

Ion PGM™ Hi-Q™ View Sequencing Kit

USER GUIDE

for use with:

Ion PGM™ Hi-Q™ View Sequencing Kit

Ion PGM™ System

Ion 318™ Chip v2 BC

Ion 316™ Chip v2 BC

Ion 314™ Chip v2 BC

Catalog Number A30044

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Revision C.0

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

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Revision	Date	Description of change
C.0	6 January 2017	<ul style="list-style-type: none">Information in appendices C–G moved to the <i>Ion PGM™ System Reference Guide</i> (Pub. No. MAN0009783)Updated web links and references
B.0	31 August 2016	<ul style="list-style-type: none">Correction of minor errors in component names and amount provided.Recommendation for the time that can elapse between initialization and the start of the final run changed from 27 hours to 24 hours
A.0	8 April 2016	New User Guide, which includes instructions to use the new Ion PGM™ Hi-Q™ View Sequencing Kit with the Ion PGM™ System

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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 PGM™ Hi-Q™ View Sequencing Kit (Cat. No. A30044) includes reagents and materials for sequencing up to ~400-bp inserts using the following chips on the Ion PGM™ System:

- Ion 318™ Chip v2 BC
- Ion 316™ Chip v2 BC
- Ion 314™ Chip v2 BC

The Ion PGM™ Hi-Q™ View Sequencing Kit is designed for use with the Ion PGM™ Hi-Q™ View OT2 Kit, which introduces colored Ion PGM™ Hi-Q™ View ISPs for easier sample visualization. In addition, the Ion PGM™ Hi-Q™ View Sequencing Solutions show improved performance in terms of reduced strand bias in amplicon sequencing.

Note: The kit and chips may also be used for Research Use Only experiments on the Ion PGM™ Dx System running Torrent Suite™ Assay Development Software^[1].

The kit includes components for 4 initializations. The number of sequencing runs per kit depends on the length of the base reads, as shown in the following table.

Sequencing type	Number of flows	Number of runs per kit	Maximum number of runs per initialization ^[1]
500-base-read ^[2]	1100	4	1
400-base read	850	4	1
200-base read	500	8	2
≤100-base read	260	12	3

^[1] For best results, begin the run within 1 hour after initialization.

^[2] For ISPs prepared using the Ion PGM™ Template IA 500 Kit

^[1] The Ion PGM™ Dx System with Torrent Suite™ Assay Development Software is For Research Use Only. Not for use in diagnostic procedures.

Intended use

The Ion PGM™ Sequencer performs real-time measurements of hydrogen ions produced during DNA replication.

Library kit compatibility

This sequencing kit can be used with all library types prepared using any Ion library kit.

Template kit compatibility

The Ion PGM™ Hi-Q™ View Sequencing Kit is compatible with the following template preparation kit:

- Ion PGM™ Hi-Q™ View OT2 Kit (Cat. No. A29900)

Select this template kit when creating a Planned Run.

Software and instrument system compatibility

The procedures in this guide are compatible with the following software and instrument systems:

- Torrent Suite™ Software version 5.0.4 or later on the Ion PGM™ System. To update the software, see the *Ion PGM™ System Reference Guide*. (Pub. No. MAN0009783), available at thermofisher.com.
- Torrent Suite™ Assay Development Software version 5.0.6 or later for Research Use Only experiments on the Ion PGM™ Dx System^[2]. To update the software, see the *Torrent Suite™ Assay Development Software Help*. To use this kit on the Ion PGM™ Dx System with Torrent Suite™ Assay Development Software, see the following user guides:
 - *Ion PGM™ Hi-Q™ View OT2 Kit—Assay Development Mode User Guide* (Pub. No. MAN0015753) and
 - *Ion PGM™ Hi-Q™ View Sequencing Kit—Assay Development Mode User Guide* (Pub. No. MAN0015792)

^[2] The Ion PGM™ Dx System with Torrent Suite™ Assay Development Software is For Research Use Only. Not for use in diagnostic procedures.

Kit contents and storage

The Ion PGM™ Hi-Q™ View Sequencing Kit (Cat. No. A30044) includes the following components:

Component	Amount	Storage
Ion PGM™ Sequencing Supplies (Part No. A25587)		
Wash 1 Bottle w/ label (250 mL)	1 bottle	15°C to 30°C
Wash 3 Bottle w/ label (250 mL)	1 bottle	
Ion PGM™ Reagent Bottle Sipper Tubes (blue)	16 sipper tubes	
Ion PGM™ Wash Bottle Sipper Tubes (gray, for 250-mL bottles)	8 sipper tubes	
Ion PGM™ Wash Bottle Sipper Tubes (gray, for 2-L bottles)	4 sipper tubes	
Reagent Bottles w/ labels (50 mL)	25 bottles	
Ion PGM™ Hi-Q™ View Sequencing Reagents (Part No. A30043)		
Ion PGM™ Hi-Q™ View Sequencing Polymerase (yellow cap)	36 µL	-30°C to -10°C
Ion PGM™ Hi-Q™ View Sequencing Primer (white cap)	144 µL	
Ion PGM™ Hi-Q™ View Control Ion Sphere™ Particles (clear cap)	60 µL	
Ion PGM™ Hi-Q™ View Sequencing Solutions (Part No. A30275)		
Ion PGM™ Hi-Q™ View Sequencing W2 Solution	4 × 125 mL	15°C to 30°C ^[1]
Ion Cleaning Tablet	4 tablets	
Annealing Buffer	12 mL	
Ion PGM™ Hi-Q™ View Sequencing W3 Solution	2 × 100 mL	
Ion PGM™ Hi-Q™ Sequencing dNTPs (Part No. A25590)		
Ion PGM™ Hi-Q™ Sequencing dGTP (black cap)	80 µL	-30°C to -10°C
Ion PGM™ Hi-Q™ Sequencing dCTP (blue cap)	80 µL	
Ion PGM™ Hi-Q™ Sequencing dATP (green cap)	80 µL	
Ion PGM™ Hi-Q™ Sequencing dTTP (red cap)	80 µL	

^[1] Store Ion PGM™ Hi-Q™ View Sequencing W2 Solution protected from light.

Ion PGM™ Wash 2 Bottle Kit

The Ion PGM™ Wash 2 Bottle Kit (Cat. No. A25591) is sold separately, and includes the following components:

Component	Amount	Storage
Wash 2 Bottle w/ label (2 L) Note: Must be conditioned at least 8 hours before use as described in “Condition the Wash 2 Bottle for first use” on page 21	1 bottle	15°C to 30°C
Wash 2 Bottle Conditioning Solution	1 × 125 mL	

Ion Chip kits

The following Ion Chip kits are compatible with this sequencing kit, and are sold separately:

Component	Amount	Catalog No.	Storage
Ion 318™ Chip v2 BC	4 pack	4488146	15°C to 30°C
	8 pack	4488150	
Ion 316™ Chip v2 BC	4 pack	4488145	
	8 pack	4488149	
Ion 314™ Chip v2 BC	8 pack	4488144	

Instruments and server

Components	Cat. No.
Ion PGM™ System and accessories	4462921
Ion PGM™ Torrent Server	4483643
Ion Chip™ Minifuge:	
120 VAC	4479672
230 VAC	4479673

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 may adversely affect system performance.

✓	Item	Source
	Tank of compressed nitrogen (grade 4.5, 99.995% or better) (see "Gas cylinders" on page 15)	MLS
	Multistage (dual-stage) gas regulator (0-50 PSI, 2-3 Bar output)	Fisher Scientific NC0393866 or MLS
	Choose from one of the following systems: ELGA™ PURELAB™ Flex 3 Water Purification System Equivalent 18-MΩ water purification system	4474524 MLS
	Microcentrifuge (capable of >15,500 × g, fits 1.5-mL and 0.2-mL microcentrifuge tubes)	MLS
	0.22-μm or 0.45-μm vacuum filtration system and filters (nylon or PVDF filters, 1-L volume)	MLS
	Rainin™ Pipet-Lite™ LTS Pipette L-100XLS 10–100 μL ^[1] (Alternatives from Gilson and Eppendorf may be used)	Rainin 17014384
	Rainin™ Pipet-Lite™ LTS Pipette L-20XLS 2–20 μL ^[1] (Alternatives from Gilson and Eppendorf may be used)	Rainin 17014392
	Rainin™ LTS pipette tips, 200 μL, SR-L200F (Alternatives from Gilson and Eppendorf may be used)	Rainin 17005859
	Rainin™ LTS pipette tips, 20 μL, SR-L10F ^[1] (Alternatives from Gilson and Eppendorf may be used)	Rainin 17005860
	PCR tubes, Flat Cap, 0.2-mL (do not use polystyrene tubes)	Fisher Scientific 14-222-262
	Vortexer with a rubber platform	MLS
	Thermal cycler with a heated lid	MLS
	Graduated cylinders (1 L or 2 L volume)	MLS
	Glass bottle (1 L)	MLS
	15-mL conical tubes	MLS
	NaOH (10 M), molecular biology grade	MLS
	Pipette set and filtered tips, P2, P20, P200, and P1000 μL	MLS
	Microcentrifuge tubes, 1.5-mL or 1.7-mL	MLS

✓	Item	Source
	Syringe, 10 CC, Female Luer-Lok™ (used for clearing lines)	Provided with the Ion PGM™ Sequencer, or MLS
	Ion PGM™ 2.5 L Waste Bottle ^[2]	4482565
Optional materials		
	Ion PGM™ Controls Kit v3 ^[3]	A30046
	Ion PGM™ Sequencing Sippers Kit ^[4]	4478682
	Non-interruptible Power Supply (UPS) ^[5]	MLS

^[1] Ensure tips from any vendors are low binding tips. Required for loading samples onto the Ion PGM™ Chips.

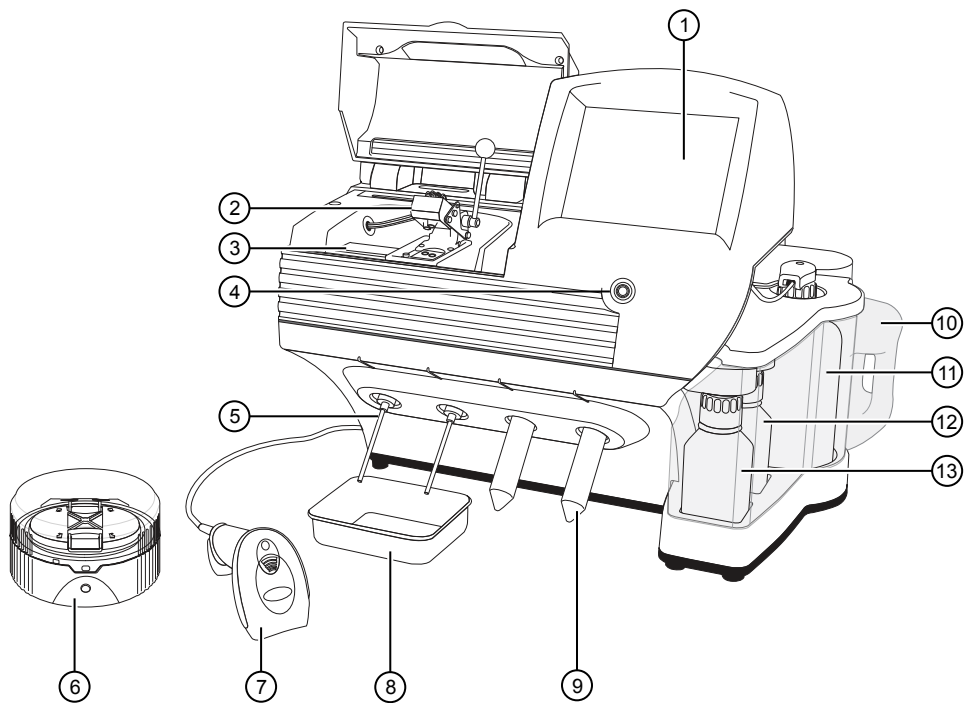
^[2] Required one-time purchase for older ("Orange") versions of the Ion PGM™ System.

