run to analyze the templating efficiency, remove the samples for testing when prompted to do so by the Ion Chef™ Instrument (approximately 10.8 hours after the start of the run).
   a. When prompted to remove the QC sample, open the instrument door.

   ![Image of Process Paused](image)

   b. Transfer the QC samples (entire volume) from Positions A and B of the Ion PI™ Hi-Q™ Chef Reagents cartridge on the instrument deck to two new labeled microcentrifuge tubes.

   **IMPORTANT!** Do not remove the Library Sample Tubes from the Ion PI™ Hi-Q™ Chef Reagents cartridge.

   **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.

   ![Diagram of Ion PI™ Hi-Q™ Chef Reagents cartridge](image)

   ① Position A (QC sample)
   ② Position B (QC sample)

   c. Analyze the QC samples.

   **Note:** For detailed protocols, see the Ion Sphere™ Quality Control Kit User Guide (Pub. No. MAN0017531) available at [thermofisher.com/order/catalog/product/4468656](thermofisher.com/order/catalog/product/4468656).

   d. 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. See “Quality control using the Guava™ easyCyte 5 Benchtop Flow Cytometer” on page 86 or “Quality control using the Attune™ Cytometer” on page 87.
e. Close the instrument door, then tap **Continue** to complete the run.

13. When the run is complete, unload the Ion Chef™ Instrument and sequence the chips immediately. 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 chip wells after the Ion Chef™ run. **DO NOT** remove any residual liquid from the wells.

**Note:** If you cannot sequence a loaded chip immediately, place the chip into a separate chip storage container and store at 4°C until you are ready to sequence it (up to 6 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.

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

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

If you cannot sequence a loaded chip immediately, place the chip into a separate chip storage container and store at 4°C until you are ready to sequence it (up to 6 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.
Clean and initialize the Ion Proton™ Sequencer

Guidelines for handling and inserting chips

- 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.
- When handling chips, as a best practice, use a bare hand to touch the grounding surface and then use the opposite hand to insert and remove chips from the chip clamp.

Insert a chip into the chip clamp

The following procedure is used at various points during cleaning, initialization, and sequencing to insert chips into the chip clamp:

1. Pull the metal tab forward to release the chip clamp.
2. If necessary, remove the chip currently in the clamp.

3. Place the appropriate 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 figure.

4. Push the metal tab back until it clicks to engage the clamp.
Prepare 1 M NaOH daily

Prepare a stock of 1 M NaOH daily by diluting 10 M NaOH with 18 MΩ water directly from the purification system. Do not use water that has been collected or stored in any other containers.

Note: You will need 32 µL for each initialization and 1 mL for each chlorite cleaning.

Clean the Ion Proton™ Sequencer

Materials required

- 18 MΩ water (prepared and used directly from a water purification system, for example, the ELGA™ PURELAB™ Flex 3 Water Purification System)
- Two 140-mL Reagent Tubes (provided with kit; label the Reagent Tubes C1 and C2 before use)
- Collection tray (provided with the Ion Proton™ Sequencer)
- Cleaning chip (leave chip on the instrument during cleaning)

Note: A cleaning chip is a used chip that you designate for cleaning. You can use this chip for cleaning for up to 1 week.

- Used Sippers (from previous run or provided with the instrument)
- New short blue Sippers
- For chlorite cleaning only:
  - Ion Cleaning Tablet (provided with kit)
  - 2 Reagent Tubes designated for chlorite cleaning (Relabel used C1 or C2 Reagent Tubes for this purpose)
  - 1 M NaOH
  - 0.22-µm or 0.45-µm vacuum filtration system and filters

Cleaning schedule

Run the cleaning program with 18 MΩ water or chlorite solution before each initialization according to the following schedule. Cleaning takes ~30 minutes.

<table>
<thead>
<tr>
<th>Clean with:</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 MΩ water</td>
<td>• Before each initialization.</td>
</tr>
<tr>
<td></td>
<td>• (Recommended) After the last run of the day if the instrument will</td>
</tr>
<tr>
<td></td>
<td>not be used within 72 hours after the last run (for example, clean</td>
</tr>
<tr>
<td></td>
<td>after the last run before a 3-day weekend).</td>
</tr>
<tr>
<td></td>
<td>• Before shutting the instrument down for an extended period.</td>
</tr>
<tr>
<td>Chlorite solution</td>
<td>• Once a week (unless the instrument has not been used since the last</td>
</tr>
<tr>
<td></td>
<td>chlorite cleaning, in which case, clean with 18 MΩ water before</td>
</tr>
<tr>
<td></td>
<td>using).</td>
</tr>
<tr>
<td></td>
<td>• If reagents have been left on the instrument for more than 48 hours</td>
</tr>
<tr>
<td></td>
<td>[for example, over the weekend].</td>
</tr>
</tbody>
</table>
IMPORTANT! For the following steps, use 18 MΩ water directly from the purification system. Do not use water that has been collected or stored in any other containers.

1. Select Clean on the Ion Proton™ Sequencer touchscreen Main Menu, then follow the instructions on the touchscreen to perform the cleaning procedure.

2. When prompted, orient the cleaning chip with the notch in the bottom-front corner, place the chip in the chip clamp, then push the metal tab back until it clicks to engage the clamp.

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 following figures. For more detailed instructions, see “Insert a chip into the chip clamp” on page 51.

3. When prompted, remove the Reagent Tubes and Wash 2 Bottle:
   a. Remove and discard all eight 140-mL Reagent Tubes.

   Note: If needed, you can reuse the same C1 and C2 Reagent Tubes for multiple cleanings for up to 1 week. If you reuse the C1 and C2 Reagent Tubes, ensure that the tubes are correctly labeled, and cap the tubes when they are not installed on the instrument.

   b. Remove the Wash 2 Bottle, discard the liquid, then save the bottle for reuse in initialization.
c. Remove the old Sippers from the C1 and C2 positions. Put on fresh gloves, then install new short blue Sippers in those positions.

**IMPORTANT!** Leave all other Sippers in place; they are used during cleaning and initialization.

4. Remove the Waste Container, empty the waste, replace the container on the instrument, then tap **Next**.

5. Rinse the C1 and C2 Reagent Tubes twice with ~110 mL of 18 MΩ water.

6. Add 110 mL of 18 MΩ water to the C1 and C2 Reagent Tubes, then install them in the C1 and C2 positions.
7. Place the collection tray on the instrument, direct all Sippers into the collection tray, then tap Next.

8. When cleaning is finished, tap Next to return to the Main Menu.

Proceed to “Initialize the Ion Proton ™ Sequencer” on page 58.

**Chlorite cleaning**

Perform chlorite cleaning weekly, and as directed in “Cleaning schedule” on page 53.

**IMPORTANT!** For the following steps, use 18 MΩ water directly from the purification system. Do not use water that has been collected or stored in any other containers.

