iontorrent

Ion PI™ Hi-Q™ Sequencing 200 Kit

USER GUIDE

for use with:
Ion Proton™ System
Ion PI™ Chip v3

Catalog Numbers A26433, A26772
Publication Number MAN0010947
Revision D.0

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ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Revision history: Pub. No. MAN0010947

Revision	Date	Description
D.0	2 July 2019	• Updated for Torrent Suite™ Software 5.12. • Corrected the number of Ion Proton™ Reagent Tubes and Reagent Tube Sippers provided in the Ion Proton™ Sequencing Supplies sub kit. • "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. • Appendix B, "Best practices for a successful Ion PI™ sequencing run" added. • Appendix C, "Sharing Planned Runs between Torrent Servers", deleted; users are referred to an updated topic "Transfer a Planned Run to an Ion Torrent™ Server with Ion Mesh" on page 61.
C.0	12 January 2017	• Volume of 50% Annealing Buffer needed to prepare per chip corrected • Recommended volume of 18 MΩ water and chlorite solution to add to C1 and C2 Reagent Tubes in the sequencer cleaning procedures increased from 100 mL to 110 mL • Web links updated • Rebranding • Graphics enhanced
B.0	18 March 2015	Product launch version
A.0	22 December 2014	Restricted release of new sequencing kit

Contents

1 Product information

CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

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

Product description

The Ion PI™ Hi-Q™ Sequencing 200 Kit includes reagents and materials for sequencing up to 200-bp average insert libraries using Ion PI™ Chips v3 on the Ion Proton™ System. The sequencing kit also includes components for cleaning and initializing the instrument.

The kit is designed to sequence templates that have been amplified on Ion PI™ Ion Sphere™ Particles (ISPs) using the Ion PI™ Hi-Q™ OT2 200 Kit (Cat. No. A26434).

Each sequencing kit (Cat. No. A26433 or A26772) provides reagents and materials for 8 sequencing runs. Cat. No. A26433 provides components for one instrument initialization for every two sequencing runs, while A26772 provides additional initialization components, allowing one initialization for every sequencing run.

Sequencing type	Number of flows[1]	Number of sequencing runs per kit	Average sequencing run time
200-base-read	500	8	2.6 hours
150-base-read	400	8	2.1 hours
100-base-read	260	8	1.4 hours

[1] Some applications allow for shorter flow times and shorter read lengths. The number of flows can be reduced in increments of 20. Contact Technical Support for more information.

Library kit compatibility

The Ion PI™ Hi-Q™ Sequencing 200 Kit is optimized for 200-base-read libraries of any type, including Ion AmpliSeq™ Exome libraries.

Template kit compatibility

The Ion PI™ Hi-Q™ Sequencing 200 Kit must be used with the Ion PI™ Hi-Q™ OT2 200 Kit (Cat. No. A26434). Select the Ion PI™ Hi-Q™ OT2 200 Kit when creating a Planned Run.

Software compatibility

This sequencing kit is compatible with Torrent Suite™ Software 4.4 and later. We recommend that you update your system software to the latest available version before using this kit.

Ion PI™ Hi-Q™ Sequencing 200 Kit

Each Ion PI™ Hi-Q™ Sequencing 200 Kit (Cat. No. A26433 or A26772) contains the boxes and components listed below.

- Catalog No. A26433 contains one of each box listed below, for one instrument initialization for every two sequencing runs.
- Catalog No. A26772 contains an additional box each of the Supplies, Solutions, and Nucleotides, which allows one initialization for every sequencing run.

Subkit	Number of boxes included	
	Cat. No. A26433	Cat. No. A26772
Ion PI™ Hi-Q™ Sequencing 200 Reagents	1	1
Ion Proton™ Sequencing Supplies	1	2
Ion PI™ Hi-Q™ Sequencing 200 Solutions	1	2
Ion PI™ Sequencing Nucleotides	1	2

Kit contents and storage

Components	Amount	Storage
Ion PI™ Hi-Q™ Sequencing 200 Reagents (Part No. A26431)		
Ion PI™ Hi-Q™ Sequencing Polymerase (yellow cap)	48 µL	–30°C to –10°C
Ion PI™ Sequencing Primer (white cap)	160 µL	
Ion PI™ Control Ion Spheres (clear cap)	40 µL	
Ion Proton™ Sequencing Supplies (Part No. 4488651)		
Ion Proton™ Reagent Tube Caps	16 caps	15°C to 30°C
Ion Proton™ Wash Bottle Sippers	4 sippers	
Ion Proton™ Reagent Tube Sippers	32 sippers	

Components	Amount	Storage
Ion Proton™ Reagent Tubes with labels (140 mL)	8 packs of 4 tubes each	15°C to 30°C
Ion PI™ Hi-Q™ Sequencing 200 Solutions (Part No. A26430)		
Ion PI™ Hi-Q™ W2 Solution	4 × 125 mL	Shipped at 15°C to 30°C Store at 2°C to 8°C (store W2 Solution protected from light)
Ion PI™ 1X W3 Solution	2 × 100 mL	
Ion Cleaning Tablet	4 tablets	
Ion PI™ Annealing Buffer	30 mL	
Ion PI™ Loading Buffer (brown cap)	80 µL	
Ion PI™ Foaming Solution (violet cap)	1 mL	
Ion PI™ Sequencing Nucleotides (Part No. A26432)		
Ion PI™ dGTP (black cap)	280 µL	–30°C to –10°C
Ion PI™ dCTP (blue cap)	280 µL	
Ion PI™ dATP (green cap)	280 µL	
Ion PI™ dTTP (red cap)	280 µL	

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).

Component	Quantity	Storage
Ion Proton™ Wash 2 Bottle with label	1 bottle	15°C to 30°C

Ion PI™ Chip Kit v3

The Ion PI™ Chip Kit v3 (Cat. No. A26771), ordered separately, is compatible with the Ion PI™ Hi-Q™ Sequencing 200 Kit.

Component	Quantity	Storage
Ion PI™ Chip Kit v3 8-pack (2 boxes[1] of 4 chips each)	8 chips	15°C to 30°C

[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.

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

Item	Source
Ion Proton™ System (instrument and server) and included accessories	4476610
Ion PI™ Controls 200 Kit [1]	4488985
Tank of compressed nitrogen (grade 4.5, 99.995% pure or better)	MLS
Multistage (dual-stage) gas regulator (0–50 psi, 2–3 Bar output)	Fisher Scientific NC0393866, or MLS
(Optional) 1/8" x 1/4" stem reducing coupler (only required if using a separate tank for the wash station)	McMaster 5779K699
Uninterruptible Power Supply (UPS) [2]	MLS
ELGA™ PURELAB™ Flex 2 Water Purification System *or* ELGA™ PURELAB™ Flex 3 Water Purification System *or* Equivalent 18 MΩ water system	ELGA or MLS
NaOH (10 M), molecular biology grade	MLS
Isopropanol (100%)	MLS
Nuclease-free water, molecular biology grade	MLS
0.22-µm or 0.45-µm vacuum filtration system and filters	MLS
Microcentrifuge[3]	MLS
25-mL or 50-mL serological pipettes *or* 100-mL graduated cylinder	MLS
(If using serological pipettes) Pipet-Aid™ XP Pipet Controller, or equivalent	Fisher Scientific 13-681-06
Rainin™ Pipet-Lite™ LTS Pipette L-20XLS 2 to 20 µL[4]	Rainin 17014392
Rainin™ Pipet-Lite™ LTS Pipette L-100XLS 10 to 100 µL[4]	Rainin 17014384
Rainin™ LTS pipette tips, 20 µL, SR-L10F[4,5]	Rainin 17005860
Rainin™ LTS pipette tips, 200 µL, SR-L200F[4]	Rainin 17005859
PCR tubes, Flat Cap, 0.2-mL (do not use polystyrene tubes)	Fisher Scientific 14-222-262
1.5-mL or 1.7-mL microcentrifuge tubes	MLS

Item	Source
Glass bottles (1 L)	MLS
Ice buckets and ice	—
Vortex mixer with a rubber platform	MLS
Thermal cycler with a heated lid	MLS
Dry bath incubator	MLS
Standard laboratory vacuum line or vacuum pump	MLS
Liquid trap	MLS
Tygon™ tubing[6]	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*.

[4] Alternatives from Gilson and Eppendorf can be used.

[5] Ensure tips from any vendors are low binding tips.

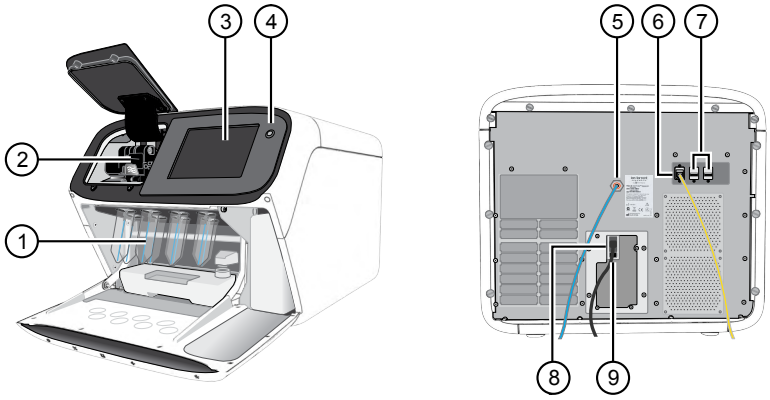
[6] If needed to connect laboratory vacuum to liquid trap and liquid trap to P200 pipette tip.

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.

Item	Catalog No.
Thermo Scientific™ Orion Star™ A111 pH Benchtop Meter Kit with electrode, electrode stand, and calibration buffers (or equivalent)	Fisher Scientific 13-645-503
1 N HCl	MLS
Magnetic stirrer (must hold 2-L bottle)	MLS
Magnetic stir bar (4 cm)	MLS
Squirt bottle	MLS

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.

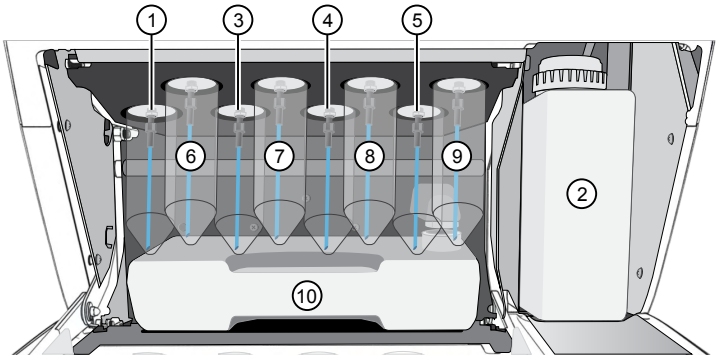
⑥ **Ethernet port** – An RJ45 port that provides Ethernet (100 Mbit) communication with the sequencer.

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**

Workflow

Use the following workflow to perform sequencing runs with the Ion Proton™ System.

"Create a Planned Run" on page 18

▼

"Clean the Ion Proton™ Sequencer" on page 23

▼

"Initialize the Ion Proton™ Sequencer" on page 28

▼

"Prepare the template-positive ISPs for sequencing" on page 36

▼

"Load the Ion PI™ Chip v3" on page 37

▼

"Start the sequencing run" on page 40

2 Before you begin

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.

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 CO_2 contamination

IMPORTANT! Dry ice (solid CO_2) 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 CO_2 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 introducing noise to instrument measurements

IMPORTANT! Install the Ion Proton™ System on a bench that is free from vibrations and that is not in contact with freezers, pumps, or other equipment that can cause vibrations. Significant vibration during sequencing may add noise and reduce the quality of the measurements.