^[3] Not commonly needed, but available for troubleshooting.

^[4] Contains additional sipper tubes; not commonly needed.

^[5] We recommend using a non-interruptible power supply (UPS) for laboratories that experience frequent power outages or line voltage fluctuations. The UPS must be compatible with 1500 W output or higher. The 1500 VA unit from APC provides ~11 minutes of backup power for an Ion PGM™ System.

Ion PGM™ System layout



- | | |
|------------------------------|-------------------------------|
| ① Touchscreen | ⑧ Collection tray |
| ② Chip clamp | ⑨ Reagent Bottle |
| ③ Grounding plate | ⑩ Waste Bottle |
| ④ Power button | ⑪ Wash 2 Bottle (W2 position) |
| ⑤ Reagent Bottle Sipper Tube | ⑫ Wash 3 Bottle (W3 position) |
| ⑥ Ion Chip™ Minifuge | ⑬ Wash 1 Bottle (W1 position) |
| ⑦ Barcode Scanner | |



Before you begin

Before you begin

For additional safety information, see Appendix D, “Safety”.

Update the software

IMPORTANT! Before proceeding, make sure that you have updated the Torrent Suite™ and Ion PGM™ System software to version 5.0.4 or later. See the *Ion PGM™ System Reference Guide* (Pub. No. MAN0009783) for more information.

Instrument installation by trained personnel only

IMPORTANT! The Ion PGM™ System is installed by trained service personnel and must not be relocated without assistance from trained service personnel. See “Customer and technical support” on page 64.

Nucleic acid contamination

IMPORTANT! A primary source of contamination is DNA fragments from previously processed samples. Do not introduce amplified DNA into the library preparation laboratory or work area.

IMPORTANT! Possible contamination can occur during the transfer of dNTPs into Reagent Tubes. Be careful to avoid cross contamination of dNTP stocks. Barrier tips are required for all pipetting steps. Change gloves after handling concentrated dNTP stocks.

CO₂ contamination

IMPORTANT! Dry ice (solid CO₂) must be kept away from areas where buffers, wash solutions, or sources of molecular biology grade water for the Ion PGM™ System are used. High air concentrations of subliming CO₂ may change 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 PGM™ System.

Instrument vibration and clearances

IMPORTANT! Significant vibration during sequencing may add noise and reduce the quality of the measurements. The Ion PGM™ System must be installed on a bench that is free from vibrations or in contact with equipment that can cause vibrations to the bench (freezers, pumps, and other similar equipment).

IMPORTANT! Position the Ion PGM™ System so that the front bezel is a minimum of 12 in. (30.5 cm) and the Reagent Tubes containing dNTPs are a minimum of 8 in. (20.3 cm) from the front of the laboratory bench. Place the instrument at least 40 in. (1 meter) away from major sources of electronic noise such as refrigerators or microwaves.

Static electricity

IMPORTANT! To avoid possible damage to the chip from static electricity, prior to handling chips, you must ground yourself on the grounding plate (located next to the chip clamp) by touching the grounding plate with your bare hand.

Do not place chips on non-grounded surfaces such as a bench.

Always use the grounding plate to hold chips that are not in the package inserted in the chip clamp or the Ion Chip™ Minifuge bucket.

Ventilation requirements



WARNING! Instrumentation must be installed and operated in a well-ventilated environment, 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.

Guidelines for sequencing runs

- When performing two sequencing runs from the same initialization, the second run must be started within 24 hours after start of initialization.
 - If you press the **Abort** button on the sequencer touchscreen, the touchscreen may freeze. You may need to restart the sequencer.
-

IMPORTANT! After aborting a run, do not open the chip clamp, reagent tubes, or wash bottles until a new run or cleaning is initiated. Doing so can cause a fluid or gas leak if the sequencer was in a pressurized state when the run was aborted. From the main menu, select either **Clean** or **Run**, then follow the touchscreen prompts to depressurize the system.

Guidelines to prevent cross-contamination

- Use good laboratory practices to minimize cross-contamination of products and reagents.
- When designing the laboratory layout, consider separating pre- and post-amplification activities. To significantly reduce the potential for contamination, dedicate laboratory supplies and/or equipment to the appropriate space.

Guidelines for pipetting

- Vortex all reagents *except* enzymes for ~5 seconds. Mix enzymes by flicking the tube with your finger 4 times. Briefly centrifuge to collect the contents before use.
- Ensure that all reagents are completely thawed at room temperature, such that no ice crystals are visible in the tube.
- Pipet viscous solutions slowly and ensure complete mixing.
- Change tips between pipetting steps.

Guidelines for chip handling and use

- Always remove gloves before transferring chips onto or off the instrument. Hold chips by their edges when handling.
- To avoid damage due to electrostatic discharge (ESD), **do not place chips directly on the bench or any other surface**. Always place chips either on the grounding plate on the sequencer or in the Ion Chip™ Minifuge bucket.
- Used chips cannot be reused for sequencing. Used chips must be discarded or clearly marked for cleaning and initialization.

Gas cylinders

You must supply the required nitrogen gas cylinder and accessories for the installation. This instrument requires a pressurized house line or one size 1-A nitrogen gas cylinder that holds approximately 7.2 m³ (257 ft³) of gas when full. Use only prepurified nitrogen of 99.995% (grade 4.5) or greater purity.



CAUTION! Damage to the instrument and its products can result from using impure gas, gases other than nitrogen, or an inadequate amount of gas.



WARNING! EXPLOSION HAZARD. 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.

Perform a leak test

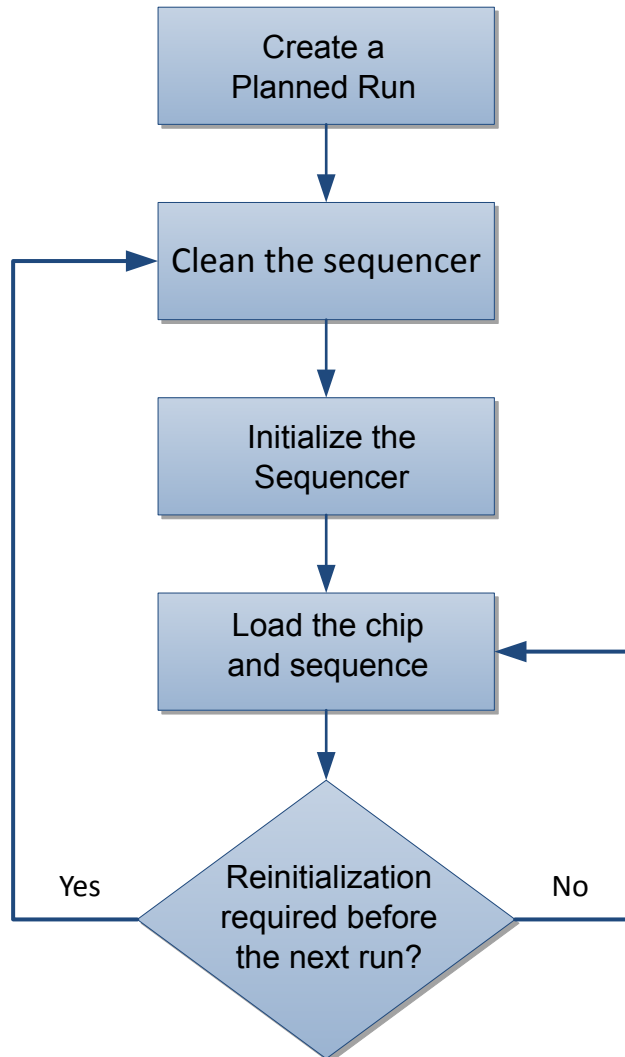
To perform a leak test on the gas cylinder:

1. Open the main tank shutoff valve. The high-pressure gauge of the gas tank regulator reads approximately 2,000–2,500 psi for a full tank.
2. Adjust the pressure to the instrument by slowly turning the pressure adjustment valve clockwise until the low-pressure gauge reads 30 psi.
3. Close the needle valve, then close the main tank valve.
4. Monitor the high-pressure gauge of the gas tank regulator for 5 minutes. There should be no noticeable drop in pressure.

If the pressure	Action
Drops in 5 minutes	There can be a leak at either the needle valve or the gas tank regulator itself. Check the fittings and resolve any problems, then continue with step 5.
Does not drop in 5 minutes	The instrument passes the leak test. Reopen the main tank valve and skip the following steps.

5. Open the main tank valve and the needle valve for at least 15 seconds to pressurize the instrument.
6. Close the main tank valve.
7. Monitor the high-pressure gas tank regulator gauge. There should be no more than a 100-psi drop in pressure after 5 minutes. Locate, then resolve any leaks. Turn the main tank valve back on.

Protocol workflow



3

Create a Planned Run

Planned Runs can be created with the following software programs for use on the following systems:

Software	Instrument System
Torrent Suite™ Software, v5.0.4 or later	Ion PGM™ System
Torrent Suite™ Assay Development Software, v5.0.6 or later	Ion PGM™ Dx System

IMPORTANT! If you are using the Ion PGM™ Dx System with Torrent Suite™ Assay Development Software^[3], see the following user guides:

- *Ion PGM™ Hi-Q™ View OT2 Kit—Assay Development Mode User Guide* (Pub. No. MAN0015753) and
- *Ion PGM™ Hi-Q™ View Sequencing Kit—Assay Development Mode User Guide* (Pub. No. MAN0015792)

Planned Run set-up and the Ion PGM™ Dx System workflow differ from those described in this user guide.

About Planned Runs

Planned Runs contain all the settings 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 or Torrent Suite™ Assay Development Software on the 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 Torrent Server and then transfer it to another server for sequencing. See “Enable Planned Run sharing” on page 55 for more information.

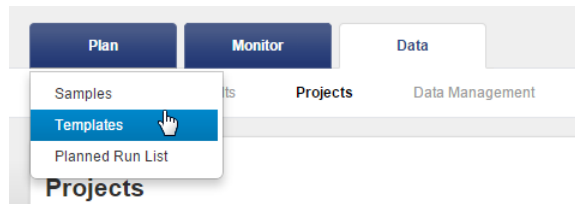
^[3] The Ion PGM™ Dx System with Torrent Suite™ Assay Development Software is For Research Use Only. Not for use in diagnostic procedures.


Create a Planned Run

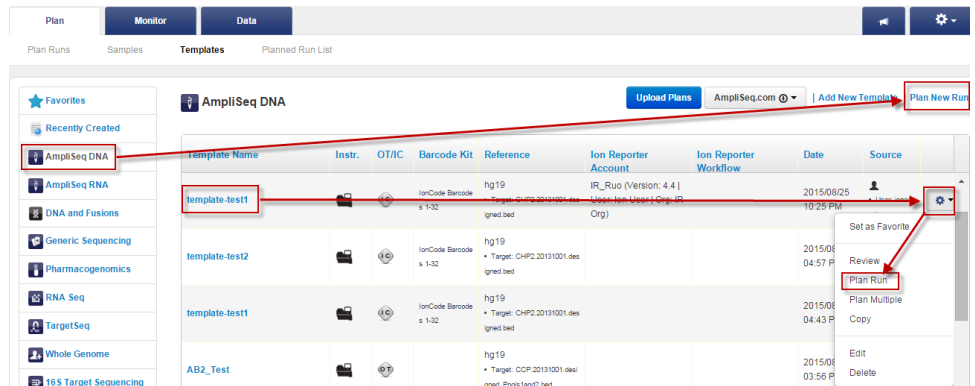
The following provides a summary of steps for creating a Planned Run in Torrent Suite™ Software, for use on the Ion PGM™ System.