1. Fill a glass bottle with 1 L of 18 MΩ water, then add an Ion Cleaning Tablet (chlorite tablet). Allow the tablet to dissolve completely (~10 minutes).

2. When the tablet has dissolved, add 1 mL of 1 M NaOH and filter the solution using a 0.22-µm or 0.45-µm filter. Use the chlorite solution within 2–3 hours. Discard any unused solution after this time.

3. Select Clean on the Ion Proton ™ Sequencer touchscreen Main Menu, then follow the instructions on the touchscreen to perform the cleaning procedure.

4. When prompted, secure a cleaning chip in the chip clamp.

5. When prompted, remove all the Reagent Tubes and the Wash 2 Bottle:
   a. Remove and save the C1 and C2 Reagent Tubes for use with chlorite solution. (Label these tubes for chlorite cleaning only, then discard after the chlorite cleaning cycle is completed. Do not use these tubes for 18 MΩ water cleaning.
   b. Remove and discard all other 140-mL Reagent Tubes.
   c. Remove the Wash 2 Bottle, discard the liquid, then save the bottle for reuse in initialization.
   d. Remove the old Sippers from the C1 and C2 positions. Put on fresh gloves, then install new short blue Sippers in those positions.

**IMPORTANT!** Leave all other Sippers in place; they are used during cleaning and initialization.
6. Remove the Waste Container, empty the waste, replace the container on the instrument, then tap Next.

7. Add 110 mL of filtered chlorite solution to each of the two Reagent Tubes designated for chlorite cleaning.

8. On the Ion Proton™ Sequencer, install the tubes containing chlorite solution in the C1 and C2 positions.

9. Place the collection tray on the instrument, then direct all Sippers into the collection tray. Tap Next to start cleaning.

10. When cleaning is finished tap Next to return to the Main Menu.

11. Remove and discard the Reagent Tubes and Sippers used for chlorite solution from the C1 and C2 positions.  
    **Note:** If needed, you can reuse the same Reagent Tubes for multiple chlorite cleanings for up to 1 month. If you reuse the chlorite cleaning tubes, ensure that the tubes are correctly labeled, and cap the tubes when they are not installed on the instrument. Do not reuse chlorite cleaning tubes for 18 MΩ water cleaning.

12. Put on fresh gloves, then install new short blue Sippers in the C1 and C2 positions.

13. Rinse new C1 and C2 Reagent Tubes twice with ~110 mL of 18 MΩ water.

14. Fill the C1 and C2 Reagent Tubes with 110 mL of 18 MΩ water, then install the tubes into the corresponding positions.

15. Select Clean on the touchscreen Main Menu, then tap Next to advance through the instrument prompts until the cleaning procedure starts.

16. When the post-chlorite water rinse is complete, tap Next to return to the Main Menu.

Proceed to “Initialize the Ion Proton™ Sequencer”.

Initialize the Ion Proton™ Sequencer

Initialization takes ~90 minutes.

Materials required

Materials provided in the kit

- 140-mL Reagent Tubes for W1, W3, and dNTP reagents
  
  Note: Use the labels provided with the kit to label the Reagent Tubes.

- Long gray and short blue Sippers for Wash Bottles and Reagent Tubes

- Ion PI™ dGTP, Ion PI™ dCTP, Ion PI™ dATP, and Ion PI™ dTTP

- Ion PI™ Hi-Q™ W2 Solution

- Ion PI™ 1X W3 Solution

Other materials and equipment

- Used Ion PI™ Chip v3

  IMPORTANT! Initialize the instrument with a Ion PI™ Chip v3 that has previously been used for sequencing. Do not use a cleaning chip.

- Ion Proton™ Wash 2 Bottle (2-L)

- 18 MΩ water

- 1 M NaOH (prepared fresh daily)

- Ice

- Filtered pipette tips and pipettes

- Vortex mixer

- Microcentrifuge

- (If needed to adjust pH manually) pH meter, multipoint pH calibration reagents, pH probe, and probe stand, magnetic stirrer, stir bar, and squirt bottle

Initialization guidelines

IMPORTANT! Handle nucleotides carefully to avoid cross-contamination. Always discard gloves after removing used Sippers from the sequencer in order to avoid cross-contamination of the nucleotides. Always discard gloves after handling concentrated dNTP stocks. Barrier tips are required for all dNTP pipetting steps.

- Start a sequencing run within 1 hour of initialization.

- If you are performing more than one sequencing run per initialization, we recommend starting the second run on the same day. However, the second run can be started up to 24 hours after initialization.

- Replace the Reagent Tubes and Sippers every time you initialize.

- Replace the Ion Proton™ Wash 2 Bottle after 40 uses (20 initializations) or 3 months, whichever comes first.

- Check for updates to Torrent Suite™ Software, then install the updates if available.
Before you begin

Remove the dNTP stock solutions from the freezer and begin thawing on ice.

Start the initialization

1. Remove the Sippers from the W1, W2, and W3 positions. Do not remove the used Sippers from the dNTP ports until instructed to do so.

2. Select Initialize on the touchscreen main menu.

3. When prompted, scan or enter the barcode on the W2 Solution bottle, or select the Ion PI™ Hi-Q™ Sequencing 200 Kit from the dropdown list.
   
   **Note:** If you are using a barcode scanner, tap Enter barcode in the touchscreen before scanning the W2 Solution barcode.

4. Secure a used chip from an old sequencing run in the chip clamp (do not use a cleaning chip), then tap Next.
   
   The system verifies the gas pressure. If the gas pressure is low, tap Yes to retry gas-pressure verification. If the gas pressure remains low, see “Error Message: Confirm Instrument Has Gas Pressure” on page 75.

5. Tap Next to start the initialization.

Prepare and install the Wash 1 and Wash 3 Reagent Tubes

Prepare the Wash 1 and Wash 3 Reagent Tubes.

1. Add 32 µL of 1 M NaOH solution to the Wash 1 Reagent Tube.

2. Add 40–50 mL of the 1X W3 Solution from the kit to the Wash 3 Reagent Tube, measured using a serological pipette or graduated cylinder.

3. With fresh gloves, install new short Sippers in the W1 and W3 positions. **Do not let the new Sippers touch other Sippers on the instrument or any other surfaces.**

4. Install the Wash 1 and Wash 3 Reagent Tubes into the W1 and W3 positions of the Ion Proton™ Sequencer, place the collection tray beneath the dNTP Sippers, then tap Next.
1. Rinse the Wash 2 Bottle three times with 200 mL of 18 MΩ water, directly from the water purification system. Do not use water that is stored in other containers.

2. Extend the spigot from the water purification system into the neck of the Wash 2 Bottle, then add 18 MΩ water up to the groove on the bottle (marked in the following figure).