See the *Ion Proton™ System Site Preparation Guide* (Pub No. 4478733) for Ion Proton™ System space requirements and clearances.

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.

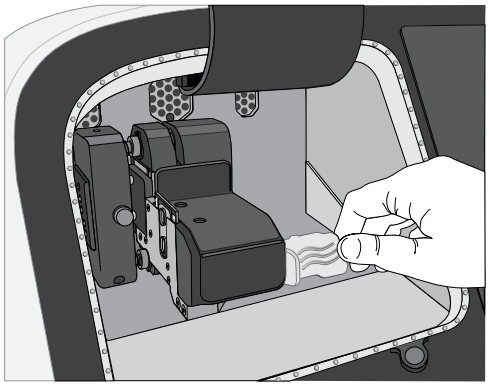
Guidelines for handling and inserting chips

- 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.

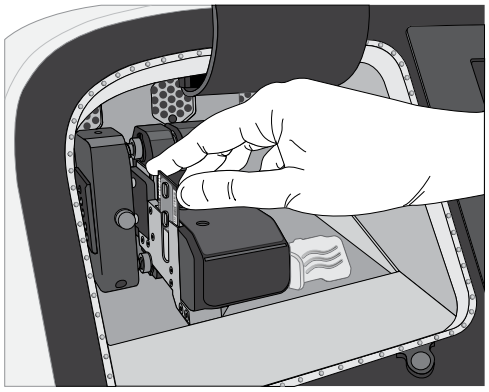
Insert a chip into the chip clamp

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.

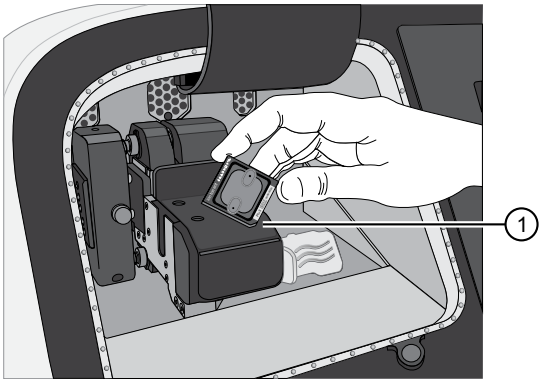


2. If necessary, remove the chip currently in the clamp.



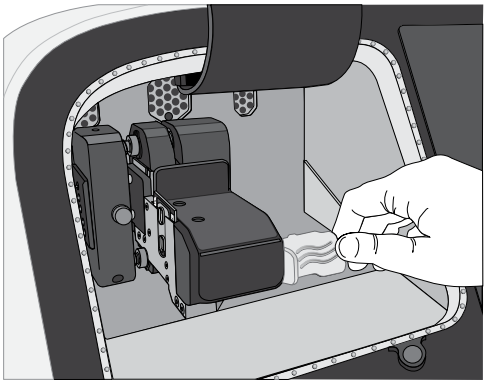
3. Place the appropriate chip in the chip clamp with the chip notch in the bottom-front corner.

   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.



① Notch

4. Push the metal tab back until it clicks to engage the clamp.



3 Create a Planned Run

About Planned Runs

Planned Runs contain all the settings that are used in a sequencing run, including number of flows, kit types, barcodes, sample information, and reference files (if any). They are used to track samples, chips, and reagents throughout the sequencing workflow, from template preparation through sequencing and subsequent data analysis.

You can create a Planned Run in the Torrent Suite™ Software on the Ion Torrent™ Server connected to your sequencer, and then select the appropriate plan on the sequencer touchscreen when you start the run.

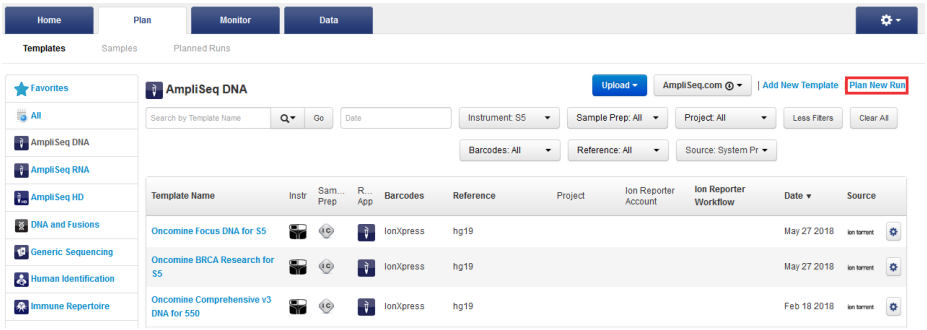
You can also create a Planned Run on one Ion Torrent™ Server and then transfer it to another server for sequencing. For more information, see "Transfer a Planned Run to an Ion Torrent™ Server with Ion Mesh" on page 61.

Create a Planned Run

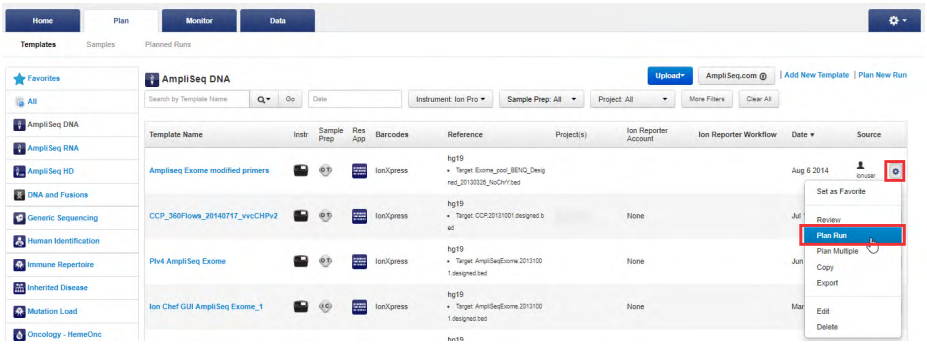
The following provides a summary of steps for creating a Planned Run in Torrent Suite™ Software, for use on the Ion Proton™ System. Ensure that you have updated to the latest version of Torrent Suite™ Software before starting a run.

For more detailed instructions, see the *Torrent Suite™ Software Help*.

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 create a new Planned Run using the generic template for the selected application.



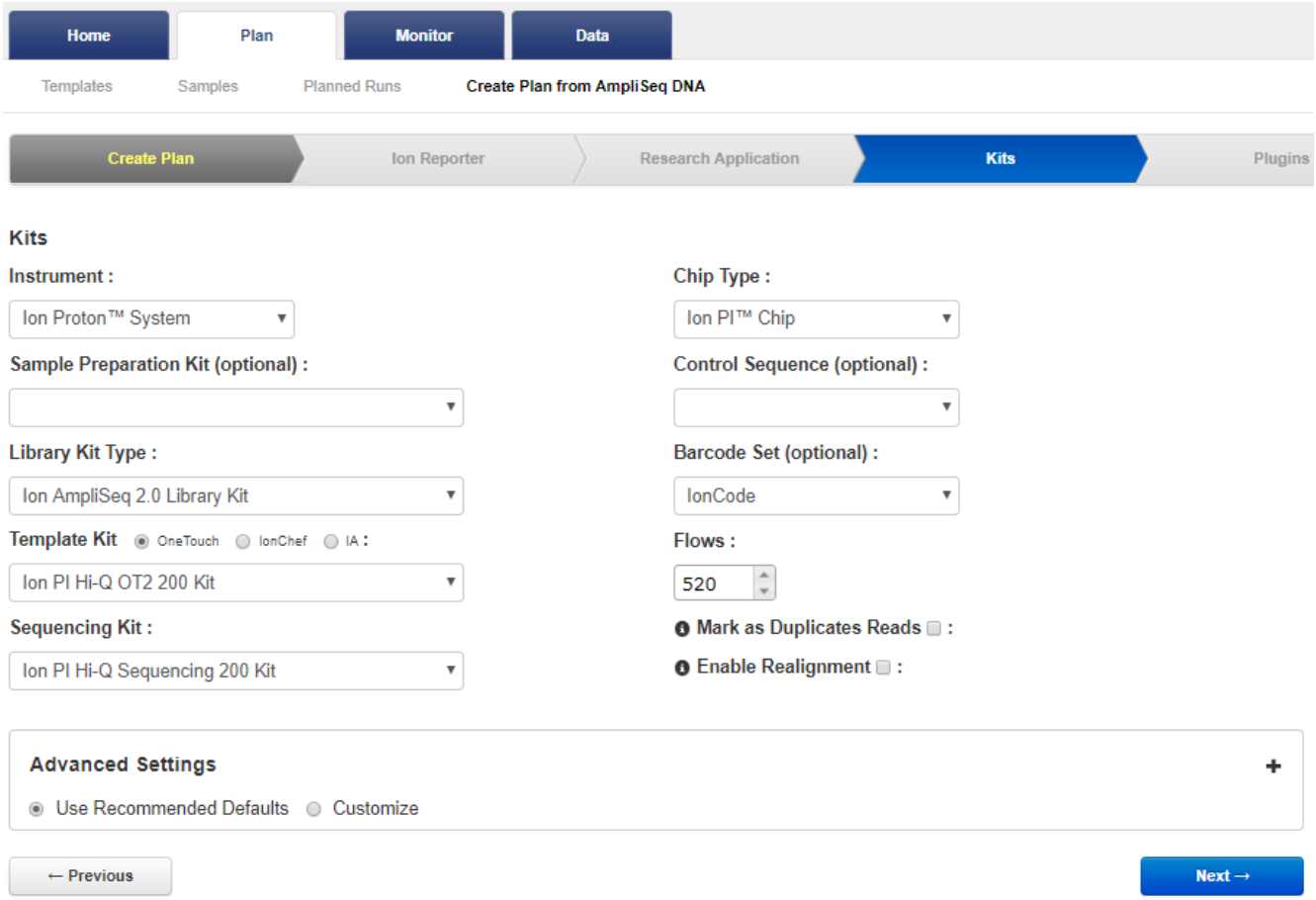
   - **Plan Run** in the dropdown menu under the **Settings** ⚙ tab to the right of an existing template to create a new Planned Run using that template.



3. In the Planned Run wizard, review the **Ion Reporter** and **Research Application** steps, 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 **OneTouch** for Template Kit, then select **Ion PI Hi-Q OT2 200 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 OT2 200 Kit** from the **Sequencing Kit** dropdown list.



g. 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.

h. Enter the appropriate number of **Flows**.