For more detailed instructions, see the *Torrent Suite™ Software Help Guide*, available at thermofisher.com/ion-pgm-docs.

1. Open the Torrent Browser on a computer connected to your sequencing system.
2. Select the **Plan** tab, then select **Templates**.

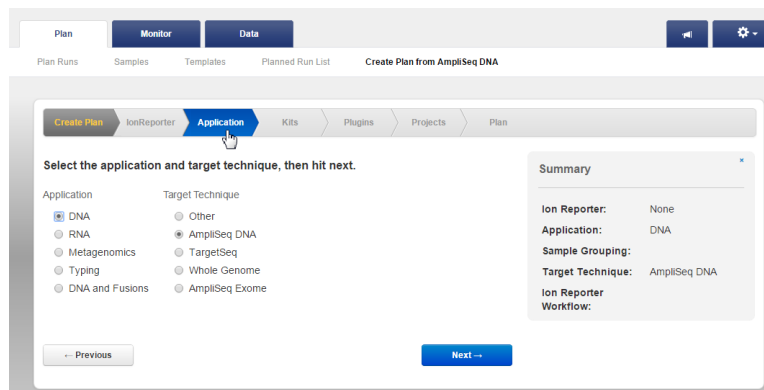


3. Select the application in the left navigation bar (for example, **AmpliSeq DNA**). A list of existing Planned Run templates for that application will be displayed. Select one of the following options to create a new plan:
 - To create a new Planned Run without using an existing template, click on **Plan New Run**.
 - To create a new Planned Run from an existing template, click the  button for the template and select **Plan Run** from the drop-down menu.
 - Other options may be available depending on the selected application, such as downloading templates from AmpliSeq.com.



4. In the wizard, make your selections on each screen, then click **Next** to proceed to the next screen.

Note: For a complete description of each option, see the *Torrent Suite™ Software Help Guide*.



5. When you have completed your selections, click **Plan Run**. The run will be listed on the Planned Runs screen under the name you specified, and is available on the sequencer when you are setting up the run.



Clean and initialize

Condition the Wash 2 Bottle for first use

New Wash 2 Bottles must be conditioned with Wash 2 Bottle Conditioning Solution for at least 8 hours before first use.

Note: If necessary, you can reuse an existing Wash 2 Bottle while you condition a new bottle. Bottles can be used for sequencing up to 40 times before they must be replaced.

To condition the Wash 2 Bottle:

1. Fill the bottle to the mold line with 18 M Ω water, add the entire container of Wash 2 Bottle Conditioning Solution, then cap the bottle and invert it 5 times to mix.
2. Allow the bottle to sit at room temperature for at least 8 hours and preferably overnight, then dispose of the contents. The bottle is now ready for use.

Clean the Ion PGM™ System

Materials required

- 18 M Ω water (e.g., the ELGA™ PURELAB™ Flex Water Purification System)
- Cleaning bottles and collection trays (provided with the Ion PGM™ System)
- Old chip that has been used for sequencing, marked for cleaning
- Used sipper tubes (from the previous run)
- Squirt bottle
- **Chlorite cleaning:** Ion Cleaning Tablet (provided in the kit)
- **Chlorite cleaning:** 1 M NaOH, diluted fresh each week from 10 M NaOH
- **Chlorite cleaning:** Glass bottle (1 L)
- **Chlorite cleaning:** 0.22- μ m or 0.45- μ m vacuum filtration system and filters

Cleaning schedule The Ion PGM™ Sequencer requires cleaning with either 18-MΩ water or a chlorite solution every time the instrument is initialized.

Clean with	Schedule
18 MΩ water	<ul style="list-style-type: none"> • Daily, when instrument is in use (e.g., not necessary on weekends) • After one or more runs totaling $\leq 1,100$ flows • If more than 27 hours but less than 48 hours have elapsed between the last cleaning/initialization and the start of a run • If you cleaned with chlorite a week ago and have not used the instrument since then
Chlorite solution	<ul style="list-style-type: none"> • 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 the instrument has been left with reagents for more than 48 hours (for example, over the weekend)

Cleaning setup

IMPORTANT! For all 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.

- Remove any wash and reagent bottles that are attached to the Ion PGM™ System before cleaning.
- Do not remove old sippers before cleaning. The sippers are used as part of the cleaning procedure.
- Old chips that have been used for sequencing can be marked and used in the cleaning procedure.
- Wash bottles (250 mL and 2 L) provided as part of instrument installation can be marked and used for cleaning. After you have used the wash bottles provided with the sequencing kit for the specified number of runs, you can use them as extra cleaning bottles. Mark them for cleaning use only.

18 MΩ water cleaning

1. Empty any remaining solution from each cleaning bottle (two 250-mL bottles and one 2-L bottle) and rinse each bottle twice with ~100 mL of 18 MΩ water.
2. Press **Clean** on the touchscreen, and select the **18-MO Ω water cleaning** checkbox. Press **Next**.
3. Using ungloved hands, secure a used chip designated for cleaning in the chip clamp.

IMPORTANT! Always make sure that both red rubber gasket port fittings are securely in place when securing chips with the chip clamp. Failure to do so can result in a spill hazard and instrument damage.

4. Remove all wash and reagent bottles attached to the instrument. Keep the sippers in place at all positions. Press **Next**.
5. Add 250 mL of 18 MΩ water to an empty 250-mL cleaning bottle.

6. Rinse the outside of the sipper tube in the W1 position on the instrument with a squirt bottle containing 18 MΩ water.
7. Attach the 250-mL bottle containing 18 MΩ water to the W1 position, ensuring that the W1 cap is screwed on tightly. Press **Next**.
8. Place the empty 2-L cleaning bottle in the W2 position and the empty 250-mL bottle in the W3 position, and insert the sippers into the bottles. Do not screw on the caps.
9. Place collection trays below the reagent sippers in the dNTP positions. Press **Next** to begin cleaning.
10. When cleaning is complete, remove the bottles and sippers from the W1, W2 and W3 positions. Leave the reagent sippers and collection trays in place. Press **Next** to return to the main menu and proceed to initialization.

Chlorite cleaning

Note: Prepare a stock of 1 M NaOH each week by diluting 10 M NaOH with 18 MΩ water.

1. Empty any remaining solution from each cleaning bottle (two 250-mL bottles and one 2-L bottle), then rinse each bottle twice with ~100 mL of 18 MΩ water.
2. 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).
3. 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.
4. Press **Clean** on the touchscreen, then select the **Chlorite cleaning** checkbox. Press **Next**.
5. Using ungloved hands, secure a used chip designated for cleaning in the chip clamp.

IMPORTANT! Always ensure that both red rubber gasket port fittings are securely in place when securing chips with the chip clamp. Failure to do so can result in a spill hazard and instrument damage.

6. Remove all wash and reagent bottles that are attached to the instrument. Keep the sippers in place at all positions. Press **Next**.
7. Add 250 mL of the filtered chlorite solution to an empty 250-mL cleaning bottle.
8. Rinse the outside of the sipper tube in the W1 position on the instrument with a squirt bottle containing 18 MΩ water.
9. Attach the 250-mL bottle with the filtered chlorite solution to the W1 position. Ensure that the W1 cap is tight. Press **Next**.
10. Place the empty 2-L cleaning bottle in the W2 position and the empty 250-mL bottle in the W3 position, then insert the sippers into the bottles. Do not screw on the caps.

11. Place collection trays below the reagent sippers in the dNTP positions. Press **Next** to start cleaning.
12. When prompted, remove the bottle containing the chlorite solution from the W1 position.
13. Rinse the outside of the W1 sipper tube with a squirt bottle containing 18 MΩ water.
14. Fill a clean 250-mL bottle with 250 mL of 18 MΩ water, then attach the bottle in the W1 position. Ensure the cap is tight. Press **Next** to start the water rinse.
15. When cleaning is complete, remove the bottles and sippers from the W1, W2 and W3 positions. Leave the reagent sippers and collection trays in place. Press **Next** to return to the main menu, then proceed to initialization.

Initialize the Ion PGM™ System

Initialization takes ~1 hour. As part of the initialization process, first prepare the Wash and Reagent Bottles as described in this section.

Materials required

Materials provided in the kit

- Ion PGM™ Hi-Q™ Sequencing dGTP
- Ion PGM™ Hi-Q™ Sequencing dCTP
- Ion PGM™ Hi-Q™ Sequencing dATP
- Ion PGM™ Hi-Q™ Sequencing dTTP
- Ion PGM™ Hi-Q™ View Sequencing W2 Solution (stored protected from light)
- Ion PGM™ Hi-Q™ View Sequencing W3 Solution
- Wash 1 and Wash 3 Bottles and sipper tubes
- Wash 2 Bottle and sipper tubes (bottle must be conditioned prior to first use, as described in “Condition the Wash 2 Bottle for first use” on page 21)
- Wash 2 Bottle Conditioning Solution
- Reagent Bottles and sipper tubes

Other materials and equipment

- Used chip (leave chip on the instrument during initialization)
- 18 MΩ water
- 100 mM NaOH (prepared daily)
- Ice
- 5-mL and 25-mL pipettes
- Filtered and unfiltered pipette tips and pipettes
- Vortex mixer
- Microcentrifuge
- **Optional:** Ion PGM™ Sequencing Sippers Kit (Cat. No. 4478682)

Initialization guidelines

IMPORTANT! Handle nucleotides carefully to avoid cross-contamination. Always change gloves after removing used sipper tubes from the Ion PGM™ System to avoid cross contamination of the nucleotides. Also change gloves after handling concentrated dNTP stocks.

For each initialization, the first run should be started within 1 hour after initialization, and the last run must be started within 24 hours after initialization.

Bottle usage

- Wash 2 Bottles can be used for up to 40 initializations, after which you can use them in the cleaning procedure.
- Wash 1 and Wash 3 Bottles can be used for up to 4 initializations, after which you can reuse them in the cleaning procedure.
- Replace the Reagent Bottles and sipper tubes every time you initialize.

Before initialization

1. Remove the dNTP stock solutions from the freezer and begin thawing on ice.
2. Check the tank pressure for the nitrogen gas. When the tank pressure drops below 500 psi, change the tank.

Prepare the Wash 2 Bottle

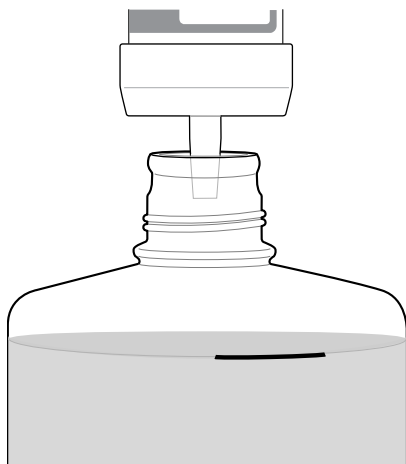
Note:

- Do not remove the old sippers from the dNTP ports until instructed to do so.
- Load the bottles as quickly as possible to prevent atmospheric CO₂ from reducing the pH of the Wash 2 solution.
- For all the following steps, pour the 18 MΩ water directly from the purification system into the Wash 2 Bottle. Do not use water that has been collected or measured in any other containers.

IMPORTANT! Do not let the new sippers touch any surfaces.

1. Rinse the Wash 2 Bottle (2 L) 3 times with 200 mL of 18 MΩ water.
2. Prepare 500 μL of 100 mM NaOH by diluting 50 μL of 1 M NaOH in 450 μL of nuclease-free water.