3. Add the entire bottle of Ion PI™ Hi-Q™ W2 Solution to the Wash 2 Bottle. Immediately cap the bottle securely and invert five times to mix.

   **IMPORTANT!** To prevent air exchange, keep the bottle tightly capped until it is attached to the Ion Proton™ Sequencer.

4. Following the on-screen prompts, use fresh gloves to install a new long Sipper in the cap for the Wash 2 Bottle. **Do not let the Sipper touch any surfaces.**
5. Immediately attach the prepared Wash 2 Bottle, then tighten the cap. Ensure that the cap is screwed on tightly, then place the Wash 2 Bottle in the reagent compartment before you continue.

6. Direct sippers to the collection tray, then tap **Next** to continue initialization. The instrument tests the tubes for leaks, fills the Wash 1 Reagent Tube, adjusts the pH of the W2 Solution, then dilutes the Wash 1 Reagent Tube solution to the optimal concentration for the sequencing run. This procedure takes ~40 minutes.

**IMPORTANT!** In the following steps, handle the nucleotides carefully to avoid cross-contamination and ensure that the correct dNTP solution is installed in each position on the Ion Proton™ Sequencer.

1. After each deoxyribonucleotide (dNTP) stock solution has thawed, vortex to mix and briefly centrifuge to collect the contents. Keep dNTP stock solutions on ice throughout this procedure.

2. Use the labels provided with the kit to label four new 140-mL Reagent Tubes as dGTP, dCTP, dATP, and dTTP.

3. After the wash solutions have initialized, follow the on-screen prompts to remove the used dNTP Sippers and the collection tray.

4. Using new gloves, attach a new short Sipper to each dNTP port. **Do not let the Sippers touch any surfaces.**

5. Using a new filtered pipette tip, carefully transfer 70 µL of dGTP stock solution into the bottom of the appropriate Reagent Tube, then attach the dGTP Reagent Tube to the Ion Proton™ Sequencer in the correct position (front left row) and firmly tighten.
6. Using a new pipette tip and fresh gloves for each tube, prepare and install the dCTP, dATP, and dTTP Reagent Tubes by transferring 70 µL of each dNTP stock solution to the corresponding Reagent Tube. Ensure that you install the Reagent Tubes in the correct order (dGTP, dCTP, dATP, and dTTP from left to right when facing the instrument).

**IMPORTANT!** Prepare and install the Reagent Tubes one at a time with new gloves and pipette tips each time to avoid cross-contamination.

7. Confirm that all Reagent Tubes and Wash 2 Bottle are tightly secured, then tap Next.
   - The Ion Proton™ Sequencer checks the pressure of the Reagent Tubes and Wash 2 Bottle, then adds W2 Solution to each dNTP Reagent Tube.
   - If a tube or bottle leaks, you are prompted to check that it is tightly attached to the instrument. If it continues to leak, replace it. If you replace the tube or bottle but the instrument does not pass the leak check, contact Technical Support.

8. At the end of initialization, the Ion Proton™ Sequencer measures the pH of the reagents.
   - If every reagent is in the target pH range, a Passed screen is displayed. Tap Next to return to the Main Menu. Proceed to Chapter 6, “Start the sequencing run”.
   - If a Failed screen appears, see “Error message: Reagent pH: Failed; Reagent pH is displayed” on page 81.
Start the sequencing run

Sequence the chip on the Ion Proton™ Sequencer

IMPORTANT! Observe the following when sequencing the chips:

- The Ion Proton™ Sequencer must be cleaned and initialized before sequencing the chips.
- Empty the waste container before you start the sequencing run.
- Do not use reagents from other sequencing kits for sequencing chips that are prepared by the Ion Chef™ System.
- To avoid damage due to electrostatic discharge (ESD), do not place the chip directly on the bench or any other surface. Always place the chip either on the Ion Proton™ Sequencer grounding plate or in the custom Ion centrifuge adapter/rotor bucket.
- To avoid ESD damage, do not wear gloves when transferring chips to and from the instrument.

Sequence the loaded chips on the Ion Proton™ Sequencer as soon as possible after unloading the Ion Chef™ Instrument. If not, place the chips into a chip storage container and store at 4°C until you are ready to sequence (up to 6 hours maximum).

IMPORTANT! Do not start the sequencing run with the loaded chip. Use a used chip for the line cleaning at the start of the run.

1. With the used chip from initialization still in the chip clamp, tap Run on the main menu, then tap Next, and confirm that "Cleaning fluid lines" displays on the instrument touchscreen. Observe the chip for leaks. The Run Setup screen automatically populates the fields when the Ion Proton™ Sequencer connects to the server.

2. Follow the on-screen instructions to empty the waste bottle, load the cleaning chip, then clean the fluid lines of the Ion Proton™ Sequencer. After line cleaning, tap Next.

3. When prompted, enter or scan the barcode of the chip that you intend to sequence, then tap Next.

4. Confirm that the pre-populated settings are correct. Make changes using the buttons and drop-down lists, if needed.

Note: If an error message appears, see “Error message: Not enough disk space for the necessary number of flows” on page 76.
5. Remove the used chip from the chip clamp, then secure the chip that is loaded with template-positive ISPs (see “Insert a chip into the chip clamp” on page 51). Close the chip compartment lid, wait until the Chip Status icon in the lower left corner of the screen indicates "Ready" then tap Next to start the sequencing run.

The system calibrates the chip (~1 minute), then starts the sequencing run. If chip calibration fails, see “Error message: Failed: Reseat chip, then press Next to recalibrate” on page 80.

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

6. If you are sequencing a loaded chip that you have stored, remove the chip from 4°C storage (but keep it in the chip container) at least 20 minutes before the end of the first run to warm to room temperature.

7. When the first run is complete, empty the waste container, then sequence the remaining chip as soon as possible.

**IMPORTANT!** After the first sequencing run is complete:
- Empty the waste container before you start the next sequencing run.
- Leave the first chip in the instrument while you start the second run. Load the second chip when prompted.

When the run is complete, the touchscreen returns to the main menu. Use Torrent Suite™ Software to review the results.
Clean the Ion Chef™ System

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
2. Empty Tip Cartridge v2: move to waste pipette tip station
3. Thermal cycler sample block
4. Reagents station
5. Solutions station
6. Recovery centrifuges
7. Enrichment station
8. Chip-loading centrifuge
Remove and dispose of used consumables

**IMPORTANT!**
- Do not discard the empty Tip Cartridge v2.
- Make sure to transfer the QC samples before you remove and discard 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.

3. Remove, then discard the PCR Plate from the thermal cycler sample block.

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:

5. Move the empty Tip Cartridge v2 to the waste tip position.
6. Remove, then discard the
   • Ion PI™ Hi-Q™ Chef Reagents cartridge
   • Ion PI™ Hi-Q™ Chef Solutions cartridge
   • Enrichment Cartridge v2

7. Remove, then discard the consumables from the Recovery centrifuges, including the:
   • Recovery Station Disposable Lid v2
   • Recovery Tubes 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.