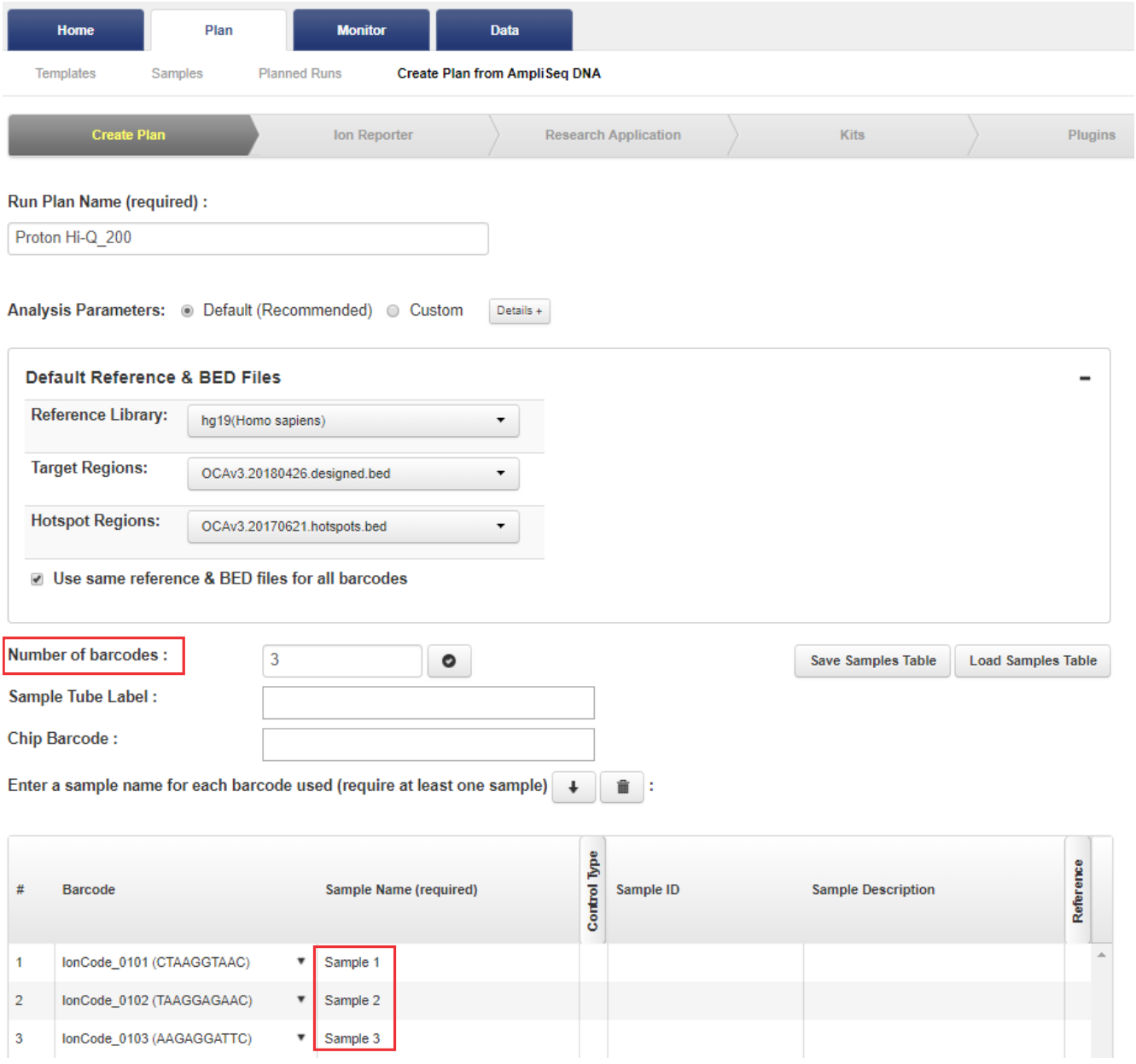
i. Expand the **Advanced Settings** field, then select or edit optional information fields appropriately for your run, if needed.

j. 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.

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 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 in the **Plan** screen, click **Plan Run**. The run is listed on the Planned Runs screen under the name you specified, and is available on the sequencer when you are setting up the run.

Planned Run workflow key fields

Field name	Description
IonReporter	If Ion Reporter™ Software is installed and enabled and you want to analyze the run data using the software, select the account and workflow.
Application	Select the sequencing application you are performing.
Instrument	Select **Ion Proton™ System**.
Chip Type	Select the chip type you are using.
Library Kit Type	Select the kit used to prepare the library.
Template Kit	Select **Ion PI Hi-Q OT2 200 Kit**.
Sequencing Kit	Select **Ion PI Hi-Q Sequencing 200 Kit**.
Barcode Set (optional)	If you are using barcodes with: • **DNA libraries**: Select the appropriate barcode set from the dropdown list • **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 BC01-16 Kit. If you are *not* using barcodes with: • **DNA libraries**: Leave the Barcode field blank. • **RNA libraries prepared using the Ion Total RNA-Seq Kit v2**: Select **RNA_Barcode_None** from the drop-down list. This will ensure that the proper trimming is performed on the resulting sequence when the RNA library does not have a barcode.
Flows	Enter the appropriate number of flows for the sequencing kit and read length.
Plugins	Select and configure the appropriate plugins for your application.

Field name	Description
Projects	Select or add a project within which to group your run data. You can include runs in multiple projects, and remove runs from a project at any time.
Run Plan Name	Enter a name for the run.
Reference Library	Select a reference library uploaded to Torrent Suite™ Software, if any.
Target Regions and Hotspot Regions	Select the **Target Regions** and/or **HotSpot Regions** BED file on the Torrent Server, if any.
Enter a sample name...	Specify the Sample Name, ID, and Description for each sample in the run (number of samples will change based on the number of barcodes and/or chips selected).
Monitoring Thresholds	Set thresholds for Bead Loading, Key Signal, and Usable Sequence. In the Torrent Suite™ Software **Monitor ▸ Runs in Progress** tab, an alert is displayed if the values for a run fall below the selected thresholds.

Clean and initialize the Ion Proton™ Sequencer

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.

Clean with:	Schedule
18 MΩ water	• Before each initialization. • (Recommended) After the last run of the day if the instrument will not be used within 72 hours after the last run (for example, clean after the last run before a 3-day weekend). • Before shutting the instrument down for an extended period.
Chlorite solution	• Once a week (unless the instrument has not been used since the last chlorite cleaning, in which case, clean with 18 MΩ water before using). • If reagents have been left on the instrument for more than 48 hours (for example, over the weekend).

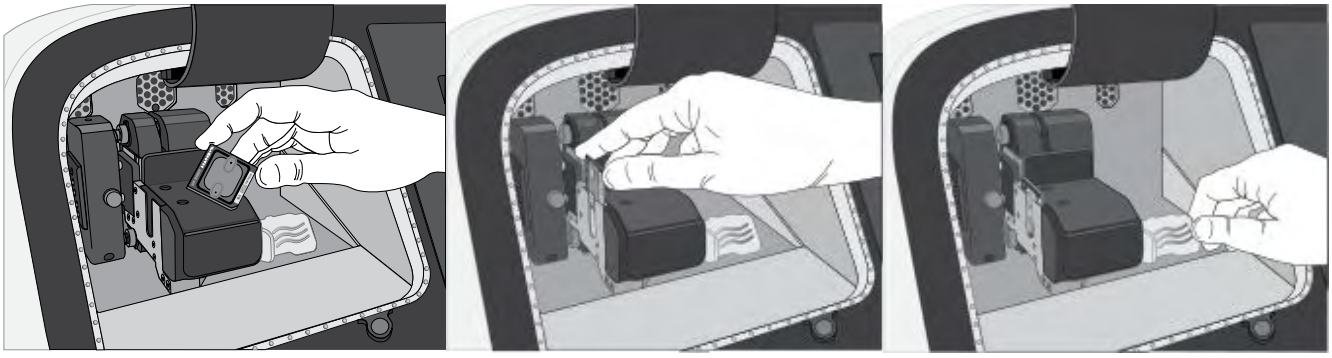
18 MΩ water cleaning

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.

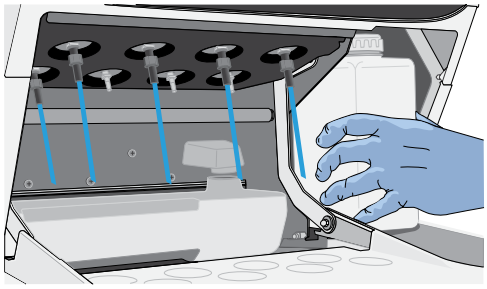
   Note: Do not force the chip into the clamp. If the chip does not fit easily in the clamp, confirm that the notch is oriented as shown in the following figures. For more detailed instructions, see "Insert a chip into the chip clamp" on page 15.



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

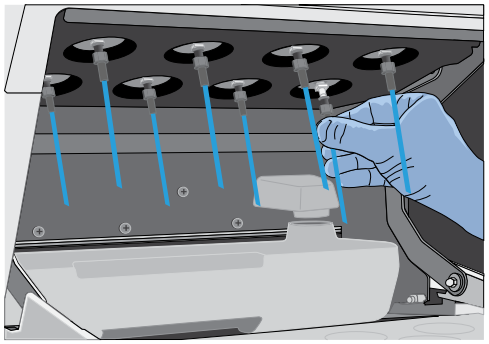
      Note: If needed, you can reuse the same C1 and C2 Reagent Tubes for multiple cleanings for up to 1 week. If you reuse the C1 and C2 Reagent Tubes, ensure that the tubes are correctly labeled, and cap the tubes when they are not installed on the instrument.

b. Remove the Wash 2 Bottle, discard the liquid, then save the bottle for reuse in initialization.

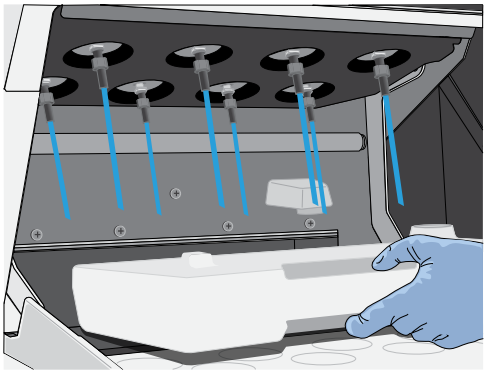


c. Remove the old Sippers from the C1 and C2 positions. Put on fresh gloves, then install new short blue Sippers in those positions.

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

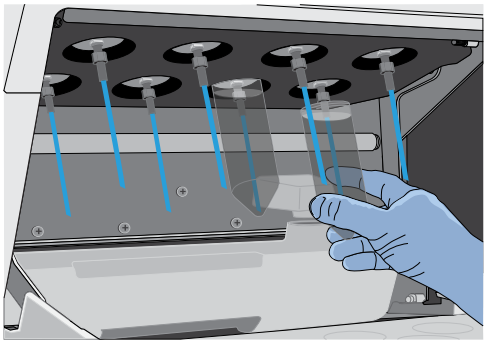


4. Remove the Waste Container, empty the waste, replace the container on the instrument, then tap **Next**.

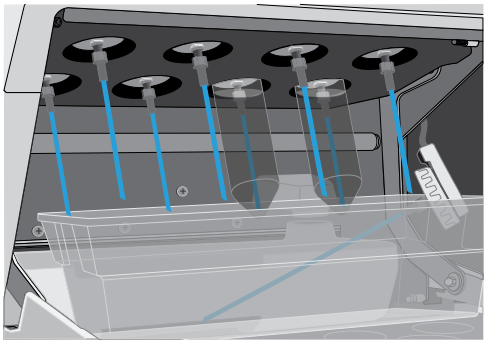


5. Rinse the C1 and C2 Reagent Tubes twice with ~110 mL of 18 MΩ water.

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 28.

Chlorite cleaning

Perform chlorite cleaning weekly, and as directed in "Cleaning schedule" on page 24.

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 thaw on ice.
- Check the tank pressure for the nitrogen gas. When the tank pressure drops below 500 psi, change the tank.

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 43.
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**.