3. If your 18 MΩ water system has a spigot, extend it into **but not below** the neck of the Wash 2 Bottle. Otherwise, position the nozzle as close to the mouth of the bottle as possible.



Note: If your water system has a digital display, ensure it reads "18 MΩ" throughout filling the bottle. If not, see Appendix A, "Troubleshooting".

4. Fill the bottle to the mold line with 18 MΩ water. The volume of water is ~2 liters. (You can mark the mold line on the bottle for clarity.)

Note: If you are preparing bottles for multiple sequencers, cap each bottle immediately after filling, and leave capped until you are ready to add Ion PGM™ Hi-Q™ View Sequencing W2 Solution.

5. Add the entire bottle of Ion PGM™ Hi-Q™ View Sequencing W2 Solution to the Wash 2 Bottle.



Note: Keep the Ion PGM™ Hi-Q™ View Sequencing W2 Solution bottle to scan the barcode during the initialization procedure.

- Using a P200 pipette, add 70 μL of 100 mM NaOH to the Wash 2 Bottle.

Note: Different sites can require adding different volumes of 100 mM NaOH. Some sites, for example, can require doubling the volume to 140 μL . See “Error message: Added too much W1 to W2” on page 52 for information on determining the volume of 100 mM NaOH to add.

- Cap the bottle and invert 5 times to mix, and immediately proceed through the remainder of the initialization procedure.

IMPORTANT! Do not store the mixed Wash 2 Bottle.

Prepare the Wash 1 and Wash 3 Bottles

Note: For the following steps, label the Wash 1 and Wash 3 Bottles to avoid confusion.

- Rinse the Wash 1 and Wash 3 Bottles 3 times with 50 mL of 18 M Ω water.
- Wash 1 Bottle:** Add 350 μL of freshly prepared 100 mM NaOH to the Wash 1 Bottle, then cap the bottle.
- Wash 3 Bottle:** Add Ion PGM™ Hi-Q™ View Sequencing W3 Solution to the 50-mL line marked on the Wash 3 Bottle, then cap the bottle.

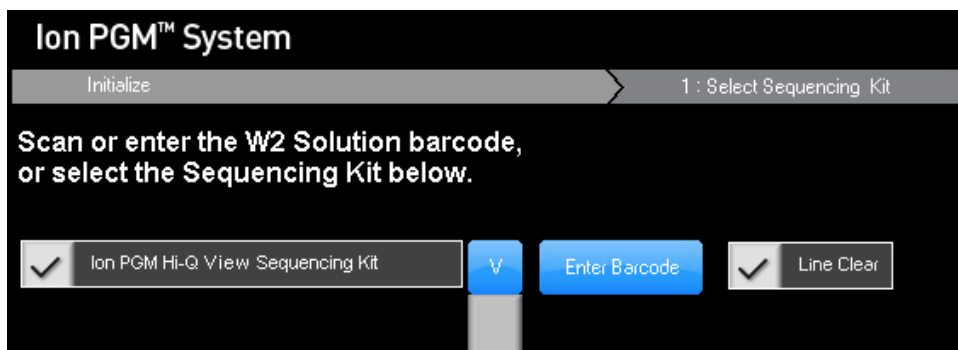
Begin the initialization

Note:

- Do not remove the old sipper tubes from the dNTP ports until instructed to do so.
- Load the bottles as quickly as possible to prevent atmospheric CO₂ from reducing the pH of the Wash 2 Bottle solution.

IMPORTANT! Do not let the new sipper tubes touch any surfaces.

1. On the main menu, press **Initialize**.
2. Make the following selections in the next screen, then press **Next**:
 - Click **Enter barcode** to scan or enter the barcode on the Ion PGM™ Hi-Q™ View Sequencing W2 Solution bottle, or the 2D barcode on the Ion PGM™ Hi-Q™ View Sequencing Solutions box.
 - Alternatively, select the checkbox for the **Ion PGM™ Hi-Q™ View Sequencing Kit** from the dropdown list.
 - In the same screen, if you routinely experience clogging during initialization, select the **Line Clear** checkbox to clear any blockage in the fluid lines before initialization. This is optional.



IMPORTANT! Be careful to select the correct kit, to ensure proper pH adjustment.

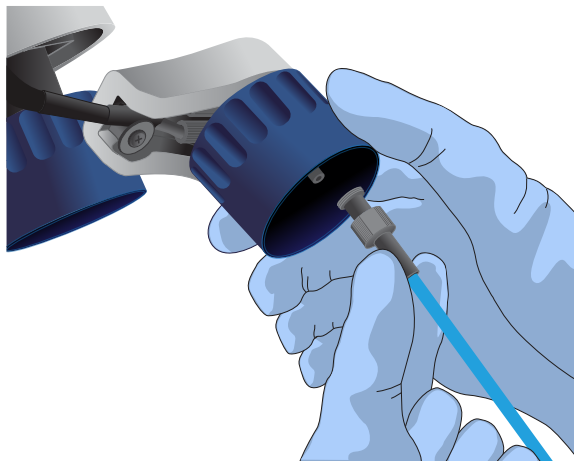
After you press **Next**, the system will check the gas pressure.

3. Following the gas pressure check:

Result	Action
If the pressure is sufficient	Ensure that the cleaning chip, reagent sipper tubes, and collection trays are in place, and press Next to start the initialization.
If the pressure is low	Press Yes to re-check the pressure. If the pressure remains low, contact Technical Support.

- Wearing clean gloves, firmly attach a new, long gray sipper to the cap in the W2 position.

IMPORTANT! Do not let the sipper touch any surfaces, and firmly attach the sipper to the port. Loosely attached sippers can adversely affect results.



- Immediately attach the prepared Wash 2 Bottle in the W2 position, then tighten the cap. Press **Next**.
- Change gloves and firmly install new sipper tubes (short gray) in the caps in the W1 and W3 positions.
- Immediately attach the prepared Wash 1 and 3 Bottles, then tighten the caps. Press **Next**.
- If you selected the **Line Clear** checkbox in the earlier screen, press **Next**, then follow the touchscreen prompts to perform the line clear procedure. At the beginning and end of the procedure, you are prompted to select one of the following:

Option	Description
Press Line Clear	To start a new line clear procedure
Press Re-flow	To retest the lines after you have performed a line clear
Press Auto pH	If the lines are clear and you are ready to continue with initialization

- Following line clear, or if you did not select that option, the sequencer begins adjusting the pH of the W2 Solution, which takes ~30 minutes. After 15 minutes, check the instrument touchscreen to confirm that initialization is proceeding normally.

Note:

- If an error occurs during the automatic pH process, note the error message and proceed to “Initialization—Auto pH errors” on page 48.
- During the process, you can start preparing the Reagent Bottles with dNTPs as described in the next section.

Prepare the 50-mL Reagent Bottles with dNTPs

1. Use the labels provided with the kit to label four new Reagent Bottles as dGTP, dCTP, dATP, and dTTP.
2. Confirm that no ice crystals are visible in each thawed dNTP stock solution. Vortex each tube to mix, and centrifuge to collect the contents. Keep the dNTP stock solutions on ice throughout this procedure.

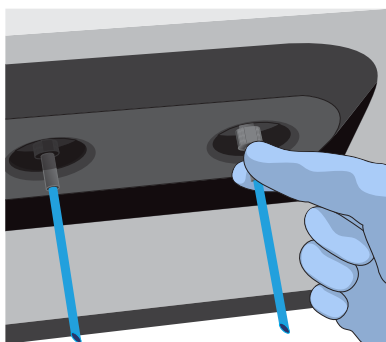
IMPORTANT! To avoid cross-contamination in the next step, open only one dNTP stock tube at a time and use a fresh pipette tip for each aliquot.

3. Using separate filtered pipette tips and clean gloves, carefully transfer 20 µL of each dNTP stock solution into its respective Reagent Bottle.
4. Cap each Reagent Bottle and store on ice until you are ready to attach it to the instrument. Place the remaining dNTP stocks back into –20°C for storage.

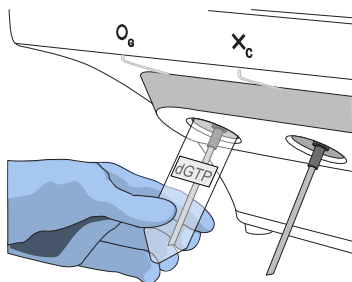
Attach the sipper tubes and Reagent Bottles

1. After the wash solutions have initialized, follow the touchscreen prompts to remove the used sipper tubes and collection trays from the dNTP ports.
2. Change gloves, then firmly insert a new sipper tube (blue) into each dNTP port. Do not let the sipper touch any surfaces.

IMPORTANT! Be careful to firmly push each sipper onto the port. Loosely attached sippers may adversely affect results.



3. Attach each prepared Reagent Bottle to the correct dNTP port (e.g., the dGTP tube on the port marked "G") and tighten firmly by hand until snug. Press **Next**.



Note: The instrument checks the pressure of the Reagent Bottles and Wash Bottles. If a bottle leaks, check that it is tightly attached to the instrument. If it continues to leak, replace it. If the instrument still does not pass the leak check, contact Technical Support.

4. Follow the touchscreen prompts to complete initialization. The instrument will fill each Reagent Bottle with 40 mL of W2 Solution.
5. At the end of initialization, Ion PGM™ System will measure the pH of the reagents:
 - If every reagent is in the target pH range, a green **Passed** screen will be displayed.
 - If a red failure screen appears, see Appendix A, “Troubleshooting”.
6. Press **Next** to finish the initialization process and return to the main menu.
7. Proceed to the appropriate sequencing protocol for your chip type.

5

Load the chip and start the sequencing run

Use the following chip loading and sequencing protocol for all Ion PGM™ chip types.

Materials required

Materials provided in the kit

- Ion PGM™ Hi-Q™ View Sequencing Primer
- Ion PGM™ Hi-Q™ View Control Ion Sphere™ Particles
- Annealing Buffer
- Ion PGM™ Hi-Q™ View Sequencing Polymerase

Other materials and equipment

- Ion Chip kit: Ion 318™ Chip v2 BC, Ion 316™ Chip v2 BC, or Ion 314™ Chip v2 BC
- Enriched template-positive ISPs
- 0.2-mL PCR tube (non-polystyrene)
- Rainin™ SR-L200F pipette and tips
- Vortex mixer
- Ion Chip™ Minifuge
- Thermal cycler with heated lid (programmed at 95°C for 2 minutes and 37°C for 2 minutes)
- Barcode scanner (included with the Ion PGM™ System)

Before starting

- Thaw the Sequencing Primer on ice.
- Make sure that you have updated the Torrent Suite™ System and Ion PGM™ System software to Version 5.0.4 or later.

Note: For each initialization, start the first run within 1 hour after initialization, and start the last run within 24 hours after initialization.

IMPORTANT! The ISPs are difficult to see. To avoid aspirating the particles:

- When centrifuging the ISPs, orient the tab of the tube lid so that it is pointing away from the center of the centrifuge, to indicate where the pellet will be formed.
 - Always remove supernatant from the tube from the top down
-

Add controls to the enriched, template-positive ISPs

1. Vortex the Control Ion Sphere™ Particles, then pulse-centrifuge in a picofuge for 2 seconds before taking aliquots.
2. Add 5 µL of Control ISPs directly to the entire volume of enriched, template-positive ISPs (prepared using your template preparation method) in a 0.2-mL non-polystyrene PCR tube.

Proceed to "Anneal the Sequencing Primer".