<table>
<thead>
<tr>
<th>Is liquid present?</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Proceed to “Start the cleaning” on page 69.</td>
</tr>
<tr>
<td>Yes</td>
<td>Clean the centrifuge bowl and buckets as described below. <strong>IMPORTANT!</strong> Clean the Recovery centrifuges occasionally, only when excess liquid is noticeable in the bowl and/or buckets. You do not need to clean the centrifuges after every run.</td>
</tr>
</tbody>
</table>

**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.

![Bucket](image1.png)  
**1** Bucket

![Lint-free wipe](image2.png)  
**2** Lint-free wipe
3. Use lint-free wipes to remove all fluid from inside the centrifuge bowl.

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.

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.

![Ion Chef Instrument touchscreen](image1)

**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.

![Clean Instrument](image2)

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.

![Clean Instrument](image3)

**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

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- **Ion Proton™ Sequencer alarms and events** ............................... 74
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- **Instrument leaks** .......................................................... 77
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- **Ion Sphere™ Assay troubleshooting table** ................................. 85

### Ion Chef™ System setup and operation

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| Instrument does not display the home screen when powered on | Multiple causes are possible. | 1. Power off the instrument, wait 30 seconds, then power on the instrument.  
2. If the instrument fails again, contact Technical Support. |
| 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. |
| The instrument stops during a run | The instrument encountered an internal error. | 1. Record the error displayed on the instrument display, then tap **OK**.  
2. Contact Technical Support to report the problem and for further assistance. |
<p>| Liquid residue is present in the Recovery centrifuge following a run | During normal instrument operation, a noticeable coating of liquid can collect on the bowl and buckets of the Recovery centrifuge following repeated runs. | Remove the residue as instructed in “Inspect and clean the Recovery centrifuges and buckets” on page 68. |</p>
<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| Instrument will not begin a run      | The instrument has encountered a Deck Scan error (one or more consumables are absent or loaded improperly). | 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 **No** when prompted then tap **Next** to cancel the run. After returning to the home screen, restart the run.  
2. If the error persists, confirm that:  
   • All buckets are seated correctly in the rotors of the Recovery and Chip-Loading centrifuges.  
   • All cartridges are loaded correctly and are level on the instrument deck.  
   • The barcodes of the Ion Chef™ Library Sample Tubes are visible and positioned correctly.  
   • All tubes are both present and uncapped on the Reagents cartridge (Library Sample Tubes, NaOH tube, and the empty tube).  
3. If the error persists after you check the consumables on the instrument deck, do one of the following:  
   • If you are confident that the Ion Chef™ Instrument is set up correctly and you are comfortable disregarding the warnings, tap **YES** following Deck Scan to proceed with the run.  
   • If the instrument cannot begin the run, contact Technical Support for further assistance. |
| The instrument has encountered an internal error |                                                                                   | 1. Record the error displayed on the instrument display, then touch **OK**.  
2. Contact Technical Support to report the problem and for further assistance. |
<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument displays one or more alerts during a run</td>
<td>The instrument detected one or more problems during the run.</td>
<td>After the instrument completes the run, contact Technical Support. If possible, capture an image of the alert or error message to help troubleshoot.</td>
</tr>
<tr>
<td>IMPORTANT!</td>
<td>The detected problem might impact the performance of the sequencing run.</td>
<td>1. Tap the Instrument status button to view the alert(s).</td>
</tr>
<tr>
<td>• Network connection to the server was interrupted.</td>
<td>2. In the Instrument status screen, confirm that the name of the Ion Torrent™ Server connection is red.</td>
<td>3. Contact your network administrator to confirm that:</td>
</tr>
<tr>
<td>• User name or password was incorrect.</td>
<td>4. The Ion Torrent™ Server can be accessed from the network port used by the Ion Chef™ Instrument. If not, troubleshoot the network connection.</td>
<td>• The user name and password used by the Ion Chef™ Instrument are valid. If not, contact the server administrator to renew the credentials.</td>
</tr>
<tr>
<td></td>
<td>5. 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.</td>
<td></td>
</tr>
</tbody>
</table>
# Ion Proton™ Sequencer alarms and events

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| Red “Alarms” and/or “Events” message in Main Menu | • Available software updates were detected.  
• Connectivity issues were detected.  
• Instrument did not detect required files or hardware. | Tap the red Alarms icon to see detailed messages.  
• If a message states "Newer Software Available":  
  **IMPORTANT!** After updates are installed, the instrument must be restarted.  
  a. In the main menu, tap Options ➔ Updates.  
  b. Select the Released Updates checkbox, then tap Update.  
  c. When installation is complete, follow the onscreen prompts to restart the instrument.  
  **Note:** In some cases, the instrument restarts automatically after software installation.  
• 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.  
• For any other messages:  
  a. Power off the instrument: In the main menu, tap Tools ➔ Shut Down ➔ Shut Down.  
  b. Wait 30 seconds, then press the button on the front of the instrument to power on the instrument.  
• If the red “Alarms” and/or “Events” message still appears in the main menu, contact Technical Support. |
## Ion Proton™ Sequencer status bar icon warnings

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chip is secured in chip clamp, but chip icon indicates no chip detected</td>
<td>• Clamp is not engaged</td>
<td>1. Remove the chip from the chip clamp.</td>
</tr>
<tr>
<td></td>
<td>• Chip is not properly seated</td>
<td>IMPORTANT! Do not disengage the chip clamp if fluid is running to the chip. If you are currently running “Clean”, “Initialize”, or “Run”, wait until the Next button on the touchscreen is active, or tap Abort to return to the Main Menu before disengaging the clamp.</td>
</tr>
<tr>
<td></td>
<td>• Chip is damaged or dirty</td>
<td>2. Examine the chip for damage, such as hairline cracks, debris, or a detached flow cell.</td>
</tr>
<tr>
<td></td>
<td>• Issue with chip socket</td>
<td>• If the chip is damaged, insert a new chip in the chip clamp and engage the clamp, look at the chip icon to confirm the chip is detected, then tap Next or make a selection in the Main Menu.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If no damage can be observed, reinsert the chip in the chip clamp and engage the clamp, look at the chip icon to confirm the chip is detected, then tap Next or make a selection in the Main Menu.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Note:</strong> Alternatively, clean the back surface of the chip with a lint-free laboratory wipe treated with isopropanol, then dry using a clean wipe.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. If the chip is not detected by the instrument, there may be a problem with the chip socket. Contact Technical Support.</td>
</tr>
</tbody>
</table>