Prepare the Wash 2 Bottle and install

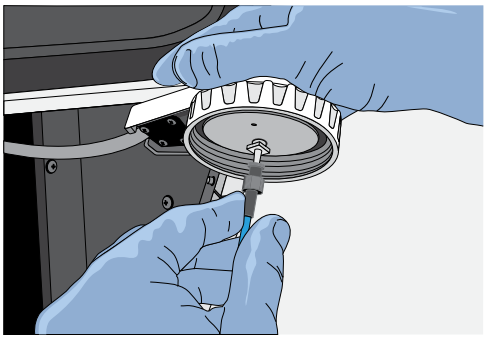
1. Rinse the Wash 2 Bottle three times with 200 mL of 18 MΩ water, directly from the water purification system. Do not use water that is stored in other containers.

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).

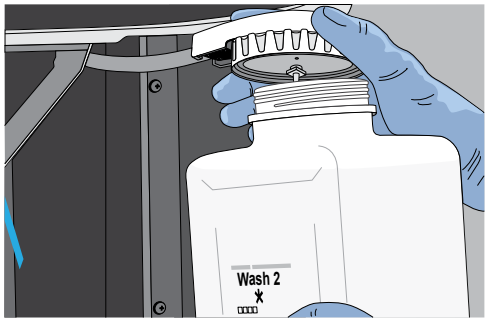
3. Add the entire bottle of Ion PI™ Hi-Q™ W2 Solution to the Wash 2 Bottle. Immediately cap the bottle securely and invert five times to mix.

   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.

Prepare and install the Reagent Tubes with dNTP solutions

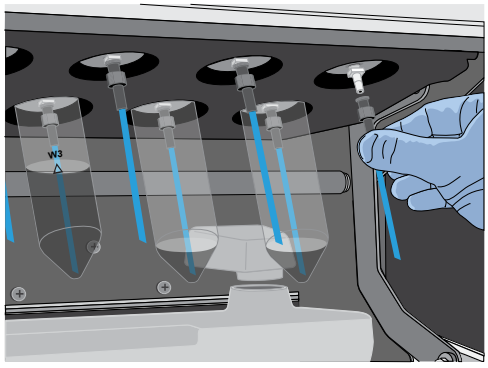
IMPORTANT! In the following steps, handle the nucleotides carefully to avoid cross-contamination and ensure that the correct dNTP solution is installed in each position on the Ion Proton™ Sequencer.

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 Reagent Tube Sippers and the collection tray.

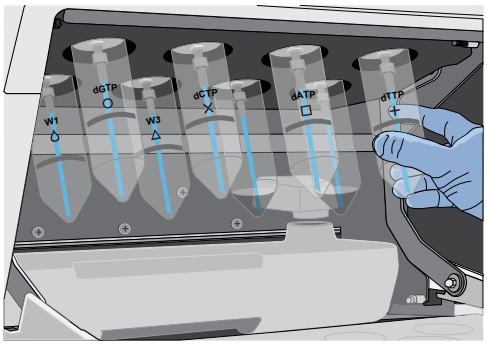
4. Using new gloves, attach a new short Sipper to each dNTP port. **Do not let the Sippers touch any surfaces.**



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, then 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. Ensure that all Reagent Tubes and the 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 5, "Load the chip and start the sequencing run".
   - If a Failed screen appears, see "Error message: Reagent pH: Failed; Reagent pH is displayed" on page 49.

5 Load the chip and start the sequencing run

Materials required

Materials provided in the Ion PI™ Hi-Q™ Sequencing 200 Kit

- Ion PI™ Control Ion Spheres
- Ion PI™ Annealing Buffer
- Ion PI™ Sequencing Primer
- Ion PI™ Loading Buffer
- Ion PI™ Foaming Solution (10% Triton™ X-100)
- Ion PI™ Hi-Q™ Sequencing Polymerase

Other required materials and equipment

- Ion PI™ Chip Kit v3 (Cat. No. A26771)
- Enriched template-positive Ion PI™ ISPs prepared with the Ion PI™ Hi-Q™ OT2 200 Kit (Cat. No. A26434)
- Standard laboratory vacuum line or vacuum pump
- Liquid trap
- Tygon™ tubing

  Note: As needed to connect laboratory vacuum to liquid trap and liquid trap to P200 pipette tip.
- Rainin™ Pipet-Lite™ XLS LTS with tips
- P200 and P10 pipette and filtered tips
- Vortex mixer
- Molecular-biology grade nuclease-free water
- 100% isopropanol

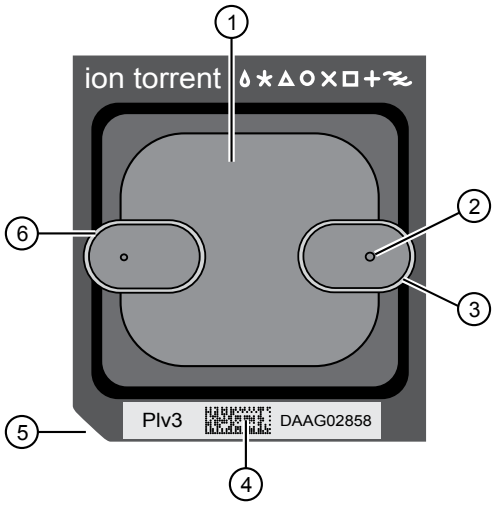
- Thermal cycler with heated lid (programmed at 95°C for 2 minutes and 37°C for 2 minutes)
- Ion Chip™ Minifuge (Cat. No. 4479672 or 4479673) equipped with Minifuge Proton Rotor and Bucket (Cat. No. 4482578)

Scheduling sequencing runs after initialization

If you are performing two sequencing runs per initialization, schedule your sequencing runs as follows:

- The first sequencing run should be started within 1 hour after initialization.
- We recommend starting the second run on the same day, but the second run may be started up to 24 hours after initialization.

Chip loading guidelines

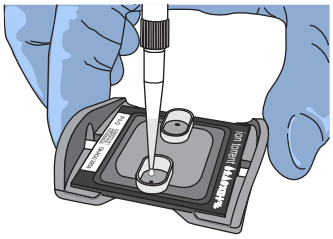


Ion PI™ Chip v3

1. Flow cell
2. Loading port
3. Loading well
4. Barcode
5. Chip notch
6. Exit well

When loading a sample:

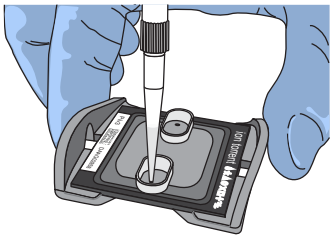
- Place the chip on a flat, stable surface such as a benchtop.



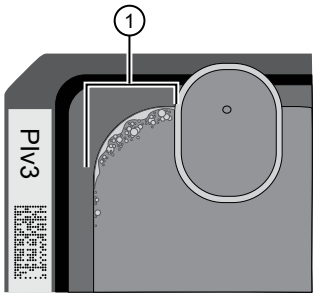
- Pipet the sample into the chip loading well.

When injecting reagents or buffers:

- Place the chip on a flat, stable surface such as a benchtop.



- With the pipette tip at a 90° angle to the chip, press the tip firmly into the circular loading port, and apply gentle pressure between the pipette tip and chip.
- Pipet carefully to avoid introducing bubbles into the chip flow cell (see example with introduced air bubbles on right).
- After each injection, remove the expelled liquid from the exit well, opposite the loading well.



① Air bubbles

Before you begin

IMPORTANT! Use enriched, template-positive Ion PI™ ISPs prepared using the Ion PI™ Hi-Q™ OT2 200 Kit.

- Thaw the Sequencing Primer.
- Prepare the following stock solutions fresh weekly or more frequently if needed:
  - **50% Annealing Buffer**: In a 1.5-mL tube, combine 0.5 mL of Ion PI™ Annealing Buffer with 0.5 mL of nuclease-free water (you need ~530 µL of 50% Annealing Buffer for each run).
  - **Flushing solution**: In a 1.5-mL tube, combine 0.5 mL of 100% isopropanol with 0.5 mL of Ion PI™ Annealing Buffer (for use in "Flush the chip and load the Ion PI™ Hi-Q™ Sequencing Polymerase" on page 39; you need 200 µL of Flushing solution for each run).

  Note: You can prepare multiple 1-mL aliquots of stock solutions at the same time, and store at room temperature. After opening an aliquot, use the contents within 1 week. Discard any opened, unused solution after 1 week.
- Check for updates to Torrent Suite™ Software and Ion Proton™ Sequencer software, and install the updates if available.
- Before using the Ion Chip™ Minifuge for the first time, perform the procedures in "Set up and test the Ion Chip™ Minifuge" on page 64.

Prepare the template-positive ISPs for sequencing

Add Ion PI™ Control Ion Spheres to the enriched ISPs

IMPORTANT! If you are performing an installation or troubleshooting run, do not use enriched ISPs. Follow the procedure in "Troubleshooting using the Ion PI™ Control Ion Spheres" on page 53 to prepare the control ISPs for the installation or troubleshooting run.

1. Vortex the Ion PI™ Control Ion Spheres for 5 seconds, then centrifuge for 2 seconds before taking aliquots.
2. Add 5 µL of control ISPs directly to the entire volume of enriched, template-positive ISPs in a 0.2-mL PCR tube (non-polystyrene), then pipet up and down to mix.

Anneal Sequencing Primer to the enriched ISPs

Note: The Ion PI™ ISPs are difficult to see. To avoid aspirating the particles in the following steps, orient the PCR tube the same way each time when centrifuging so that it is easy to know where the pellet has formed, and remove the supernatant from the top down.

1. Centrifuge the enriched, template-positive ISPs for 5 minutes at 15,500 × *g*.
2. Carefully remove the supernatant without disturbing the pellet, leaving 10 µL of supernatant in the tube (visually compare to 10 µL of liquid in a separate tube).
3. Add 15 µL of Ion PI™ Annealing Buffer for a total volume of 25 µL.
4. Add 20 µL of Ion PI™ Sequencing Primer, then confirm that the total volume is 45 µL. Add Ion PI™ Annealing Buffer if needed to bring the total volume to 45 µL.
5. Briefly vortex to mix, then centrifuge briefly to collect the contents at the bottom of the tube.
6. Program a thermal cycler for 95°C for 2 minutes and then 37°C for 2 minutes, using the heated lid option.
7. Place the tube in the thermal cycler, then run the program.
8. After cycling, add 10 µL of Ion PI™ Loading Buffer, briefly vortex to mix, then centrifuge briefly to collect the contents at the bottom of the tube.

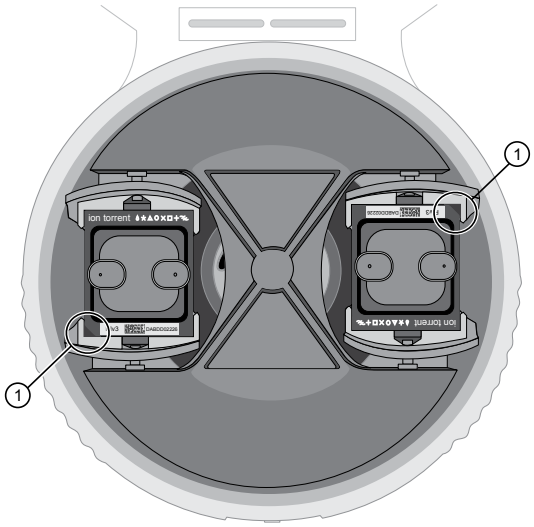
Load the Ion PI™ Chip v3

Load the sample on the chip

1. Place the Ion PI™ Chip v3 on a flat, stable surface.
2. Dispense the entire prepared sample (55 µL) into the chip loading well (not the chip loading port) of the chip.