Anneal the Sequencing Primer

1. Mix the tube containing the ISPs by thoroughly pipetting up and down.
2. Place the tube in a microcentrifuge with an appropriate tube adapter. Orient the tab of the tube lid so that it is pointing away from the center of the centrifuge, to indicate where the pellet will be formed.
3. Centrifuge for 2 minutes at $15,500 \times g$.
4. Keeping the pipette plunger depressed, insert a pipette tip into the tube containing the pelleted ISPs and carefully remove the supernatant from the top down, avoiding the side of the tube with the pink ISP pellet (that is, the side with the tab on the tube lid). Discard the supernatant. Leave ~15 µL in the tube (visually compare to 15 µL of liquid in a separate tube).

Note: ISPs are visible up until chip loading, although the intensity of the pink color decreases as ISPs are processed during the workflow.

5. Ensure that the Sequencing Primer is completely thawed before use (no ice crystals should be visible).
6. Vortex the primer for 5 seconds, then pulse-centrifuge in a picofuge for 3–5 seconds to collect the contents. Leave on ice until ready to use.
7. Add 12 µL of Sequencing Primer to the ISPs, then confirm that the total volume is 27 µL (add Annealing Buffer if needed).
8. Pipet the mixture up and down thoroughly to disrupt the pellet.

IMPORTANT! Ensure that the pipette tip is at the bottom of the tube during mixing to avoid introducing air bubbles into the sample.

9. Program a thermal cycler for 95°C for 2 minutes and then 37°C for 2 minutes, using the heated lid option.
10. Place the tube in the thermal cycler, then run the program. After cycling, the reaction can remain in the cycler at room temperature (20–30°C) while you set up the sequencing run.

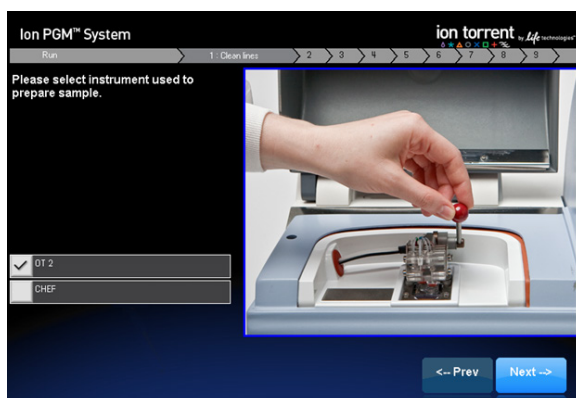
Perform Chip Check

Chip Check tests the chip and ensures that it is functioning properly before loading the sample.

IMPORTANT!

- To avoid damage due to electrostatic discharge (ESD), **do not place the chip directly on the bench or any other surface**. Always place the chip either on the grounding plate on the Ion PGM™ Sequencer or in the Ion Chip™ Minifuge adapter bucket.
 - To avoid ESD damage, **do not wear gloves** when transferring chips on and off the instrument.
-

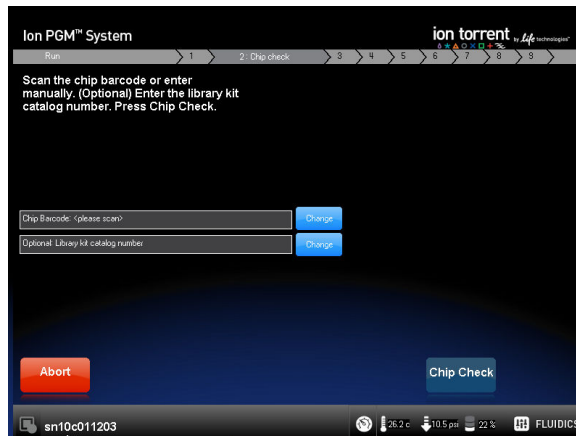
1. On the main menu of the Ion PGM™ Sequencer touchscreen, press **Run**. Remove the waste bottle and completely empty it. Press **Next**.
2. When prompted to insert a cleaning chip, use the same used chip that was used for initialization. Press **Next** to clean the fluid lines.
3. When prompted, select the instrument that you used to prepare the template-positive ISPs. Then press **Next**.



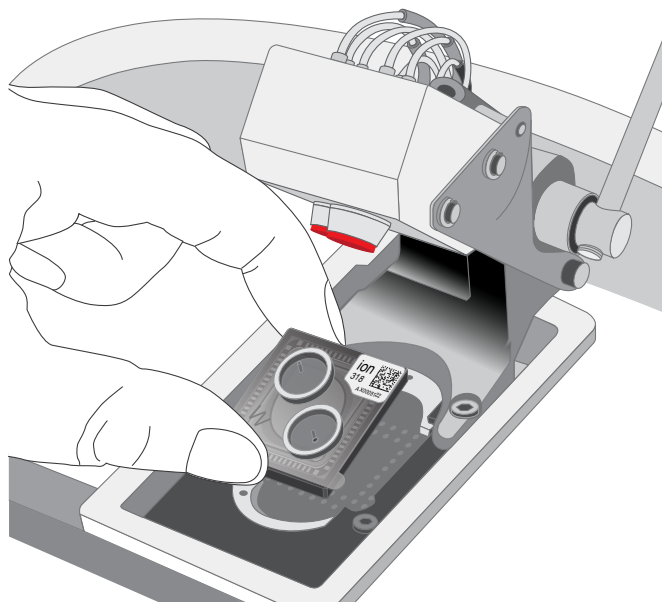
4. Remove gloves, then ground yourself by touching the grounding pad on the sequencer. Remove a new chip from its packaging, then label it to identify the experiment (save the chip package). Press **Next**.

- When prompted, use the scanner to scan the barcode located on the new chip, or press **Change** to enter the barcode manually. Optionally, you can also enter the library kit catalog number.

Note: A chip cannot be run without scanning or entering the barcode.



- Replace the old chip in the chip socket with the new one. Close the chip clamp, then press **Next**.



- Press **Chip Check**. During the initial part of Chip Check, visually inspect the chip in the clamp for leaks.

Note:

- If there is a leak, press the **Abort** button immediately to stop the flow to the chip. Proceed to Appendix A, "Troubleshooting".
- The chip socket can be damaged by rubbing or wiping its surface.** Never rub or wipe the socket to clean up leaks. See Appendix A, "Troubleshooting" for more information.

8. When Chip Check is complete:
 - If the chip passes, press **Next**.
 - If the chip fails, open the chip clamp, reseal the chip in the socket, close the clamp, and press **Calibrate** to repeat the procedure. If the chip passes, press **Next**. If the chip still fails, press **Main Menu** and restart the experiment with a new chip. See Appendix A, “Troubleshooting” for more information.

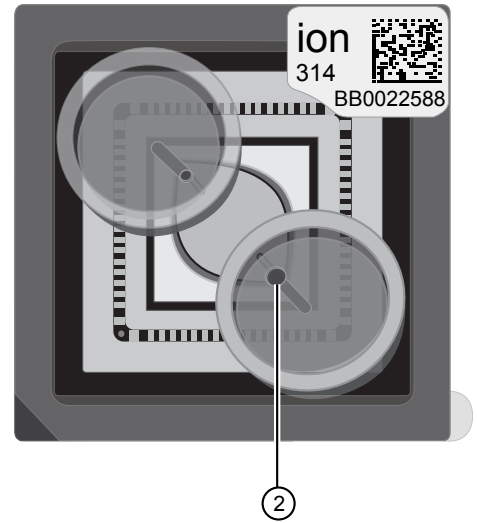
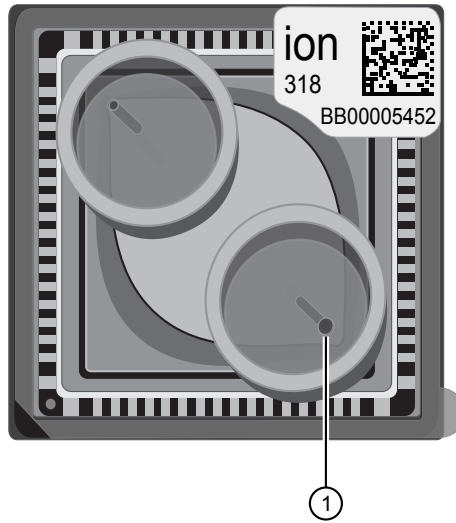
Note: To return *damaged* chips, contact Technical Support.
9. Following a successful Chip Check, empty the waste bottle, then select the **Waste bottle is empty** checkbox on the touchscreen. Press **Next**.

Bind the Sequencing Polymerase to the ISPs

1. Remove the Ion PGM™ Hi-Q™ View Sequencing Polymerase from storage and flick mix with your finger tip 4 times. Pulse-centrifuge for 3–5 seconds. Place on ice.
2. After annealing the Sequencing Primer, remove the ISPs from the thermal cycler, then add 3 μL of Ion PGM™ Hi-Q™ View Sequencing Polymerase to the ISPs, for a total final volume of 30 μL .
3. Pipet the sample up and down to mix, then incubate at room temperature for 5 minutes.

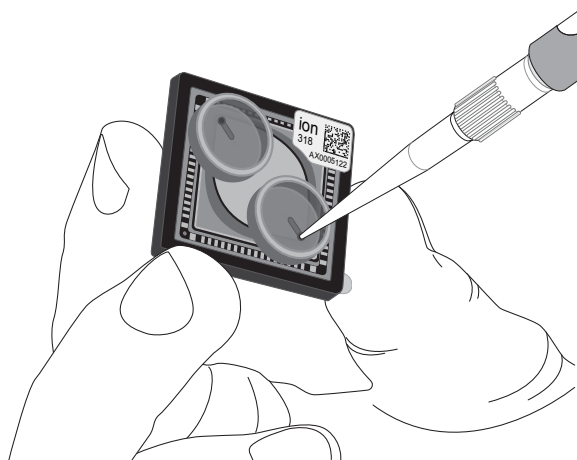
Prepare and load the chip

Remove liquid from the chip



- ① Ion 318™ Chip v2 BC/Ion 316™ Chip v2 BC ② Ion 314™ Chip v2 BC loading port

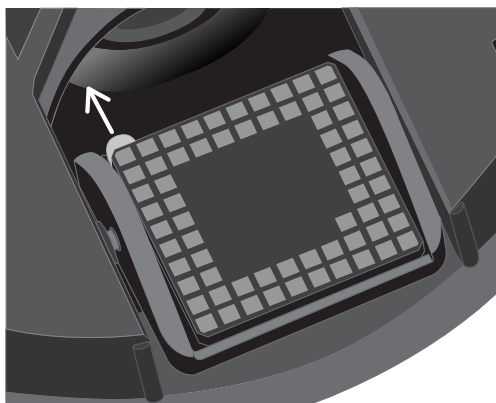
1. Following chip calibration, remove the new chip from the Ion PGM™ Sequencer. Insert a used chip in the chip clamp while loading the new chip.
2. Tilt the new chip at a 45° angle so that the loading port is the lower port.



3. Insert the pipette tip firmly into the loading port, then remove as much liquid as possible from the loading port. Discard the liquid.

IMPORTANT! For the next steps, if you are preparing one chip at a time, balance the chip in the Ion Chip™ Minifuge rotor with a used chip of the same chip type and orientation. Be careful to balance an upside-down chip with another upside-down chip. Mark the used chip with a laboratory marker to differentiate it from the new chip.

- Place the chip **upside-down** in the minifuge bucket, then transfer the bucket **with the chip tab pointing in** (toward the center of the minifuge). Balance the bucket with another chip.



- Centrifuge for 5 seconds to empty the chip completely.



CAUTION! Allow the minifuge to come to a complete stop before opening the lid.

- Remove the chip from the bucket, then wipe the bucket with a disposable wipe to remove any liquid. Place the chip right-side up in the bucket.
- Place the chip in the bucket on a firm, flat surface. Following polymerase incubation, load the chip with following volume of prepared ISPs using the listed pipettes, or equivalent, depending on your chip type. We recommend using a P20 pipette for Ion 314™ Chips for optimal loading.