### Error Message: Confirm Instrument Has Gas Pressure and/or Pressure icon indicates low gas pressure (50 PSI)

**Note:** The correct operating pressure is 10.5 psi.

1. Replace the gas tank if empty.
2. If tank is not empty, confirm that the cylinder has at least 500 psi and 30 psi at the outlet of the regulator. Confirm that all valves between the cylinder and the Ion Proton™ Sequencer are open, then press Yes to retry verification of gas pressure.
3. If the pressure test continues to fail, contact Technical Support.
<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| Temperature icon indicates chip compartment temperature is out of range    | Thermistor in chip compartment is damaged                                         | Contact Technical Support.  
**Note:** Do not perform sequencing runs until this problem is corrected; non-optimal temperatures in the chip compartment may affect sequencing. |
| Error message: Not enough disk space for the necessary number of flows     | Data normally transfer automatically from the hard drive to the Torrent Server,  
(The sequencer hard drive does not contain enough space for the Planned Run) and/or  
Hard drive icon indicates hard drive is almost full | 1. Check for connectivity or network issues, for example, unplug and replug the ethernet cable, confirm that the router is operational, and verify that the network is up and running.  
2. If in "Select Planned Run", select **Data Management** in the touch screen, otherwise select **Tools**  
**Data Management** from the Main Menu.  
3. In the Data Management screen, select **All**, then review the runs. If there are runs that do not need to be transferred to the Torrent Server (for example test or aborted runs), select the checkbox next to the run names, then press **Delete Sel**.  
4. If there are runs that you do want to transfer, you may need to wait until connectivity is restored for the run to transfer and then autodelete. |
| On instrument analysis icon indicates error                                 | Corrupt data files or file system, for example, SSD file array is corrupted      | 1. Power off the instrument: In the Main Menu, select **Tools**  
**Shut Down**  
**Shut Down**.  
2. Wait 30 seconds, then press the button on the front of the instrument to power on the instrument. |
## Instrument leaks

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| Liquid in drip pan below chip clamp | • Chip leak  
• Cracked chip  
• Chip clamp not closed properly  
• Leaky fluidic seal  
• Problem with the chip clamp or socket | 1. Tap **Abort** to return to the Main Menu.  
2. Use a lab wipe to absorb the liquid in the drip pan.  
3. Open the chip clamp, remove the chip, and gently dab the chip with a lab wipe to dry.  
4. Look for damage to the chip, such as hairline cracks, debris, or a detached flow cell.   
• If the chip is damaged, insert a new chip in the chip clamp and engage the clamp.  
• If no damage can be observed, reinsert the chip in the chip clamp and engage the clamp.  
5. From the Main Menu, make the appropriate selection (**Clean**, **Initialize**, or **Run**) to start from the beginning of the process.  
6. If the leak persists, contact Technical Support. |
| Leak from bottom of instrument | • Waste container not emptied  
• Reagent Tubes or Wash 2 Bottle not securely installed | 1. Tap **Abort** to return to the Main Menu.  
2. Clean up all liquid beneath the instrument.  
3. Confirm that the waste container is not overflowing, and empty if needed.  
4. Confirm that all Reagent Tubes and Wash 2 Bottle are securely fastened on the instrument.  
5. If the leak is due to the waste container, Reagent Tubes, or Wash 2 Bottle, re-start the procedure after correcting the problem.  
6. If there are no observable issues with the waste container, Reagent Tubes, or Wash 2 Bottle, contact Technical Support. |
| Leak on instrument tubing (Fluid on fluid lines entering clamp) | Fluid lines running to chip clamp are damaged or are not secured correctly | Contact Technical Support. |
### Touchscreen

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Touchscreen not registering input in correct location</td>
<td>Touchscreen needs to be calibrated</td>
<td>• In Main Menu, select Tools &gt; Screen Cal, then follow the onscreen prompts.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If the touchscreen continues to malfunction after Screen Cal, contact Technical Support.</td>
</tr>
<tr>
<td>Touchscreen inoperable</td>
<td>Damaged or defective touchscreen</td>
<td>Contact Technical Support.</td>
</tr>
</tbody>
</table>