   Note: Some sample enters the flow cell at this point by capillary action. The remaining sample is loaded into the flow cell by centrifugation.

3. Transfer the chip to a bucket in the Ion Chip™ Minifuge with the chip notch pointing **out**, away from the center of the minifuge. Place a used chip in the opposite bucket with the chip notch also pointing out.



① Chip notch

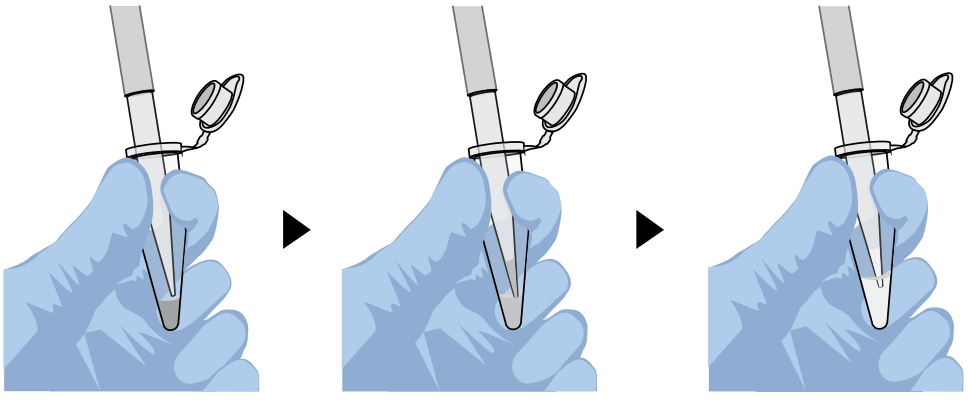
4. Centrifuge for 10 minutes.

5. In a 1.5-mL tube, combine 49 µL of 50% Annealing Buffer with 1 µL of Foaming Solution (10% Triton™ X-100).

   Note: You can prepare a bulk mixture by combining 4.9 mL of 50% Annealing Buffer with 100 µL of Foaming Solution. The mix can be stored at 4°C and used for up to 6 months.

6. Create foam by injecting air into the 50-µL mixture from the previous step using a Rainin™ SR-L200F pipette set to dispense 100 µL. Next, break the large bubbles into smaller bubbles by rapidly pipetting for ~5 seconds. Repeat this step one more time.

   Note: Do not over-inject the air; the final volume of foam should be approximately 250 µL.



7. Place the chip on a stable surface such as a benchtop, then inject 100 µL of foam into the chip loading port. Remove the expelled liquid from the opposite port.

8. Dispense 55 µL of 50% Annealing buffer into the chip loading well (not the chip loading port).
9. Place the chip back in the minifuge with the chip notch pointing out, then centrifuge for 30 seconds.
10. Place the chip on a stable surface such as a benchtop. Remove the liquid that has accumulated in both of the chip loading wells.
11. Briefly "re-foam" the foam sample by pipetting rapidly for ~5 seconds, then inject 100 µL of foam into the chip loading port. Remove the expelled liquid from the opposite port.
12. Dispense 55 µL of 50% Annealing buffer into the chip loading well (not the chip loading port).
13. Place the chip back in the minifuge with the chip notch pointing out, then centrifuge for 30 seconds. Then proceed to flushing the chip.

Flush the chip and load the Ion PI™ Hi-Q™ Sequencing Polymerase

1. Inject 100 µL of the Flushing solution into the chip loading port 2 times. After each injection, discard the solution that is expelled from the opposite port.
2. Inject 100 µL of 50% Annealing Buffer into the chip loading port 3 times. Do not introduce air bubbles. After each injection, remove the expelled liquid from the opposite port.
3. Combine 6 µL of Ion PI™ Hi-Q™ Sequencing Polymerase with 60 µL of 50% Annealing buffer.
4. Inject 65 µL of the polymerase solution into the chip loading port and remove the expelled liquid from the exit port. Be careful to avoid introducing air bubbles.
5. Allow the chip to incubate for 5 minutes, then immediately proceed to “Start the sequencing run“.

Start the sequencing run

IMPORTANT! Do not start the sequencing run with the loaded chip. Use a used Ion PI™ Chip v3 for the line cleaning at the start of the run.

1. With the used Ion PI™ Chip v3 from initialization still in the chip clamp, tap **Run** on the Main Menu, then tap **Next**. Confirm that "Cleaning fluid lines" displays on the instrument touchscreen. Observe the chip for leaks.

   Note: Never open the chip clamp during line cleaning. If there is a leak, press the **Abort** button immediately to stop the flow to the chip, then see “Liquid in drip pan below chip clamp“ on page 45.

2. After line cleaning, tap **Next**.

3. In the dropdown list, select a Planned Run that you created in the Torrent Suite™ Software, then tap **Next**.

4. Ensure that the run settings are correct, or make changes using the buttons and dropdown lists if needed.

   Note: If an error message appears, see “Error message: Not enough disk space for the necessary number of flows“ on page 44.

5. Remove the used Ion PI™ Chip v3 from the chip clamp, then secure the Ion PI™ Chip v3 loaded with template-positive Ion PI™ ISPs. 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 48.

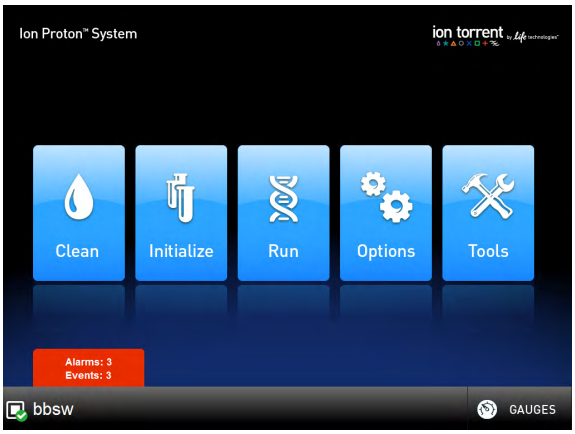
   IMPORTANT! During a run, do not open the chip compartment lid or reagent compartment door, and avoid touching the instrument. Touching the instrument during the sequencing run can reduce the quality of the measurements.

When the run is complete, the touchscreen returns to the Main Menu. Use Torrent Suite™ Software to review your results. Clean and initialize the instrument before starting a new run. See Chapter 4, “Clean and initialize the Ion Proton™ Sequencer“. If the instrument will not be used for more than three days, see “Powering off“ on page 60.

A

Troubleshooting

Ion Proton™ Sequencer alarms and events

Observation	Possible cause	Recommended action
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

Observation	Possible cause	Recommended action
Chip is secured in chip clamp, but chip icon indicates no chip detected 	• Clamp is not engaged • Chip is not properly seated • Chip is damaged or dirty • Issue with chip socket	1. Remove the chip from the chip clamp. **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. 2. Examine the chip for damage, 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, look at the chip icon to confirm the chip is detected, then tap **Next** or make a selection in the Main Menu. • 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. **Note:** Alternatively, clean the back surface of the chip with a lint-free laboratory wipe treated with isopropanol, then dry using a clean wipe. 3. If the chip is not detected by the instrument, there may be a problem with the chip socket. Contact Technical Support.
Error Message: Confirm Instrument Has Gas Pressure and/or Pressure icon indicates low gas pressure (0 PSI) **Note:** The correct operating pressure is 10.5 psi.	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.

Observation	Possible cause	Recommended action
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 (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.
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

Observation	Possible cause	Recommended action

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

Observation	Possible cause	Recommended action
Touchscreen not registering input in correct location	Touchscreen needs to be calibrated	• In Main Menu, select **Tools ▸ Screen Cal**, then follow the onscreen prompts. • If the touchscreen continues to malfunction after Screen Cal, contact Technical Support.
Touchscreen inoperable	Damaged or defective touchscreen	Contact Technical Support.

Instrument error messages

Note: Error messages are listed in alphabetical order.

Observation	Possible cause	Recommended action
Error message: Added too much W1 to W2	• Poor water quality (18 MΩ water not used directly from water purifier, or exposed to air for too long) • Too much W2 Solution used to prepare Wash 2 Bottle • Incorrect solution added to the Wash 2 Bottle • Too little NaOH added to Wash 1 Reagent Tube • Damaged chip	1. Confirm high water quality and correct preparation of the 1 M NaOH and Wash 2 Bottle. 2. If solution preparation is incorrect or water quality is poor, correctly prepare the solution(s) and/or use high-quality water. 3. Clean the instrument. 4. Repeat instrument initialization with fresh reagents and a new (unused) chip. (The new chip can be used for sequencing after initialization completes.) **Note:** 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 the W2 Solution.
Error Message: Check Wash1 for leaks	• W1 Reagent Tube seal is not tight • Tube may be damaged or defective	1. Remove the Wash 1 Reagent Tube, then replace in the W1 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.

Observation	Possible cause	Recommended action
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 (0 PSI) **Note:** The correct operating pressure is 10.5 psi.	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.

Observation	Possible cause	Recommended action

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.

Observation	Possible cause	Recommended action
Error message: OVERSHOT TARGET PH: W2 pH = n.nn Failed	Auto-pH added more NaOH from the Wash 1 Reagent Tube to the Wash 2 Bottle than was needed	1. Reinitialize the instrument using new reagents and a new chip (the chip can then be used for sequencing). 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. 3. If the pH is consistent with the pH of the previous chip, manually adjust the pH of the W2 Solution. **Note:** 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.
Error message: Please insert a chip and press Start	Instrument cannot detect the chip in chip clamp	See recommended action for "Chip is secured in chip clamp, but chip icon indicates no chip detected" on page 43.
Error message: Reagent pH: Failed; Reagent pH is displayed **Note:** Message displays on touchscreen after Reagent Check at end of Initialization procedure.	One or more reagents are not within the target pH	1. Tap **Restart** to restart auto pH and confirm the measurement. 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). 3. If any reagents fail, clean and reinitialize the instrument with fresh reagents and a new chip.

Observation	Possible cause	Recommended action
Error message: Reagent pH: Failed; Reagent pH is not displayed **Note:** Message displays on touchscreen after Reagent Check at end of Initialization procedure.	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.

Observation	Possible cause	Recommended action
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 51. 2. Press **Restart** to re-start auto-pH. If the "Undershot 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.

Observation	Possible cause	Recommended action
Error message: W2 average not stable. Try reseating/replacing chip	The waste lines may be blocked	See first recommended action 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 51.
	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 52.

Sample loading or sequencing

Observation	Possible cause	Recommended action
Ion PI™ Control Ion Spheres are not present in the Test Fragment Report section of the run report and library sequencing is poor	• Poor chip loading • Ion PI™ Control Ion Spheres were not added to the sample • Chip is damaged • Instrument failure	1. Confirm that the Ion PI™ Control Ion Spheres (included in this sequencing kit) were added. 2. If controls were added, contact Technical Support.
Sample results were obtained, but poor or no results for Ion PI™ Control Ion Spheres in Test Fragment report	• No Ion PI™ Control Ion Spheres added to the sample • Ion PI™ Control Ion Spheres are past expiry date	Check Ion PI™ Control Ion Spheres expiry date.

Observation	Possible cause	Recommended action
Good conversion rate for Ion PI™ Control Ion Spheres, but poor sample loading and sequencing	Problems with library or template preparation	Verify the quantity and quality of the library and template preparations.