Load the chip

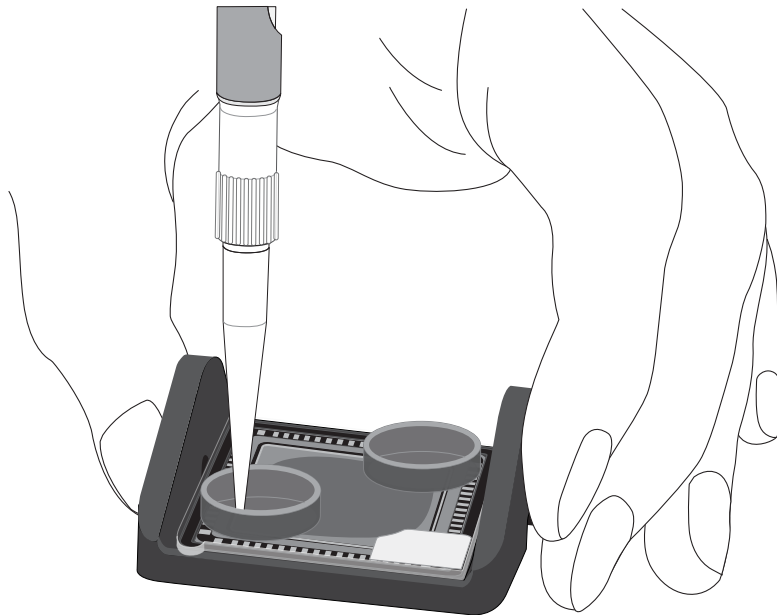
Chip	Volume to load	Recommended pipette ^[1]
Ion 316™ or Ion 318™ Chip	Entire volume (~30 µL)	Rainin™ Pipet-Lite™ LTS L-100XLS, 10–100 µL
Ion 314™ Chip	10 µL	Rainin™ Pipet-Lite™ LTS L-20XLS, 2–20 µL

^[1] Alternatives from Gilson and Eppendorf can be used.

Note: For Ion 314™ Chips, the remaining volume of ISPs can be used to prepare another chip, provided the chip can be loaded and sequenced in parallel with the first chip. After polymerase incubation, ISPs should be loaded and sequencing initiated within ~15 minutes.

2. Insert the tip firmly into the loading port of the chip.

Note: When loading the ISPs into the chip, keep 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.



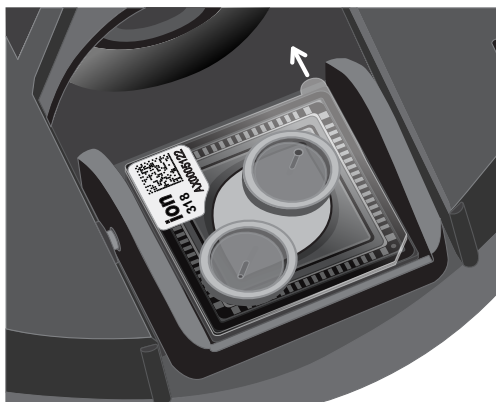
3. With the pipette unlocked, apply gentle pressure between the tip and chip and slowly dial down the pipette (~1 μL per second) to deposit the ISPs. To avoid introducing bubbles into the chip, leave a small amount in the pipette tip (~0.5 μL).



Note: Do not remove the pipette tip from the port during the dial-down process, because removal can introduce air bubbles and inhibit loading.

4. Remove, then discard any displaced liquid from the other port of the chip.

- Transfer the chip in the bucket to the minifuge with the chip tab **pointing in** (toward the center of the minifuge), then centrifuge for 30 seconds.



- Turn the chip so that the chip tab is **pointing out** (away from the center of the minifuge), then centrifuge for 30 seconds.



- Remove the bucket from the minifuge, then place it on a flat surface. Set the volume of the pipettor as follows, depending on your chip type:
 - **Ion 316™ or Ion 318™ Chip:** 25 μ L
 - **Ion 314™ Chip:** 5 μ L
- Tilt the chip at a 45° angle so that the loading port is the lower port, then insert the pipette tip into the loading port.
- Without removing the tip, slowly pipet the sample out and then back into the chip one time. **Pipet slowly to avoid creating bubbles.**
- Slowly remove as much liquid as possible from the chip by dialing the pipette. Discard the liquid.
- Turn the chip upside-down in the bucket, transfer it back to the minifuge, then centrifuge upside-down for 5 seconds. Remove and discard any liquid.

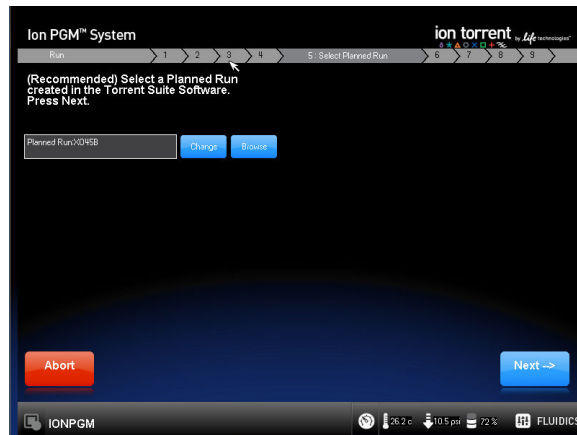
12. If some liquid remains in the chip, lightly and rapidly tap the point of the chip tab against the benchtop a few times, then remove and discard any collected liquid. Do not flush the chip.
13. When chip loading is complete, press **Next** on the touchscreen, then proceed immediately to performing the run.

Select the Planned Run and perform the run

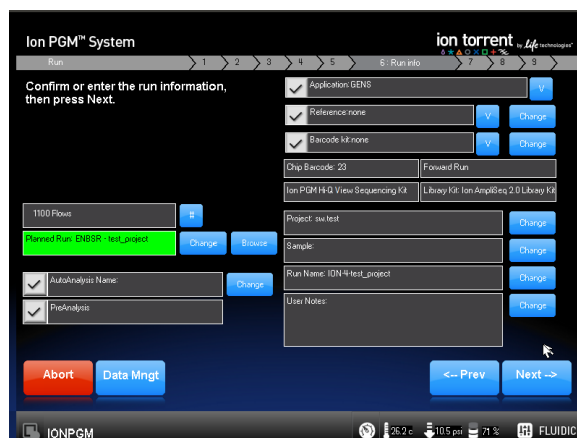
Select the Planned Run

1. Press **Browse** next to the **Planned Run** field and select the name of the plan you created, then touch **Next**.

Note: The Ion PGM™ Sequencer automatically populates this field for barcoded Ion chips.



2. Confirm that the settings are correct. If necessary, make any changes using the touchscreen controls.



Note: If the number of flows (cycles) to be run cannot be selected, there may not be enough disk space to store the experiment data. Touch **Data Mngt** to start the Data Management application (this can also be accessed from the Tools Menu) and delete old runs from the Ion PGM™ System.

Perform the run

1. After you enter the Planned Run, press **Next** to verify the experimental setup. Press **OK** to confirm the settings or press **Cancel** to return to the touchscreen to adjust the settings.
2. When prompted by the instrument, load and clamp the chip, then press **Next**.
3. At the beginning of the run, visually inspect the chip in the clamp for leaks before closing the cover. The instrument will flush any loose ISPs from the chip and begin calibrating the chip.
4. When the calibration is complete (~1 minute), the touchscreen will indicate whether calibration was successful.
 - If the chip passes calibration, press **Next** to proceed with the sequencing run.
 - If the chip fails calibration, see “Error message: Calibration FAILED” on page 47.
5. After 60 seconds, the run will automatically begin, or press **Next** to begin the run immediately.

IMPORTANT! During a run, avoid touching the instrument and any of the attached bottles or tubes, as this may reduce the quality of the measurements.


6. When the run is complete, leave the chip in place, then touch **Next** to return to the Main Menu. You can then remove the chip and proceed with another run or perform a cleaning/initializing if required.

Note: See “Cleaning schedule” on page 22 to determine whether cleaning is required after the run.

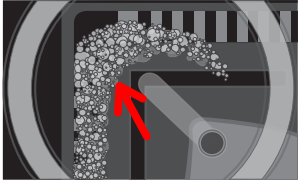


Troubleshooting

Chip Check

Observation	Possible cause	Recommended action
Chip Check fails	<ul style="list-style-type: none">• Clamp is not closed.• Chip is not properly seated.• Debris is present on the chip socket.• Chip is damaged.	<ol style="list-style-type: none">1. Open the chip clamp, remove the chip, and look for signs of water outside the flow cell: 2. If the chip appears damaged, replace it with a new one.3. Look for debris on the chip socket. Remove any debris by rinsing with 18-MΩ water and gently dabbing the socket with a lab wipe tissue. IMPORTANT! Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail.4. Close the clamp and repeat the Chip Check.5. If the chip passes, click Next. If the chip fails, replace it with a new chip, scan the barcode of the new chip, then press Chip Check.6. If Chip Check continues to fail, there could be a problem with the chip socket. Contact Technical Support.

Chip calibration (before loading sample)

Observation	Possible cause	Recommended action
Leak of unknown origin occurs	<ul style="list-style-type: none"> • Chip is leaking. • Chip clamp is not closed properly. • Problem exists with the chip clamp or socket. 	<ol style="list-style-type: none"> 1. Press Main Menu. 2. Open the chip clamp, remove the chip, and gently dab the chip socket with a lab wipe tissue to absorb any fluid. IMPORTANT! Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 3. Rinse the socket with 18-MΩ water and gently absorb most of the water with the lab wipe. 4. Repeat the rinse, then gently dab the chip socket until dry. 5. Place a lab wipe on the grounding plate and dampen it with 18-MΩ water. Wipe the bottom of the chip on this wipe to remove salts from the chip contacts. 6. Remove the wipe, dry the grounding plate, and place chip on grounding plate. Ensure that there is no condensation outside the flow cell:  7. Replace the chip with a new (unused) one if needed. Note: The new chip can be used for sequencing after initialization completes. 8. Press Run to restart the experiment. 9. When prompted to install the new chip, ensure that the chip clamp is fully closed. 10. If the chip leaks again, clean the chip socket as described above. Continued leaking, even with new chips, can indicate a chip clamp or socket problem. Contact Technical support.



Observation	Possible cause	Recommended action
Error message: Calibration FAILED	<ul style="list-style-type: none"> • Chip is not seated in socket correctly. • Chip is damaged. 	<ol style="list-style-type: none"> 1. Remove the chip and confirm that there is no leakage or debris on the chip socket. If leaking or debris is seen, follow the procedure for inspecting the chip and clearing debris as described under "Chip Check fails" and/or "Leak of unknown origin" above. If no leaking or debris is seen, reseal the chip in the socket. 2. Press Calibrate to repeat the calibration. 3. If the chip passes, press Next. If the chip still fails return to the main menu and restart the experiment with a new chip. 4. If you continue to have chip calibration issues, there may be an issue with the chip socket. Contact Technical Support.