### Instrument error messages

**Note:** Error messages are listed in alphabetical order.

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error message: Added too much W1 to W2</td>
<td>• Poor water quality (18 MΩ water not used directly from water purifier, or exposed to air for too long)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Too much W2 Solution used to prepare Wash 2 Bottle</td>
<td>1. Confirm high water quality and correct preparation of the 1 M NaOH and Wash 2 Bottle.</td>
</tr>
<tr>
<td></td>
<td>• Incorrect solution added to the Wash 2 Bottle</td>
<td>2. If solution preparation is incorrect or water quality is poor, correctly prepare the solution(s) and/or use high-quality water.</td>
</tr>
<tr>
<td></td>
<td>• Too little NaOH added to Wash 1 Reagent Tube</td>
<td>3. Clean the instrument.</td>
</tr>
<tr>
<td></td>
<td>• Damaged chip</td>
<td>4. Repeat instrument initialization with fresh reagents and a new (unused) chip. (The new chip can be used for sequencing after initialization completes.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Note:</strong> Once the system has added too much NaOH, the only recourse is to clean the Ion Proton™ Sequencer and restart initialization or to manually adjust the pH of the W2 Solution.</td>
</tr>
<tr>
<td>Error Message: Check Wash1 for leaks</td>
<td>• W1 Reagent Tube seal is not tight</td>
<td>1. Remove the Wash 1 Reagent Tube, then replace in the W1 position.</td>
</tr>
<tr>
<td></td>
<td>• Tube may be damaged or defective</td>
<td>2. Make sure that the tube is securely tightened (finger-tighten).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. If the tube continues to leak, replace the tube.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. If leak check continues to fail, contact Technical Support.</td>
</tr>
<tr>
<td>Observation</td>
<td>Possible cause</td>
<td>Recommended action</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-----------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Error Message: Check Wash2 for leaks | • Wash 2 Bottle seal is not tight  
• Bottle may be damaged or defective | 1. Remove the Wash 2 Bottle, then check the o-ring inside the Wash 2 Bottle lid. If there is any visible damage, contact Technical Support.  
2. If there is no visible damage to the o-ring, replace the Wash 2 Bottle on the instrument. Make sure that the bottle is securely tightened (finger-tighten).  
3. If the bottle continues to leak, replace the bottle.  
4. If leak check continues to fail, contact Technical Support. |
| Error Message: Check Wash3 for leaks | • W3 Reagent Tube seal is not tight  
• Tube may be damaged or defective | 1. Remove the Wash 3 Reagent Tube, then replace in the W3 position.  
2. Make sure that the tube is securely tightened (finger-tighten).  
3. If the tube continues to leak, replace the tube.  
4. If leak check continues to fail, contact Technical Support. |
| Error message: Chip reading inconsistent. Please replace chip and try again. | • pH response of the chip is not uniform or reliable  
• Ran out of 1X W3 Solution or volume too low | 1. Verify that there is enough 1X W3 Solution (approximately 50 mL) in the Wash 3 Reagent Tube and that the sipper is secure.  
2. If needed, loosen the 1X W3 Solution, tighten the sipper, and add more 1X W3 Solution to fill to 50 mL. Since the system pressurization gas flows when the reagent tube is loose, perform these operations as quickly as possible. (The gas is not harmful to the 1X W3 Solution and is not a hazard.)  
3. If there is enough 1X W3 Solution, replace the chip with a new (unused) one. Secure the chip in the chip clamp, then tap Start.  
   **Note:** The new chip can be used for sequencing after initialization completes. |
| Error Message: Confirm Instrument Has Gas Pressure and/or Pressure icon indicates low gas pressure | Nitrogen gas cylinder may be turned off or empty | 1. Replace the gas tank if empty.  
2. If tank is not empty, confirm that the cylinder has at least 500 psi and 30 psi at the outlet of the regulator. Confirm that all valves between the cylinder and the Ion Proton™ Sequencer are open, then press Yes to retry verification of gas pressure.  
3. If the pressure test continues to fail, contact Technical Support. |
<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| Error message: Failed: Reseat chip, then press Next to recalibrate  
(New chip fails calibration.) | • Reagents not loaded  
• Clamp is not engaged  
• Chip is not properly seated  
• Chip is damaged or dirty  
• Issue with chip socket | 1. Confirm the required reagents are loaded on the instrument.  
2. Press Start to re-run calibration.  
3. If calibration fails, remove the chip from the clamp and look for damage to the chip, such as hairline cracks, debris, or a detached flow cell.  
• If the chip is damaged, insert a new chip in the chip clamp and engage the clamp, then press Start to re-run calibration.  
• If no damage can be observed, reinsert the chip in the chip clamp and engage the clamp, then press Start to re-run calibration.  
4. If calibration fails, run reagent check: Press Abort, select Tools > Reagent Check, then press Start.  
• If the reagent check fails, re-initialize the instrument before beginning a sequencing run.  
• If reagent check passes, contact Technical Support. |
| Error message: Not enough disk space for the necessary number of flows  
(The sequencer hard drive does not contain enough space for the Planned Run)  
and/or  
Hard drive icon indicates hard drive is almost full | Data normally transfer automatically from the hard drive to the Torrent Server, however this may not happen in the case of:  
• Data transfer manually aborted by user  
• Issue with connectivity or network  
• Incorrect configuration of the Torrent Server | 1. Check for connectivity or network issues, for example, unplug and replug the ethernet cable, confirm that the router is operational, and verify that the network is up and running.  
2. If in “Select Planned Run”, select Data Management in the touch screen, otherwise select Tools > Data Management from the Main Menu.  
3. In the Data Management screen, select All, then review the runs. If there are runs that do not need to be transferred to the Torrent Server (for example test or aborted runs), select the checkbox next to the run names, then press Delete Sel.  
4. If there are runs that you do want to transfer, you may need to wait until connectivity is restored for the run to transfer and then autodelete. |
<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error message: OVERSHOT</td>
<td>Auto-pH added more NaOH from the Wash 1 Reagent Tube to the Wash 2 Bottle than was needed</td>
<td>1. Reinitialize the instrument using new reagents and a new chip (the chip can then be used for sequencing).</td>
</tr>
<tr>
<td>TARGET PH: W2 pH = n.nn Failed</td>
<td></td>
<td>2. Prepare 50 μL of 100 mM HCl. If you are in the auto-pH screen, tap Overshoot. Follow the on-screen prompts to add 50 μL of 100 mM HCl to the W2 Solution. This action lowers the pH of the W2 Solution. Tap Restart to restart auto-pH.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. If the pH is consistent with the pH of the previous chip, manually adjust the pH of the W2 Solution.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Note:</strong> If the Ion Proton™ System consistently overshoots the pH target, add 50 μL of 100 mM HCl to the Wash 2 Bottle before installing it on the instrument. If you continue to experience overshoot problems even after the addition of the HCl, contact Technical Support for further help.</td>
</tr>
<tr>
<td>Error message: Please insert a chip and press Start</td>
<td>Instrument cannot detect the chip in chip clamp</td>
<td>See recommended action for “Chip is secured in chip clamp, but chip icon indicates no chip detected” on page 75.</td>
</tr>
<tr>
<td>Error message: Reagent pH: Failed; Reagent pH is displayed</td>
<td>One or more reagents are not within the target pH</td>
<td>1. Tap Restart to restart auto pH and confirm the measurement.</td>
</tr>
<tr>
<td>Note: Message displays on touchscreen after Reagent Check at end of Initialization procedure.</td>
<td></td>
<td>2. If any reagents fail, replace the chip with a new (unused) one. Insert the chip in the socket, then tap Restart to restart auto pH (the new chip can be used for sequencing after initialization completes).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. If any reagents fail, clean and reinitialize the instrument with fresh reagents and a new chip.</td>
</tr>
<tr>
<td>Observation</td>
<td>Possible cause</td>
<td>Recommended action</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>---------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Error message: Reagent pH: Failed; Reagent pH is not displayed | Chip did not calibrate               | 1. Remove the chip from the clamp and look for damage to the chip, such as hairline cracks, debris, or a detached flow cell.  
   • If the chip is damaged, insert a new chip in the chip clamp and engage the clamp, then press **Start** to re-run reagent check.  
   • If no damage is observed, reinsert the chip in the chip clamp and engage the clamp, then press **Start** to re-run reagent check.  
   If reagent check fails again, replace the chip with a new (unused) one, then re-run reagent check (the new chip can be used for sequencing after initialization completes).  
   2. If the reagent check continues to fail, contact Technical Support. |
| Error Message: Remove Conical tubes              | • dNTP Reagent Tube seal is not tight  
   • Tube may be damaged or defective         | 1. Remove and reinstall each dNTP Reagent Tube.  
   2. Make sure that each tube is securely tightened (finger-tighten), the tap **OK** to re-check pressure.  
   3. If the error message persists, set up dNTPs in new tubes, secure new tubes on instrument, then tap **OK**.  
   4. If leak check continues to fail, contact Technical Support. |
<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| Error message: There may be a blockage or no NaOH in W1. Please check W1 and run line clear then try again | The waste lines may be blocked                                                 | 1. If you are in the auto pH screen, tap **Line Clear**, otherwise select **Tools \ Auto pH \ Line Clear** from the Main Menu.  
2. Follow the on-screen prompts and use the syringe that is provided with the Ion Proton™ System.  
3. *(Optional)* To confirm that the Line Clear procedure was successful, select **Flow Check**, then confirm that liquid flows from both waste lines.  
4. If the flow rates are still not normal, perform **Line Clear** one more time.  
5. If one or more lines remain blocked, contact Technical Support. Otherwise, if initialization was interrupted, restart initialization from the beginning. |
| No NaOH added to the Wash 1 Reagent Tube, so chip does not detect large enough pH difference between the NaOH and W2 Solution. |                                                                                   | 1. Remove the Wash 1 Reagent Tube, rinse with 18 MΩ water, then add 32 µL of 1 M NaOH.  
2. Replace the Wash 1 Reagent Tube and securely tighten.  
3. Tap **Restart** to restart auto-pH. |
| Wash 1 or Wash 2 sipper is loose.                                            |                                                                                   | 1. Loosen the Wash 1 Reagent Tube, tighten the sipper, then replace the Wash 1 Reagent Tube and securely tighten.  
Tighten the sipper as quickly as possible to minimize the gas flow that occurs when the tube is removed. (The gas is not harmful to the NaOH solution and is not a hazard.)  
2. Loosen the Wash 2 Bottle cap and retighten the sipper. Tighten the sipper as quickly as possible to minimize the gas flow that occurs when the bottle is removed. (The gas is not harmful to the W2 Solution and is not a hazard.)  
3. Tap **Restart** to restart the auto-pH process. |
| Error message: UNDERSHOT TARGET PH: W2 pH = n.nn Failed                      | Auto-pH could not add enough Wash 1 to the Wash 2 before the maximum iterations, 10, occurred | 1. A blockage may have occurred. Follow the procedure for “Error message: There may be a blockage or no NaOH in W1. Please check W1 and run line clear then try again” on page 83.  
2. Press **Restart** to re-start auto-pH. If the “Undershoot target pH” error appears again, replace the chip with a new (unused) chip and restart auto pH.  
**Note:** The new chip can be used for sequencing after initialization completes. |
<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error message: W2 average not stable. Try reseating/replacing chip</td>
<td>The waste lines may be blocked</td>
<td>See first recommended action for &quot;Error message: There may be a blockage or no NaOH in W1. Please check W1 and run line clear then try again&quot; on page 83.</td>
</tr>
</tbody>
</table>
| Reading for W2 Solution is not stabilizing quickly enough | | 1. Remove the chip from the clamp and look for damage to the chip, such as hairline cracks, debris, or a detached flow cell.  
• If the chip is damaged, insert a new chip in the chip clamp and engage the clamp (the new chip can be used for sequencing after initialization completes).  
• If no damage can be observed, reinsert the chip in the chip clamp and engage the clamp.  
2. Loosen the Wash 2 Bottle cap and retighten the sipper. Tighten the sipper as quickly as possible to minimize the gas flow that occurs when the bottle is removed. (The gas is not harmful to the W2 Solution and is not a hazard.)  
3. Press Restart to restart auto-pH. If auto-pH fails even after running auto-pH with a new chip, contact Technical Support and manually adjust the pH of the W2 Solution. |
| Error message: W2 out of range | • Chip measurements very unstable  
• Chip is damaged | See recommended actions for “Error message: W2 average not stable. Try reseating/replacing chip” on page 84. |