Troubleshooting using the Ion PI™ Control Ion Spheres

To prepare Ion PI™ Control Ion Spheres for an installation or troubleshooting sequencing run:

1. Create a Planned Run and clean, then initialize the Ion Proton™ Sequencer as usual.
2. When you are ready to load the chip, vortex the Ion PI™ Control Ion Spheres for 5 seconds, then centrifuge for 2 seconds before taking aliquots.
3. Add 66 µL of control ISPs to an empty 0.2-mL PCR tube (non-polystyrene).
4. Add 150 µL of Ion PI™ Annealing Buffer to the tube.
5. Proceed to step 1 of "Anneal Sequencing Primer to the enriched ISPs" on page 37, substituting the control ISPs prepared as described for the enriched template-positive ISPs. Centrifuge the control ISPs, add annealing buffer and sequencing primer to a total volume of 45 µL, then proceed with annealing, chip loading, and sequencing as described.

B

Best practices for a successful Ion PI™ sequencing run

The following guidelines and tips are critical for optimal performance of the Ion PI™ Hi-Q™ Sequencing 200 Kit and Ion PI™ Chips v3 with the Ion Proton™ System. These specifications and recommendations are also found elsewhere in this guide, in the *Ion Proton™ System Specifications Sheet*, and the *Ion Proton™ System Site Preparation Guide*. Follow these guidelines carefully to achieve the best results with Ion PI™ products on the Ion Proton™ System.

General guidelines for using the Ion Proton™ System

- **Maintain a working environment temperature 20°C–25°C with non-condensing relative humidity of 40–60%.**
  - Operation outside of these working conditions creates a risk to product performance and the long-term health of instrument.
- **Provide Ion Proton™ Sequencer clearance of 30.5 cm at the rear of the instrument, and 10 cm at the sides.**
  - Instrument clearance that is below specification results in improper temperature control of the sequencer. Clearance below specification creates a risk to instrument health and to run performance. Clearance at or above specification is optimal for Ion PI™ sequencing runs. Ensure that the sequencer is clear of the air flow from other ventilated laboratory equipment, including the Ion Torrent™ Server.
- **Maintain the Ion Proton™ Sequencer gas supply at 30 psi, and instrument pressure at 10.5 psi.**
  - The Ion Proton™ Sequencer was designed to run at 10.5 psi, which allows for optimal reagent flow rates. Instrument pressure below 10.5 psi introduces sequencing errors. Instrument pressure above 10.5 psi causes the instrument to deplete sequencing reagents prematurely.
- **Keep all doors of the Ion Proton™ Sequencer closed during a run and between runs.**
  - To ensure proper temperature moderation and air flow within the Ion Proton™ Sequencer, both the chip bay door and reagent door must be closed during a run. The chip bay temperature is maintained by a heater that is located in the bay. The chip bay door should be open only when installing or removing a chip. Failure to maintain chip bay temperature results in reduced performance.
  - Optimal airflow within the Ion Proton™ Sequencer is achieved with the reagent bay door closed. The reagent bay door should be opened only during instrument cleaning and initialization. Keeping the reagent bay door closed during and after sequencing limits instrument exposure to additional noise or other environmental factors.

Guidelines for a successful Ion PI™ sequencing run

- **Prepare libraries for Ion PI™ sequencing with no longer than 200 base-read average insert length.**
  - The Ion PI™ Hi-Q™ OT2 200 Kit and the Ion PI™ Hi-Q™ Sequencing 200 Kit have been optimized for sequencing libraries with up to 200 base-read average insert length. Insert lengths above this range result in reduced performance. Use of libraries with average insert length longer than 200 base-reads is not supported.
- **Follow these recommended Ion PI™ library preparation best practices:**
  - Prepare libraries that are free of adapter dimers. For example, use the Agencourt™ AMPure™ XP Reagent (Fisher Scientific Cat. No. NC9959336) as a cleanup step.
  - Dilute libraries in Low TE (10 mM Tris-HCl, 0.1 mM EDTA), or nuclease-free water.
  - For long-term storage, store concentrated libraries at –20°C.
  - For short-term storage, store diluted libraries at 4°C.
  - Handle and store all libraries in low DNA binding tubes.

Item	Source
Eppendorf™ DNA LoBind™ Microcentrifuge Tubes	Fisher Scientific 13-698-791
PCR tubes, Flat Cap, 0.2-mL (do not use polystyrene tubes)	Fisher Scientific 14-222-262

- **Titrate library input to determine the library input that produces optimal sequencing results.**
  - A good starting point for library titration is 360×10^6–480×10^6 molecules, or 6–8 pM, as outlined in the *Ion PI™ Hi-Q™ OT2 200 Kit User Guide* (Pub. No. MAN0010857). One µL of a 100 pM library contains 60×10^6 molecules.
  - We recommend using Applied Biosystems™ TaqMan® Assays to quantify library concentration. The Agilent™ 2100 Bioanalyzer™ instrument and Qubit™ Fluorometer can also be used.
- **Use the recommended Rainin™ Pipet-Lite™ LTS Pipette L-100XLS 10–100 µL and tips for chip loading.**
  - This pipettor has been extensively used in kit and chip development, and is the preferred pipettor for loading of ISPs on an Ion PI™ Chip v3. Use low-retention pipette tips.

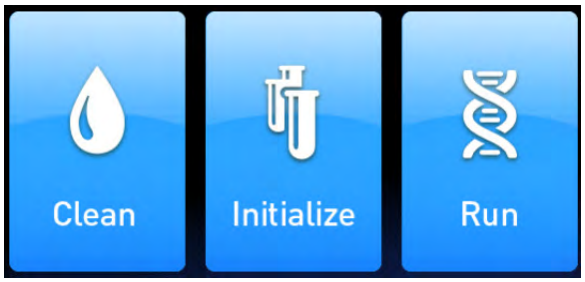
Item	Source
Rainin™ Pipet-Lite™ LTS Pipette L-100XLS 10 to 100 µL	Rainin 17014384
Rainin™ LTS pipette tips, 200 µL, SR-L200F	Rainin 17005859

C

Touchscreen Reference

Main menu

Clean, Initialize, and Run



The **Clean**, **Initialize**, and **Run** programs lead you through the necessary steps to prepare the instrument for sequencing and start a sequencing run.

- The **Clean** program can be used to perform either 18 MΩ water or chlorite solution cleaning. Cleaning must be performed before each initialization to ensure that the reagents from the previous run are cleared from the fluid lines. Abbreviated instructions are provided on the touchscreen; for the complete cleaning schedule and detailed instructions, see "Clean the Ion Proton™ Sequencer" on page 23.
- The **Initialize** program must be performed before each run to prepare the run reagents. The Initialize program walks you through the following steps:
  - Specifying the sequencing kit.
  - Setting up wash solutions. (After this step, the instrument adjusts and checks the pH of the Wash 2 Bottle solution.)
  - Setting up dNTPs. (After this step, the instrument checks the pH of all of the reagents.)

  Abbreviated instructions are provided on the touchscreen; for detailed instructions, see "Initialize the Ion Proton™ Sequencer" on page 28.
- The **Run** program walks you through steps leading up to and through sequencing, including:
  - Calibrating a chip before loading the sample on the chip.
  - Placing a loaded chip on the instrument.
  - Calibrating the loaded chip.
  - Selecting a Planned Run.
  - Performing sequencing.

  For detailed instructions, see Chapter 5, "Load the chip and start the sequencing run"

Options and Tools

- The **Options** menu gives you access to software updates. See "Update Ion Proton™ Sequencer software" on page 61 for details.
- The **Tools** menu gives you access to troubleshooting tools and to the instrument and commands. See the following table for details.

Tools menu options

Item	Description	When to use
Auto pH	Adjusts the pH of the Wash 2 Bottle solution and checks the pH. This task is normally performed by the instrument as part of the Initialize program.	If directed to do so by Technical Support or as part of a troubleshooting procedure (see Appendix A, "Troubleshooting").
Chip Cal	Runs the chip calibration portion of the Run program. Chip calibration is performed by the instrument as part of the Run program, both before and after sample is loaded on a chip.	If you need to calibrate chips without using the Run program.
Data Mgnt	Allows you to manually delete run data or transfer the data the server. Under normal conditions, run data are automatically transferred to the Ion Proton™ Torrent Server, then deleted from the instrument hard drive.	To troubleshoot data management problems. See "Error message: Not enough disk space for the necessary number of flows" on page 44.
Noise Screen	Provides real-time measurement of electrical noise readings on the chip.	For troubleshooting if directed to do so by Technical Support.
Pressure Cal	Allows you to calibrate the gas pressure after installing a new gas tank or to troubleshoot gas pressure error messages.	If seeing gas pressure error messages, see "Error Message: Confirm Instrument Has Gas Pressure" on page 43.
Reagent Check	Measures the pH of all reagents on the instrument. This task is normally performed by the instrument as part of the Initialize program.	If directed to do so by Technical Support or as part of a troubleshooting procedure (see Appendix A, "Troubleshooting").
Screen Cal	Calibrates the touchscreen.	If the touchscreen is not registering pressure in the correct location. See "Touchscreen not registering input in correct location" on page 46.
Shut Down	Access to "Shut Down" and "Reboot" commands. **Note:** It is not necessary/recommended to power off the instrument overnight or over the weekend. If you need to power off the instrument, see "Powering off" on page 60.	If directed to do so as part of a troubleshooting procedure.

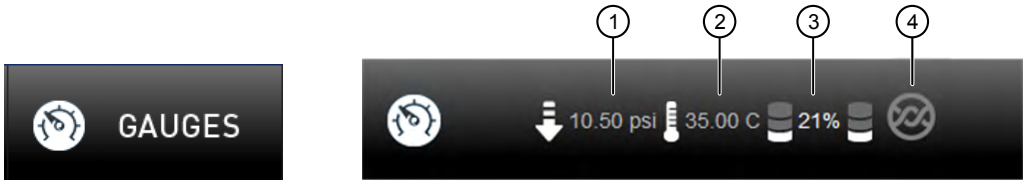
Chip Status icon

The chip status icon in the lower-left corner of the indicates that the chip is communicating with the instrument and alerts you to chip problems. Shortly after you secure a chip in the chip clamp, the chip status updates to "Ready". If the instrument does not detect the chip, see "Chip is secured in chip clamp, but chip icon indicates no chip detected" on page 43.

Ready	Online	Imaging	Sleeping	No chip detected
				

Touchscreen gauges

Press the Gauges icon in the lower right corner of the touchscreen to show or hide the instrument gauges.