Chip calibration (after loading sample)

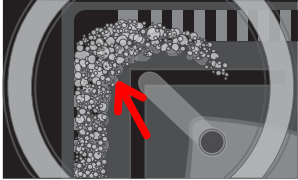
Observation	Possible cause	Recommended action
Leak of unknown origin occurs	<ul style="list-style-type: none"> Chip is leaking. Chip clamp is not closed properly. 	<ol style="list-style-type: none"> Press the Abort button. Open the chip clamp, remove the chip, and gently dab the chip socket with a lab wipe tissue to absorb any fluid. Do not rub or wipe the chip socket. Rinse the socket with 18 MΩ water and gently absorb most of the water with the lab wipe tissue. Repeat the rinse, then gently dab the chip socket until dry. Place a lab wipe tissue on the grounding plate and dampen it with 18 MΩ water. Wipe the bottom of the chip on this wipe to remove salts from the chip contacts. Remove the wipe, dry the grounding plate, and place the chip on the grounding plate. Check for condensation outside the flow cell: <div data-bbox="924 930 1219 1108" data-label="Image"> </div> If there is condensation or fluid, the chip is damaged and cannot be run. If there is no condensation or fluid, press Calibrate to restart the calibration procedure. If calibration passes and no leaks are visible, press Next to start the experiment. If the chip leaks again, clean the chip and chip socket as described above. Continued leaking can indicate a chip clamp or socket problem. Contact Technical Support.

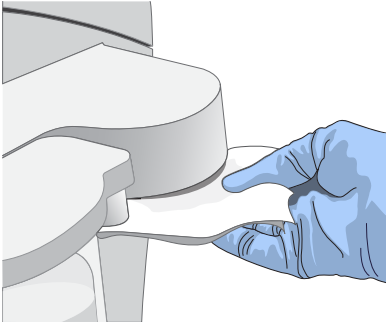
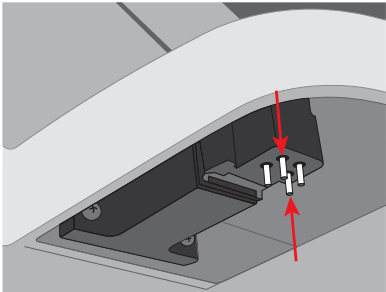
Observation	Possible cause	Recommended action
Error message: Calibration FAILED	<ul style="list-style-type: none"> Chip is not seated in socket correctly. Chip is damaged. 	<ol style="list-style-type: none"> 1. Remove the chip and check for leaks and/or debris on the chip socket, following the procedures described in "Chip Check fails" and/or "Leak of unknown origin," above. If no leaks or debris are visible, reseal the chip in the socket. 2. Press Calibrate. 3. If the chip passes, press Next to start the experiment. If the chip still fails, you can try resealing the chip multiple times and pressing Calibrate. If you are still unable to pass calibration, press Next to start the run anyhow—you may still get some data on your sample. 4. If you continue to have chip calibration issues, there may be an issue with the chip or chip socket. Contact Technical Support.

Initialization—General errors

Observation	Possible cause	Recommended action
Error message: Confirm instrument has gas pressure	Gas cylinder may be turned off or empty.	<ol style="list-style-type: none"> 1. Verify 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 PGM™ Sequencer are open. 2. Once you confirm gas pressure leading into the instrument, press Yes to retry verification of gas pressure. If the test continues to fail, contact Technical Support.
Bottle leak check fails	<ul style="list-style-type: none"> Bottle seal is not tight. Bottle may be damaged / defective. 	<ol style="list-style-type: none"> 1. Finger-tighten the bottles. 2. If the bottle continues to leak, replace the bottle. 3. If leak check continues to fail, contact Technical Support.

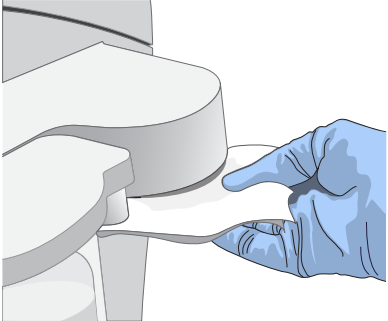
Initialization—Auto pH errors

Observation	Possible cause	Recommended action
Error message: Please insert a chip and press Start	Instrument cannot detect the chip in chip socket.	<ol style="list-style-type: none"> 1. Open the chip clamp and remove the chip. 2. Check for debris under the chip or in the chip socket. Remove any debris by rinsing with 18-MΩ water and gently dabbing the socket with a lab wipe tissue. IMPORTANT! Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 3. Look for liquid outside the flow cell of the chip:  4. If you see liquid, replace the chip with a new (unused) one. Wash the new chip once with 100% isopropanol and twice with SEQ Sample Buffer before using. Note: The new chip can be used for sequencing after initialization completes. 5. Close the clamp, then press Start to restart the process. 6. If the new chip also fails, there could be a problem with the chip socket. Contact Technical Support.
Error message: Chip calibration failed	<ul style="list-style-type: none"> • Chip is not seated in socket correctly. • Chip is damaged. • Sipper is loose. 	<p>Follow the procedure for "Error message: Please insert a chip and press Start."</p> <p>Follow the procedure for "Error message: Wash 2 average not stable."</p>

Observation	Possible cause	Recommended action
<p>Error message: The system did not reach the target W2 pH and/or has a clog</p>	<p>The waste lines can be clogged.</p>	<ol style="list-style-type: none"> 1. Press the Troubleshoot button. <p>Note: You can skip the Troubleshoot button and change the chip to restart the Auto-pH routine.</p> <ol style="list-style-type: none"> 2. Remove the waste bottle. 3. Place lab wipes under the waste arm. 4. Gently wipe the waste arm with a lab wipe to clear liquid near the waste line.  <ol style="list-style-type: none"> 5. Press Next to start buffer flow. Observe flow rates from both waste lines. One line should drip slightly faster than the other. Following the flow rate check, one of 3 results is possible: <ol style="list-style-type: none"> a. If flow rate appears normal, press Cancel and test another chip. If Auto pH failure persists, contact Technical Support. b. If flow is blocked, press Line Clear to run the standard Line Clear procedure. If the line is unable to clear, contact Technical Support. c. If the result of the flow rate check is uncertain, press Re-flow to re-flow the buffer and re-test the flow. 




Observation	Possible cause	Recommended action
Error message: The system did not reach the target W2 pH (<i>continued</i>)	Wash 1 or Wash 2 sipper may be loose.	<ol style="list-style-type: none">Loosen the Wash 1 cap and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the NaOH solution and is not a hazard.)Loosen the Wash 2 cap and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the W2 Solution and is not a hazard.)Press Start to re-start the auto-pH process.
	Forgot to add NaOH to the Wash 1 Bottle.	<ol style="list-style-type: none">If there is no NaOH in the Wash 1 Bottle, loosen the cap and add 350 μL of 100 mM NaOH to the Wash 1 Bottle. (The flowing gas is not harmful to the NaOH solution and is not a hazard.)Recap the bottle and shake gently to mix.Press Start to restart auto-pH.
	Chip is damaged.	<ol style="list-style-type: none">Replace the chip with a new (unused) one. Insert the chip in the socket, then press Start. Note: The new chip can be used for sequencing after initialization completes.If the error persists, there could be a problem with the chip clamp. Contact Technical Support.

Observation	Possible cause	Recommended action
<p>Error message: W2 average not stable. Try reseating/replacing chip</p>	<p>Reading for W2 solution is not stabilizing quickly enough.</p>	<ol style="list-style-type: none"> 1. Remove the waste bottle and gently wipe excess fluid from the waste lines with a lab wipe.  <ol style="list-style-type: none"> 2. Check for leaks and reseat the chip (see troubleshooting for "Chip Check" and "Chip calibration" above). Replace the chip with a new (unused) one if needed. <p>Note: The new chip can be used for sequencing after initialization completes.</p> 3. Loosen the cap in the W2 position and re-tighten the sipper. Because the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful and not a hazard.) 4. After performing one or more above steps, press Start to re-start auto-pH. If auto-pH fails even after replacing the chip, contact Technical Support and manually adjust the pH of the Wash 2 Bottle as described in "Manually adjust W2 pH" in the <i>Ion PGM™ System Reference Guide</i> (Pub. No. MAN0009783).
<p>Error message: W2 out of range</p>	<ul style="list-style-type: none"> • Chip measurements are very unstable. • Chip is damaged. 	<p>See troubleshooting tips for "W2 average not stable" above.</p>
<p>Error message: Chip reading inconsistent. Please replace chip and try again</p>	<ul style="list-style-type: none"> • pH response of the chip is not uniform or reliable. • Ran out of W3 Solution or volume too low. 	<ol style="list-style-type: none"> 1. Verify that there is enough W3 Solution (>25 mL) in the Wash 3 Bottle and that the sipper is secure. 2. If necessary, loosen the Wash 3 Bottle cap, tighten the sipper, and add more W3 Solution to fill to 50 mL. Since the gas flows when the cap is loose, perform these operations as quickly as possible. (The gas is not harmful to the W3 Solution and is not a hazard.) 3. If there is enough W3 Solution, replace the chip with a new (unused) one. Insert the chip in the socket, then press Start. <p>Note: The new chip can be used for sequencing after initialization completes.</p>



Observation	Possible cause	Recommended action
<p>Error message: Added too much W1 to W2</p>	<ul style="list-style-type: none"> • Water quality is poor. • 18 MΩ water was exposed to air for too long. • Incorrect solution was added to the Wash 2 Bottle. • Too little NaOH was added to Wash 1 Bottle. • Chip is damaged. 	<ol style="list-style-type: none"> 1. Check whether the water meets the 18 MΩ specification and 100 mM NaOH and W2 Solution were added correctly. 2. If solutions are incorrect or water does not meet specifications, correctly prepare the solution(s) and/or use high-quality water. Abort the initialization and restart using correct solutions/water. 3. If solutions are correct and water meets specifications, abort the initialization, return to the main menu, and proceed to the next steps. 4. Leave the Wash 2 Bottle on the instrument. 5. Remove the Wash 1 Bottle, leaving the sipper on the W1 port. Empty the bottle, and rinse the bottle twice with 18 MΩ water. 6. Add 350 μL of 100 mM NaOH to the Wash 1 Bottle and reinstall on the instrument. 7. Press Initialize, select the kit type, and keep pressing the Next button to skip all bottle prep steps until the instrument begins purging air from the bottle. Then proceed through the touchscreens as normal to complete the initialization. 8. The next time you initialize the instrument, add 140 μL of 100 mM NaOH to the Wash 2 Bottle instead of 70 μL. Continue to use this larger volume for subsequent initializations until you receive an "Overshot Target" error message at the first auto-pH iteration, at which point follow the troubleshooting steps in "Error message: The system overshot the target W2 pH." on page 53 on the following page and then return to adding 70 μL of 100 mM NaOH. 9. If you still receive the same initialization error ("Added too much W1 to W2"), contact Technical Support.
<p>Error message: UNDERSHOT TARGET PH: W2 pH = n.nn Failed</p>	<p>Auto-pH couldn't add enough Wash 1 to the Wash 2 before the maximum iterations, 10, occurred.</p>	<ol style="list-style-type: none"> 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." 2. Press Start to re-start auto-pH. If you still get the "Undershot target pH" error, try replacing the chip with a new (unused) chip and restarting auto-pH. <p>Note: The new chip can be used for sequencing after initialization completes.</p>

Observation	Possible cause	Recommended action
Error message: The system overshot the target W2 pH.	Auto-pH added more NaOH from the Wash 1 Bottle to the Wash 2 Bottle than was needed, and reports the pH value.	<ol style="list-style-type: none"> 1. Press the Overshoot button to proceed with W2 pH adjustment. 2. Unscrew the cap of the Wash 2 Bottle. Without removing the sipper from the bottle, lift the cap high enough to pipette 15 μL of 100 mM HCl into the Wash 2 Bottle, close and tighten cap.  <ol style="list-style-type: none"> 3. Press Next to re-pressurize the Wash 2 Bottle and mix the W2 solution. 4. Press Start to retry auto-pH.