### Sample loading or sequencing

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| Control Ion Sphere™ Particles are not present in the Test Fragment Report section of the run report and library sequencing is poor | • Poor chip loading  
• Ion Sphere™ Particles were not added to the sample  
• Chip is damaged  
• Instrument failure | 1. Confirm that the Ion Sphere™ Particles (included in the Ion PI™ Controls 200 Kit) were added.  
2. If controls were added, contact Technical Support. |
| Sample results were obtained, but poor or no results for Control Ion Sphere™ Particles in Test Fragment report | • No Control Ion Sphere™ Particles added to the sample  
• Control Ion Sphere™ Particles are past expiry date | Check Control Ion Sphere™ Particles expiry date. |
### Ion Sphere™ Assay troubleshooting table

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

**Note:** Successful runs that meet chip throughput specifications have been performed with a lower percentage (5–10%) of templated ISPs. Use the following recommendations as guidelines only to titrate library input for optimal results, or troubleshoot problems with library preparation.

<table>
<thead>
<tr>
<th>Qubit™ Fluorometer[1] observation</th>
<th>Ion Proton™ System observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| <10% Templated ISPs               | • Lower loading                | Too little library input into template preparation. | • Increase library input to target 10–25% templated ISPs.  
  or  
  • Continue with sequencing; expect lower throughput. |
|                                   | • Lower % enriched             |                |                     |
|                                   | • Lower key signal             |                |                     |
|                                   | • Lower throughput             |                |                     |
| >25% Templated ISPs, but <70%     | Increased number of filtered out reads | Too much library input into template preparation. | • Decrease library input to target 10–25% templated ISPs.  
  or  
  • Continue with sequencing; expect lower throughput. |
|                                   |                                |                |                     |
| >70% Templated ISPs               | • Increased % primer dimer filtered out reads | Adapter dimer contaminating library, more likely in short amplicon libraries, Ion AmpliSeq™ libraries, or miRNA libraries. | • Check Agilent™ 2100 Bioanalyzer™ traces for adapter dimer peak (Amplicon library or Ion AmpliSeq library peak around 70 bp; miRNA library peak around 60bp).  
  • Re-purify library using Agencourt™ AMPure™ XP Kit clean-up steps as outlined in the appropriate user guides. |
|                                   | • Lower throughput             |                |                     |
| Ion Chef™ System underperformance | Low loading                    | Ion Chef™ System underperformance | Troubleshoot with Technical Support or a Field Application Scientist. |
|                                   | Low % enriched                 |                |                     |
|                                   | Lower throughput               |                |                     |
|                                   | High % filtered out reads      |                |                     |

[1] Qubit™ 2.0 Fluorometer or later.
Quality control of Ion PI™ ISPs

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 Proton™ Sequencer.

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

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

Acceptance criteria for unenriched Ion PI™ 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.

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 85.

<table>
<thead>
<tr>
<th>Percent Templated ISPs</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10%</td>
<td>Sample contains an insufficient number of templated ISPs to achieve optimal loading density on the Ion Chip™.</td>
</tr>
<tr>
<td>10–25%</td>
<td>Optimal amount of library.</td>
</tr>
<tr>
<td>&gt;25%</td>
<td>Sample will yield multi-templated ISPs (mixed reads).</td>
</tr>
</tbody>
</table>

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 the Ion Proton™ Sequencer.

- **Unenriched samples** – Obtain the QC samples from the corresponding Library Sample Tubes on the Ion PI™ Hi-Q™ 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.

The Applied Biosystems™ Attune™ Acoustic Focusing Cytometer can be used for quality assessment of unenriched and enriched Ion Sphere™ Particles generated for up to 200 base-read sequencing on the Ion Proton™ Sequencer.

- **Unenriched samples** – Obtain the QC samples from the corresponding Library Sample Tubes on the Hi-Q™ 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.