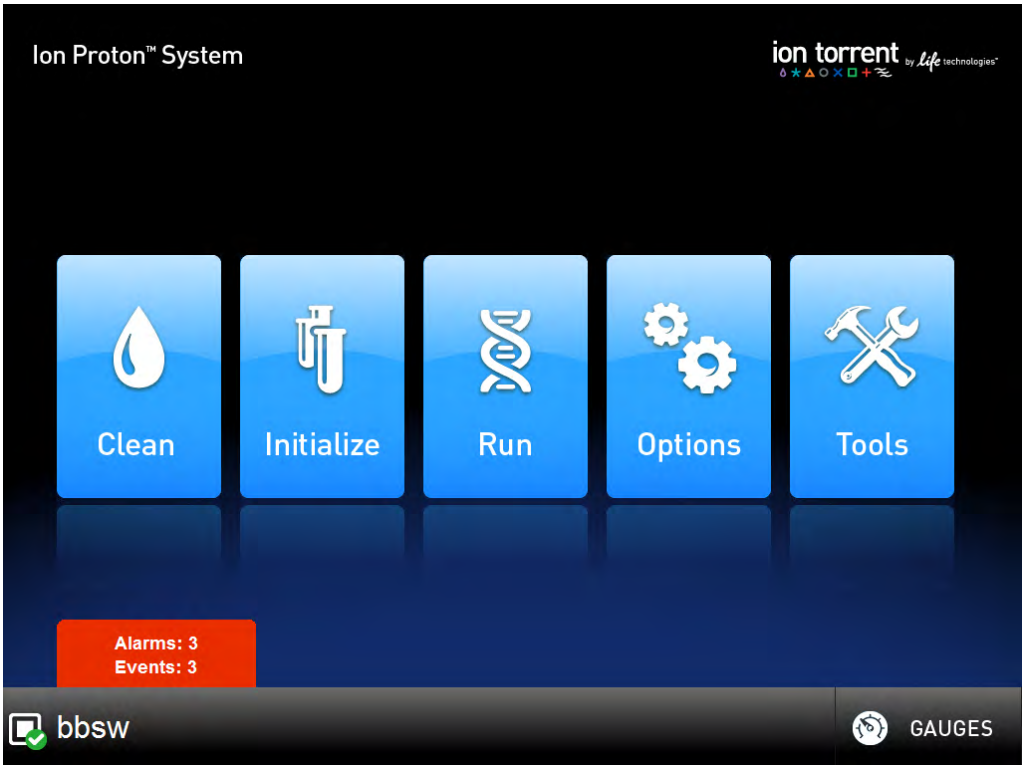


① Instrument gas pressure
② Chip compartment temperature
③ Percent instrument hard drive in use
④ On-instrument analysis

Icon	Description
	Instrument gas pressure. The expected value is 10.50 psi during cleaning and initialization, and 8.0 during a sequencing run. If the icon is red, see "Error Message: Confirm Instrument Has Gas Pressure" on page 43.
	Chip compartment temperature. The expected value when the lid is closed is 35.00 C. If the icon is red, see "Temperature icon indicates chip compartment temperature is out of range" on page 44.
	Percent instrument hard drive and SSD in use. If the icon is red, see "Error message: Not enough disk space for the necessary number of flows" on page 44.
	On-instrument analysis.  indicates on-instrument analysis is in progress.  indicates no current on-instrument analysis. If the icon displays a red "X", see "On instrument analysis icon indicates error" on page 44.

Alarms/Events pop-up

If the red Alarms/Events pop-up appears, press the pop-up to see detailed messages. To troubleshoot alarms and events, see “Red "Alarms" and/or "Events" message in Main Menu“ on page 42.



Supplemental procedures

Power instrument on or off

Powering on

Note: If the instrument is powered on, and the touchscreen is blank, touch the screen to "wake" the touchscreen.

1. Locate the power switch on the back of the instrument, then power on the instrument.
2. Press the power button on the front of the instrument.
   When the instrument touchscreen Main Menu first comes up, it will appear dim. After a few minutes, the main menu will be brightly lit, indicating that the instrument is ready for use.
3. If reagents were left on the machine for more than 48 hours, and the Clean program was not run with 18 MΩ water before powering off, run the Clean program with chlorite before initializing the instrument and performing a sequencing run.

Powering off

It is not necessary to power off the instrument overnight or over the weekend. If the instrument will not be used for more than 3 days, then power off the instrument as follows:

1. If powering off for more than 3 days, run the Clean program with 18 MΩ water before powering off.
2. In the Main Menu, select **Tools ▸ Shut Down ▸ Shut Down**.

   Note: Select **Tools ▸ Shut Down ▸ Reboot** if you want the instrument to shut down and immediately restart.

Update Ion Proton™ Sequencer software

IMPORTANT! After updates are installed, the instrument must be restarted.

If an update to the Ion Proton™ Sequencer software is available, the red "Alarms and Events" pop-up appears in the touchscreen Main Menu to alert you. Click the red pop-up to see the detailed messages. If a message states `New Software Available`, update the software by following these steps:

1. In the Main Menu, select **Options ▸ Updates**.
2. Select the **Released Updates** checkbox, then tap **Check**.
3. When the message `Press Update to begin update process` appears, tap **Update**.

   Note: If the message `All Software Current` appears, tap **Back** twice to return to the main menu.
4. When the message `Installing Completed` displays, follow the onscreen prompts to restart the instrument.

   Note: In some cases, the instrument restarts automatically after software installation.

Transfer a Planned Run to an Ion Torrent™ Server with Ion Mesh

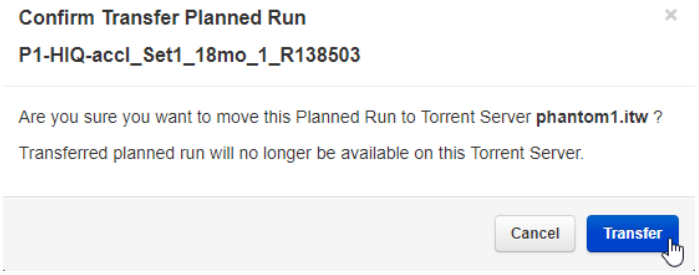
You can transfer Planned Runs that are created on one Ion Torrent™ Server and transfer them to another Ion Torrent™ Server by using Ion Mesh. This is useful if the sequencer connected directly to the origin Ion Torrent™ Server is offline or busy.

Before Planned Run transfer, first connect the Ion Torrent™ Server with Ion Mesh. For more information, see "Set up flexible workflows" in the *Torrent Suite™ Software 5.12 User Guide* (Pub. No. MAN0017972) or online *Torrent Suite™ Software 5.12 Help*.

IMPORTANT! The Ion Torrent™ Server must have the same version of Torrent Suite™ Software installed in order for a Planned Run transfer to be successful.

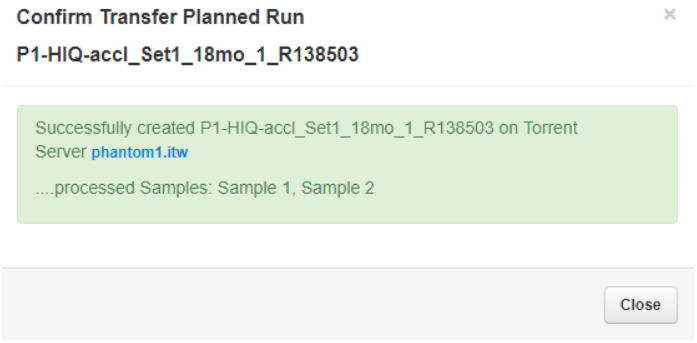
1. Sign in to Torrent Suite™ Software on the origin Ion Torrent™ Server.
2. In the **Plan** tab, click **Planned Runs**.
3. Find the row of the Planned Run that you want to transfer, then click ⚙ **(Actions) ▸ Transfer**.
4. Select the Ion Torrent™ Server that you want to receive the Planned Run.

5. In the confirmation dialog box, confirm the information, then click **Transfer**.
   You can no longer access this Planned Run on the origin server after it has transferred.



A status appears in a message box with the results of the transfer.

- A green box lists the Planned Runs that are successfully transferred.



- A red box lists any failed Planned Run transfers.

Manually adjust the pH of the W2 Solution

Materials and equipment needed

- Orion Star™ A111 pH Benchtop Meter Kit, or equivalent
- Nitrogen gas tank, tube, and flow meter
- 1 M NaOH (prepared fresh daily)
- Pipette tips and pipette
- Magnetic stirrer and stir bar
- 100 mM HCl
- Squirt bottle

If an error message during the automatic pH process indicates that there is a problem adjusting the pH of the W2 Solution, use the following procedure to adjust the pH of the W2 Solution in the Wash 2 Bottle manually.

1. Before proceeding, rinse an empty Wash 2 Bottle and have it ready next to the instrument. Also have an extra Wash 2 Bottle cap ready.

   Note: Gas will be flowing out of the Wash 2 Bottle cap, so perform the next steps as quickly as possible (flowing gas will not harm the W2 Solution, and is not a hazard).

2. Remove the Wash 2 Bottle attached to the instrument, then cap the bottle.

3. Secure the empty Wash 2 Bottle (from step 1) to the instrument. Do not remove the sipper. This bottle contains the gas flowing out of the instrument while you adjust the pH of the W2 Solution and protect the sipper from contamination.

4. Move the Wash 2 Bottle containing the W2 Solution to the stir plate near the nitrogen gas tube.

5. Secure the gas tube so that it extends inside the mouth of the Wash 2 Bottle but not below the surface of the W2 Solution.

6. Set the gas flow to 0.5 lpm. Start mixing the W2 Solution fast enough for a small whirlpool to form.

7. Calibrate the pH meter using a three-point calibration. Rinse any buffering solution from the pH probe before preparing solutions.

8. Adjust the pH of the W2 Solution to 7.7 ± 0.1 by adding a small amount (approximately 1 µL) of freshly prepared 1 M NaOH to the solution, then measuring the pH using the pH meter. Add small aliquots, then allow the pH to equilibrate before adding more.

   Note: If the pH rises above 7.8, use 100 mM hydrochloric acid (HCl) to readjust the pH to 7.7 ± 0.1.

9. When the pH is stable, stop the gas flow, remove the gas line, then cap the Wash 2 Bottle.

10. Move the bottle to the instrument, remove the empty Wash 2 Bottle from the instrument, then place the sipper inside the Wash 2 Bottle containing the pH-adjusted solution.

11. Secure the cap firmly. Tap **Next** to exit the automated pH check, then continue with instrument initialization.

Set up and test the Ion Chip™ Minifuge

The Ion Chip™ Minifuge (Cat. No. 4479672 or 4479673) is used to load sequencing chips manually for use on Ion PGM™, Ion Proton™, and Ion S5™/Ion GeneStudio™ S5 sequencing platforms. To accommodate the larger chip size, Ion Proton™ and Ion S5™/Ion GeneStudio™ S5 sequencer users must:

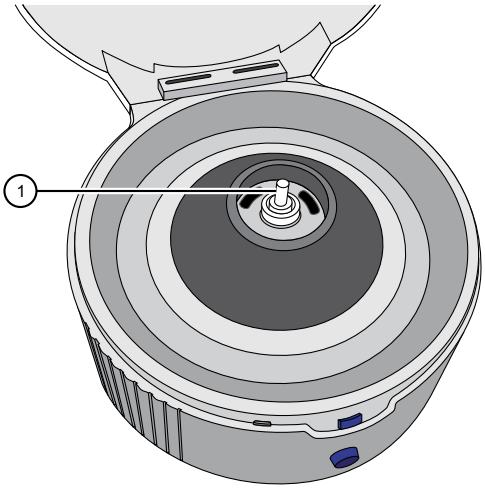
- install the Ion S5™/Ion Proton™ Rotor and Buckets (Cat. No. 4482578).
- test the minifuge to confirm that no liquid is lost during centrifugation.

before using the minifuge to load chips for the first time.

Note: The following protocols may also be used to convert the Ion Chip™ Minifuge back for use with Ion PGM™ sequencing chips.