Initialization—Reagent pH verification

Observation	Possible cause	Recommended action
Red failure screen, reagent pH is displayed	One or more reagents are not within the target pH.	<ol style="list-style-type: none"> 1. Press Start to repeat the pH measurements to confirm the measurement. 2. If any reagents still fail, try replacing the chip with a new (unused) chip and repeating. Note: The new chip can be used for sequencing after initialization completes. 3. If any reagents still fail, clean and re-initialize the instrument with fresh reagents and a new chip.
	A possible line clog exists which persisted through the Auto pH process.	<ol style="list-style-type: none"> 1. From the Tools menu, perform a W1 line clear. 2. Press Start to repeat the pH check.



Observation	Possible cause	Recommended action
Red failure screen, reagent pH is <i>not</i> displayed	Chip did not calibrate.	<ol style="list-style-type: none"> 1. Replace the chip with a new (unused) one. Note: The new chip can be used for sequencing after initialization completes. 2. Press Start to restart the pH measurement. 3. If the second test fails, contact Technical Support.

Troubleshooting using Control Ion Sphere™ Particles and control libraries

Observation	Possible cause	Recommended action
Control Ion Sphere™ Particles are not present in the Test Fragment Report section of the run report	Chip loading is poor.	Review the chip loading procedure in Chapter 5 of this user guide.
	Control Ion Sphere™ Particles were not added to the sample	<ol style="list-style-type: none"> 1. Confirm that the Control Ion Sphere™ Particles (included in this sequencing kit) were added. 2. If controls were added, contact Technical Support.
Ion PGM™ Hi-Q™ View Control Ion Sphere™ Particles are present in the run report, but AQ20 throughput is poor	<ul style="list-style-type: none"> • The quality of your library is poor. • The quality of your template is poor. 	Verify the quality of the library and template preparations using quality assessment procedures recommended in the appropriate library and template preparation user guides.
		<ol style="list-style-type: none"> 1. Use the E. coli DH10B Control 200 or 400 Library, included in the Ion PGM™ Controls Kit v3 (Cat. No. A30046), to prepare template-positive ISPs with the Ion OneTouch™ 2 Instrument. 2. Use the ISPs in an Ion PGM™ Hi-Q™ View Sequencing run. 3. If AQ20 throughput is still below specification, verify the quality of unenriched and enriched ISPs to identify a problem in template preparation. 4. If ISP quality is good, but AQ20 throughput is below specification, contact Technical Support.

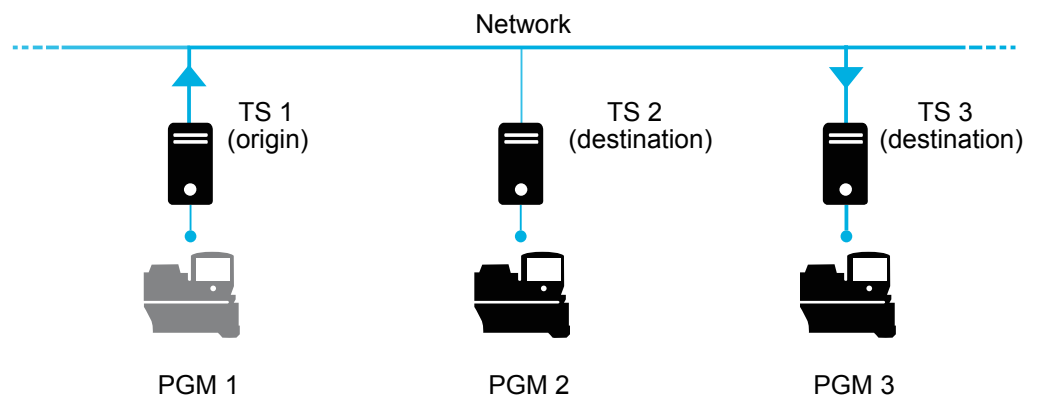


Sharing Planned Runs between Torrent Servers

Enable Planned Run sharing

Starting in Torrent Suite™ Software v4.4, Planned Runs created on one Torrent Server can be transferred to another Torrent Server. This is useful if a sequencer connected to a particular server is offline or busy.

For example, the figure below illustrates a scenario where three Torrent Servers are on the same subnet, and a Planned Run created on TS 1 (the "origin" server) is transferred to TS 3 (the "destination" server) for use on the sequencer connected to TS 3.



Requirements include:

- All Torrent Servers must be on the same subnet.
- All Torrent Servers must be running the same software version.
- All Torrent Servers must have the same genomic reference, barcode set, BED files, Variant Caller config files, etc.

Set up Server Network (admin action)

1. On the *origin* server (e.g., TS1) Site administration page, scroll down and select Shared servers. The Select shared server to change window appears.

Site administration

Auth	
Groups	Add Change
Users	Add Change
Rundb	
3' Adapters	Add Change
Analysis Args	Add Change
Analysis metrics	Add Change
Appl products	Add Change
Backup configs	Change
Backups	Change
Chips	Add Change
Content uploads	Add Change
Contents	Add Change
Crunchers	Add Change
DNA Barcodes	Add Change
Dm file sets	Add Change
Dm file stats	Add Change
Shared servers	Add Change
Support uploads	Add Change
TF metrics	Add Change
Templates	Add Change
User event logs	Add Change
User profiles	Add Change
Variant Frequencies	Add Change

Recent Actions

My Actions

- [test_plan](#)
Planned experiment
- [R_2013_04_02_16_12_55_DM...](#)
Experiment analysis settings
- [R_2013_04_02_16_12_55_DM...](#)
Experiment analysis settings
- [R_2013_04_11_10_32_06_Bar...](#)
Experiment analysis settings
- [NONE_ReportOnly_NONE/1193](#)
Experiment analysis settings
- [SharedServer object](#)
Shared server
- [SharedServer object](#)
Shared server
- [SharedServer object](#)
Shared server
- [SharedServer object](#)
Shared server
- [SharedServer object](#)
Shared server

2. If you are adding your *destination* server for the first time, click **Add shared server**.

Select shared server to change [Add shared server](#) +

Action: 0 of 2 selected

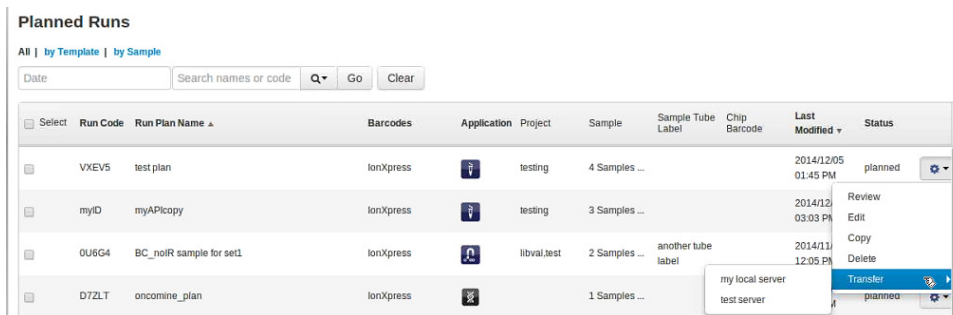
<input type="checkbox"/>	Name	Address	Username	Active
<input type="checkbox"/>	test server	6wvvpq1aε	ionadmin	<input checked="" type="checkbox"/>
<input type="checkbox"/>	my local server	127.0.0.1	ionuser	<input checked="" type="checkbox"/>

2 shared servers

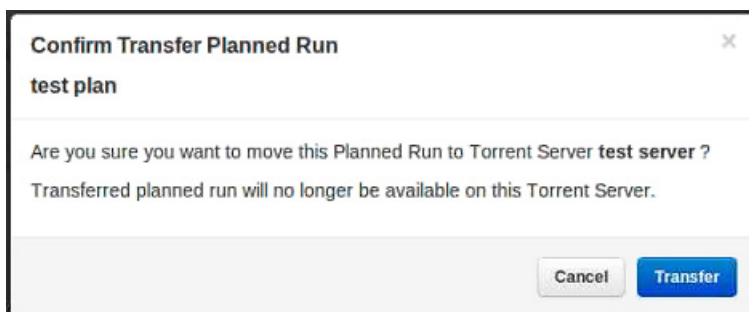
3. Define your *destination* server.
 - a. Enter the name, address (can be IP address), user name and password for the *destination* server.
 - b. Click **Active** if you want this server enabled for sharing.
 - c. (Optional) Add a comment.
 - d. Click one of the **Save** options.
4. (Optional) If you want to configure the origin Torrent Server to also be a destination server, you must go to another server and repeat these steps to set the origin server as a destination server. Once the Torrent Servers are configured, you or a user can now transfer Planned Runs between Torrent Servers.

Transfer a Planned Run

1. Using the Torrent Browser on the *origin* Torrent Server, go to **Plan ▶ Planned Run List**.
2. Open the Gear ⚙️ menu of the Planned Run you want to transfer, and select **Transfer**. Then select the *destination* Torrent Server.



3. A confirmation window appears. Check the information, then click **Transfer**.
Note: You can no longer access this Planned Run on the origin server after it has transferred.



4. A status window displays the results of the transfer:
 - The green box lists the samples successfully processed and the required target BED files found on the destination server.
 - The red box lists any required BED files or plugins that are not present on the destination server. To successfully perform the run, you will need to edit the transferred Planned Run on the destination server and manually add the missing BED files or plugins.

test plan

Successfully created test plan on Torrent Server [test server](#)

....processed Samples: Sample 2, Sample 3, Sample 1

....found BED files: target.bed

....found IR account IonEast IR (Version: 4.2 | User: Ion User | Org: IR Org)

Planned run data is incomplete, please [Edit test plan](#) to fix the following errors

Unable to find bedfile: HSMv12.1_hotspots.bed for reference: hg19

Unable to find bedfile: atarget.bed for reference: hg19

Close

5. To edit the transferred Planned Run and add missing files:
 - a. Download required files using the References tab of the Torrent browser of the destination server
 - b. Go to the Edit Plan wizard of the transferred Planned Run by selecting Edit on the gear pull-down menu to the right of the Planned Run.
 - c. Select the files or plugins as needed, then click **Update Plan**.

Note: You can also navigate to the Edit Plan wizard by clicking the **Edit test plan** link in the status page above.

Undo a Planned Run transfer (administrator)

1. On the *destination* server, delete the transferred Planned Run from either the Planned Run page or the admin page.
2. On the *origin* server, locate the plan on the /admin/rundb/plannedexperiment/page, uncheck **PlanExecuted** and change **PlanStatus** to **Planned**.

Undo a Planned Run transfer (user)

If you transferred a Planned Run in error, you can transfer it back to the origin server or to another server.

1. On the destination Torrent Server, navigate to **Plan ▶ Planned Run List** and locate the transferred Planned Run.
2. From the Gear ⚙️ menu of the Planned Run, select **Transfer**, then select the Torrent Server to which you wish to transfer the run.



Additional instrument information

See the *Ion PGM™ System Reference Guide* (Pub. No. MAN0009783) for additional information on general instrument operation and maintenance, including:

- Touchscreen reference
- How to update instrument software
- How to manually adjust W2 Solution pH
- Ion Chip™ Minifuge and Barcode Scanner operation
- Sequencing run times
- Site requirements
- Instrument safety



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, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
-

Instrument safety

For detailed information on instrument safety symbols and alerts, safety and electromagnetic compatibility standards, and general instrument safety, see the Safety appendix of the *Ion PGM™ System Reference Guide* (Pub. No. MAN0009783), available at thermofisher.com.

Chemical safety



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

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

Biological hazard safety



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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
www.cdc.gov/biosafety/publications/bmb15/BMBL.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

Customer and technical support

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- Order and web support
- Product documentation, including:
 - 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 found on Life Technologies' website 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.

For support visit [thermofisher.com/support](https://www.thermofisher.com/support) or email techsupport@lifetech.com
thermofisher.com

6 January 2017

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