**IMPORTANT!** Thermo Fisher Scientific Demonstrated Protocols have been successfully verified by Thermo Fisher Scientific for research use. There are no technical specifications for Thermo Fisher Scientific Demonstrated Protocols. Users assume all risk when using these protocols, and recognize that support for Thermo Fisher Scientific Demonstrated Protocols occurs through community discussion.

### 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.

- Install a firmware update
- Change the instrument name
- Replace the ultraviolet lamp
- Perform an XYO calibration
- Perform a Z calibration

**Note:** You must install the Ion Chef™ Instrument firmware update in Torrent Suite™ Software 5.12 or later to access the XYO calibration and Z calibration routines.
Symbols on this instrument ......................................................... 88
Location of safety labels on this instrument ................................. 90
Safety information for instruments not manufactured by Thermo Fisher Scientific ...................................................... 90
Instrument safety ........................................................................ 90
Safety and electromagnetic compatibility (EMC) standards ............. 92
Chemical safety ........................................................................... 94
Biological hazard safety .............................................................. 95

⚠️ **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, see the “Documentation and Support” section in this document.

---

**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.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>English</th>
<th>Français</th>
</tr>
</thead>
<tbody>
<tr>
<td>🟢⚠️</td>
<td>Caution, risk of danger</td>
<td>Attention, risque de danger</td>
</tr>
<tr>
<td></td>
<td>Consult the manual for further safety information.</td>
<td>Consulter le manuel pour d’autres renseignements de sécurité.</td>
</tr>
<tr>
<td>🟢⚠️</td>
<td>Moving parts</td>
<td>Parties mobiles</td>
</tr>
<tr>
<td>🟢⚠️</td>
<td>Caution, hot surface</td>
<td>Attention, surface chaude</td>
</tr>
<tr>
<td>🟢⚠️</td>
<td>Ultraviolet light</td>
<td>Rayonnement ultraviolet</td>
</tr>
<tr>
<td>⚫️</td>
<td>On/Off</td>
<td>On/Off (marche/arrêt)</td>
</tr>
<tr>
<td>⚫️</td>
<td>Standby</td>
<td>En attente</td>
</tr>
<tr>
<td>⚫️</td>
<td>Protective conductor terminal (main ground)</td>
<td>Borne de conducteur de protection (mise à la terre principale)</td>
</tr>
<tr>
<td>⚫️</td>
<td>Terminal that can receive or supply alternating current or voltage</td>
<td>Borne pouvant recevoir ou envoyer une tension ou un courant de type alternatif</td>
</tr>
<tr>
<td>⚫️</td>
<td>Do not dispose of this product in unsorted municipal waste</td>
<td>Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.</td>
</tr>
</tbody>
</table>

**CAUTION!** 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.

**MISE EN GARDE !** 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

<table>
<thead>
<tr>
<th>Conformity mark</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Conformity mark]</td>
<td>Indicates conformity with safety requirements for Canada and U.S.A.</td>
</tr>
<tr>
<td>![Conformity mark]</td>
<td>Indicates conformity with Australian standards for electromagnetic compatibility.</td>
</tr>
</tbody>
</table>

Location of safety labels on this instrument

![Safety labels on the instrument](image)

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!](image) 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.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IEC 61010-1</td>
<td>Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements</td>
</tr>
<tr>
<td>EN 61010-1</td>
<td>SAFETY REQUIREMENTS FOR ELECTRICAL EQUIPMENT FOR MEASUREMENT, CONTROL, AND LABORATORY USE – PART 1: GENERAL REQUIREMENTS</td>
</tr>
<tr>
<td>UL 61010-1</td>
<td>SAFETY REQUIREMENTS FOR ELECTRICAL EQUIPMENT FOR MEASUREMENT, CONTROL, AND LABORATORY USE – PART 1: GENERAL REQUIREMENTS</td>
</tr>
<tr>
<td>CSA C22.2 No. 61010-1</td>
<td>SAFETY REQUIREMENTS FOR ELECTRICAL EQUIPMENT FOR MEASUREMENT, CONTROL, AND LABORATORY USE – PART 1: GENERAL REQUIREMENTS</td>
</tr>
<tr>
<td>IEC 61010-2-010</td>
<td>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials</td>
</tr>
<tr>
<td>EN 61010-2-010</td>
<td>SAFETY REQUIREMENTS FOR ELECTRICAL EQUIPMENT FOR MEASUREMENT, CONTROL AND LABORATORY USE – PART 2-010: PARTICULAR REQUIREMENTS FOR LABORATORY EQUIPMENT FOR THE HEATING OF MATERIALS</td>
</tr>
<tr>
<td>IEC/EN 61010-2-020</td>
<td>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-020: Particular requirements for laboratory centrifuges</td>
</tr>
<tr>
<td>IEC 61010-2-081</td>
<td>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</td>
</tr>
<tr>
<td>EN 61010-2-081</td>
<td>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</td>
</tr>
</tbody>
</table>
### EMC

<table>
<thead>
<tr>
<th>Reference</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Directive 2014/30/EU</td>
<td>European Union &quot;EMC Directive&quot;</td>
</tr>
<tr>
<td>FCC Part 15</td>
<td>U.S. Standard &quot;Industrial, Scientific, and Medical Equipment&quot;</td>
</tr>
<tr>
<td>AS/NZS 2064</td>
<td>Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment</td>
</tr>
<tr>
<td>ICES-001, Issue 3</td>
<td>Industrial, Scientific and Medical (ISM) Radio Frequency Generators</td>
</tr>
</tbody>
</table>

### Environmental design

<table>
<thead>
<tr>
<th>Reference</th>
<th>Description</th>
</tr>
</thead>
</table>
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.

Documentation and support

Related documentation

The following related user documentation is available for download at thermofisher.com.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion PI™ Hi-Q™ Chef Kit Quick Reference</td>
<td>MAN0010968</td>
</tr>
<tr>
<td>Ion Chef™ Instrument User Guide</td>
<td>MAN0018668</td>
</tr>
<tr>
<td>Ion Chef™ Instrument Network Connection User Guide</td>
<td>MAN0013444</td>
</tr>
<tr>
<td>Ion Chef™ System Site Preparation Guide</td>
<td>MAN0007956</td>
</tr>
<tr>
<td>Torrent Suite™ Software 5.12 User Guide</td>
<td>MAN0017972</td>
</tr>
<tr>
<td>Ion Sphere™ Quality Control Kit User Guide</td>
<td>MAN0017531</td>
</tr>
<tr>
<td>Ion Sphere™ Particles Quality Assessment for the Ion Proton™ and Ion S5™ Systems Using the Guava™ easyCyte 5 Benchtop Flow Cytometer User Bulletin</td>
<td>MAN0007496</td>
</tr>
</tbody>
</table>

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

Visit 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)

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

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. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.