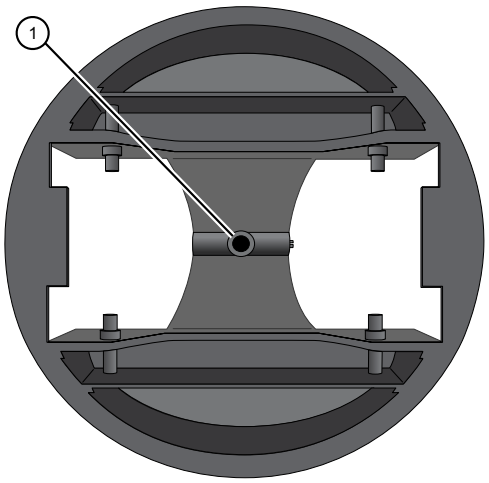
Install the Ion S5™ /Ion Proton™ Rotor and Buckets

1. Grasp the existing rotor and pull straight up to remove the rotor from the motor shaft.



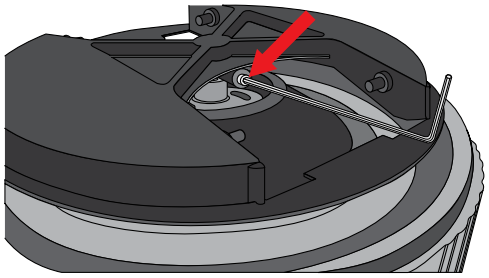
① Motor shaft

2. Press the Ion S5™/Ion Proton™ Rotor down onto the motor shaft to install.



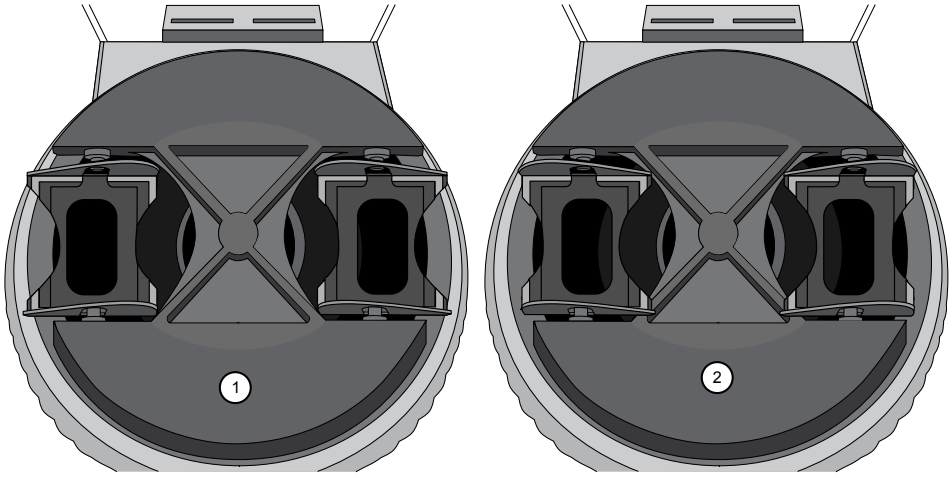
① Insert motor shaft here

3. Tighten the set screw (arrow) with a 1.5-mm hex wrench.



Note: A newer version of the rotor lacks a set screw. In this case, simply press the rotor firmly onto the motor shaft to install.

4. Install the two buckets. Position the buckets with the larger semi-circular cut-outs facing out, and ensure that the buckets hang freely.



① Correct orientation
② Incorrect orientation

Test the minifuge

1. Prepare two previously-used chips:
   a. Inject 100 µL of isopropanol two times into the loading port of each chip. After each injection, remove the expelled liquid from the opposite port.

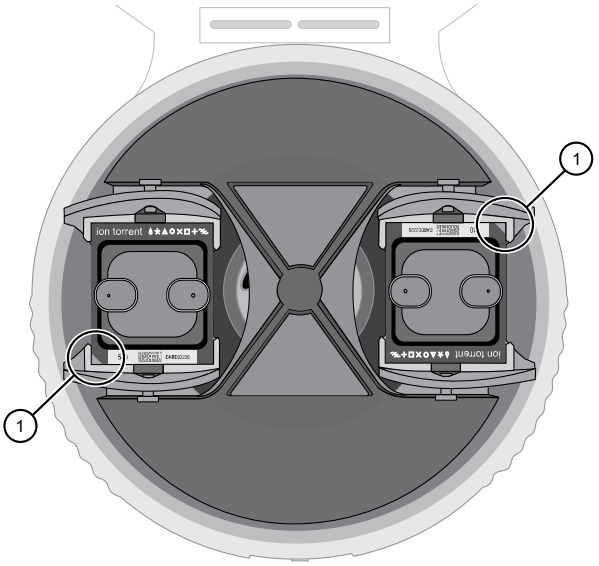
      Note: Use 50 µL volume of isopropanol if testing Ion PGM™ sequencing chips.

   b. Aspirate the remaining isopropanol from the flow cells for 5–10 seconds. Confirm that the chips are dry.

      Note: To aspirate the isopropanol, attach a P200 pipette tip to a vacuum line, then place the pipette tip in the chip loading port.

2. Place the two chips prepared in step 1 in the centrifuge buckets, with the chip notch pointing out. Add 55 µL of nuclease-free water to each chip loading well (do not inject into the chip loading port).

   Note: Use 35 µL volume of nuclease-free water if testing Ion PGM™ sequencing chips.



① Chip notch

3. Centrifuge for 5–10 seconds, then examine each chip.

   The flow cell in each chip should be completely filled with liquid with no air bubbles. A small volume of liquid will remain in the loading well; this is normal.

Result	Action
The chips are NOT completely filled...	Contact Technical Support.
The chips ARE completely filled...	Centrifuge the chips for an additional 10 minutes, then check the chips again for air bubbles, especially near the inlet and outlet ports.
The chips have air bubbles after the additional 10 minute centrifugation...	Contact Technical Support.
The chips remain completely filled...	The centrifuge is ready to use for chip loading.

Safety

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.

Equipment use

The Ion Proton™ System is for performing sequencing of amplified DNA, and should only be used for life science research applications. The Ion Proton™ System should only be used by professionals trained in laboratory techniques and who have studied the instructions for use of this instrument. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

IMPORTANT! If you use and/or install the Ion Proton™ System in an unspecified manner, you may impair the protection provided by the equipment.

Symbols on this instrument

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

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

Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
	Do not dispose of this product in unsorted municipal waste  **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.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.  **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 on this instrument

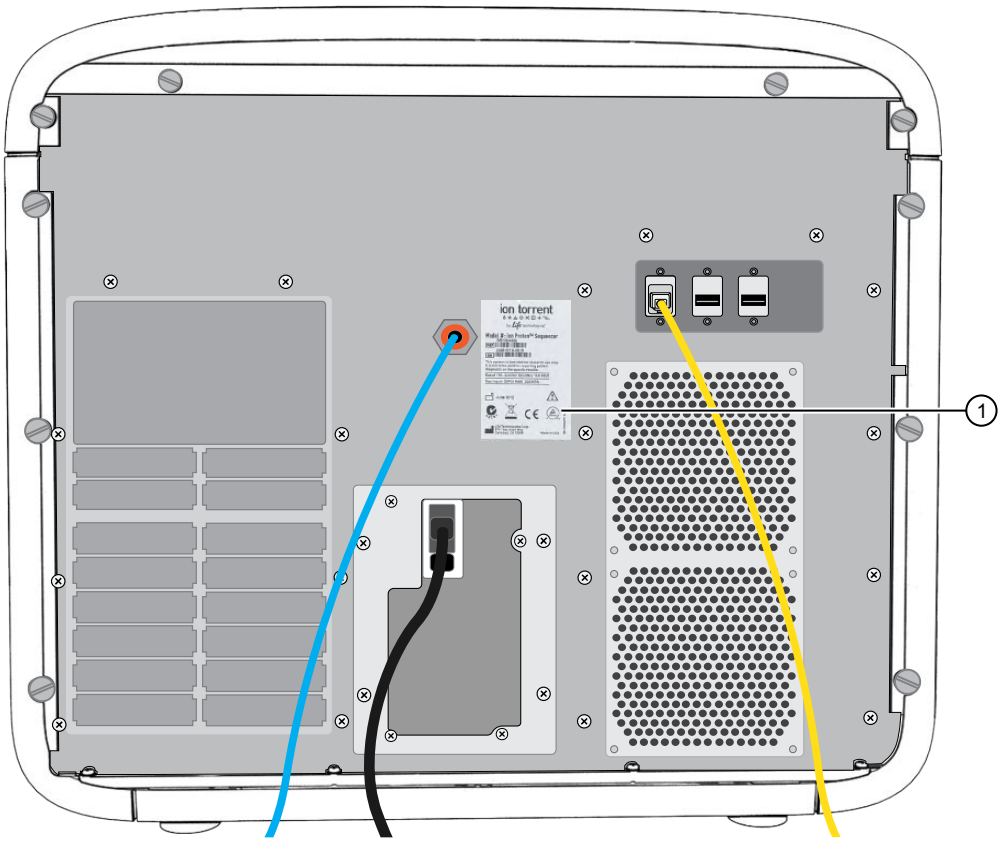
Conformity mark	English	Français
	Indicates conformity with safety requirements for Canada and U.S.A.	Indique la conformité avec les normes de sécurité en vigueur au Canada et aux États-Unis.
CE	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.	Indique la conformité avec les exigences de l'Union européenne en matière de sécurité et de compatibilité électromagnétique.
	Indicates conformity with Australian standards for electromagnetic compatibility.	Indique la conformité avec les normes australiennes régissant la compatibilité électromagnétique.

Safety alerts on this instrument

Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

English	Français
⚠ **CAUTION! Hazardous chemicals.** Read the Safety Data Sheets (SDSs) before handling.	⚠ **MISE EN GARDE ! Produits chimiques dangereux.** Lire les fiches signalétiques (FS) avant de manipuler les produits.
⚠ **CAUTION! Hazardous waste.** Refer to SDS(s) and local regulations for handling and disposal.	⚠ **MISE EN GARDE ! Déchets dangereux.** Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l'élimination des déchets.

Safety symbols used on the instrument



① Safety and regulatory symbols

Safety information for instruments not manufactured by Thermo Fisher Scientific

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

Instrument safety

General

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

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.

Gas safety

Verify that your installation room can accommodate gas cylinders.

WARNING! Instrumentation must 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 instruments will be installed and operated in an environment with sufficient ventilation.

WARNING! Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use, and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

WARNING! Gas cylinders are heavy and may topple over, potentially causing personal injury and tank damage. Cylinders should be firmly secured to a wall or work surface. Please contact your Environmental Health and Safety Coordinator for guidance on the proper installation of a gas cylinder.

Safety and electromagnetic compatibility (EMC) standards

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

Safety

Reference	Description
EU Directive 2006/95/EC	European Union "Low Voltage Directive"
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010 EN 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*

EMC

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

Environmental design

Reference	Description
Directive 2002/96/EC	European Union "WEEE Directive" – Waste electrical and electronic equipment

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.

Biological hazard safety

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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

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

Document	Pub. No.
Ion PI™ Hi-Q™ Sequencing 200 Kit Quick Reference	MAN0010948
Ion PI™ Hi-Q™ Sequencing 200 Kit Product Information Sheet	MAN0010949
Ion PI™ Hi-Q™ OT2 200 Kit User Guide	MAN0010857
Ion PI™ Hi-Q™ OT2 200 Kit Quick Reference	MAN0010858
Ion Proton™ System Site Preparation Guide	4478733
Torrent Suite™ Software 5.12 User Guide	MAN0017972
Ion Sphere™ Quality Control Kit User Guide	MAN0017531
Ion Sphere™ Particles Quality Assessment for the Ion Proton™ and Ion S5™ Systems Using the Guava™ easyCyte 5 Benchtop Flow Cytometer User Bulletin	MAN0007496

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**.