

Ion PGM™ Hi-Q™ View Sequencing Kit

Catalog Numbers A30044

Pub. No. MAN0014584 Rev. C.0

Note: For safety and biohazard guidelines, see the “Safety” appendix in the *Ion PGM™ Hi-Q™ View Sequencing Kit User Guide* (Pub. No. MAN0014583). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

■ Create a Planned Run	1
■ Condition the Wash 2 Bottle for first use	1
■ Clean the Ion PGM™ System	2
■ Initialize the Ion PGM™ System	3
■ Load the chip and start the sequencing run	4
■ Limited product warranty	6

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^[1], 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.

Create a Planned Run

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.
5. When you have completed your selections, click **Plan Run**.

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.

To condition the Wash 2 Bottle:

1. Fill the bottle to the mold line with 18 MΩ 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.

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

Clean the Ion PGM™ System

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_hm 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

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

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

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.
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.)
5. Add the entire bottle of Ion PGM™ Hi-Q™ View Sequencing W2 Solution to the Wash 2 Bottle.
6. Using a P200 pipette, add 70 µL of 100 mM NaOH to the Wash 2 Bottle.
7. 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

1. Rinse the Wash 1 and Wash 3 Bottles 3 times with 50 mL of 18 MΩ water.
2. **Wash 1 Bottle:** Add 350 µL of freshly prepared 100 mM NaOH to the Wash 1 Bottle, then cap the bottle.
3. **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

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.

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.

4. Wearing clean gloves, firmly attach a new, long gray sipper to the cap in the W2 position.
5. Immediately attach the prepared Wash 2 Bottle in the W2 position, then tighten the cap. Press **Next**.
6. Change gloves and firmly install new sipper tubes (short gray) in the caps in the W1 and W3 positions.
7. Immediately attach the prepared Wash 1 and 3 Bottles, then tighten the caps. Press **Next**.
8. 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.

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.

- Using separate filtered pipette tips and clean gloves, carefully transfer 20 µL of each dNTP stock solution into its respective Reagent Bottle.
- 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

- After the wash solutions have initialized, follow the touchscreen prompts to remove the used sipper tubes and collection trays from the dNTP ports.
- Change gloves, then firmly insert a new sipper tube (blue) into each dNTP port. Do not let the sipper touch any surfaces.
- 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**.
- Follow the touchscreen prompts to complete initialization. The instrument will fill each Reagent Bottle with 40 mL of W2 Solution.
- 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 the troubleshooting section of the user guide.
- Press **Next** to finish the initialization process and return to the main menu.
- Proceed to the appropriate sequencing protocol for your chip type.

Load the chip and start the sequencing run

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

Before starting

- Thaw the Sequencing Primer on ice.

Add controls to the enriched, template-positive ISPs

- Vortex the Control Ion Sphere™ Particles, then pulse-centrifuge in a picofuge for 2 seconds before taking aliquots.
- 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

- Mix the tube containing the ISPs by thoroughly pipetting up and down.
- 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.
- Centrifuge for 2 minutes at 15,500 × g.
- 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).
- Ensure that the Sequencing Primer is completely thawed before use (no ice crystals should be visible).
- 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.
- Add 12 µL of Sequencing Primer to the ISPs, then confirm that the total volume is 27 µL (add Annealing Buffer if needed).
- Pipet the mixture up and down thoroughly to disrupt the pellet.
- Program a thermal cycler for 95°C for 2 minutes and then 37°C for 2 minutes, using the heated lid option.
- 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

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

6. Replace the old chip in the chip socket with the new one. Close the chip clamp, then press **Next**.
7. Press **Chip Check**. During the initial part of Chip Check, visually inspect the chip in the clamp for leaks.
8. 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. 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.
4. 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.
5. Centrifuge for 5 seconds to empty the chip completely.
6. 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.

Load the chip

1. 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.

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.

2. Insert the tip firmly into the loading port of the 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).
4. Remove, then discard any displaced liquid from the other port of the chip.
5. 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.
6. Turn the chip so that the chip tab is **pointing out** (away from the center of the minifuge), then centrifuge for 30 seconds.
7. 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 µL
 - **Ion 314™ Chip:** 5 µL
8. 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.
9. Without removing the tip, slowly pipet the sample out and then back into the chip one time. **Pipet slowly to avoid creating bubbles.**
10. Slowly remove as much liquid as possible from the chip by dialing the pipette. Discard the liquid.
11. 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.

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.

5. After 60 seconds, the run will automatically begin, or press **Next** to begin the run immediately.
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.

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.

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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0014584

Revision	Date	Description of change
C.0	6 January 2017	Minor corrections in component names.
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 Quick Reference

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288

©2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.