

Ion PGM™ IC 200 Kit

Catalog Number 4484080

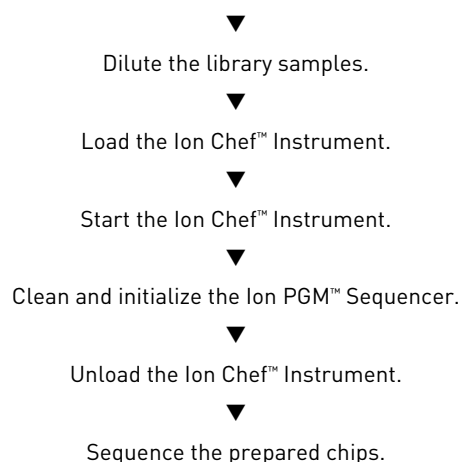
Pub. No. MAN0008320 Rev. C.0

Note: For safety and biohazard guidelines, refer to the “Safety” appendix in the *Ion PGM™ IC 200 Kit User Guide* (Pub. no. MAN0007661). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

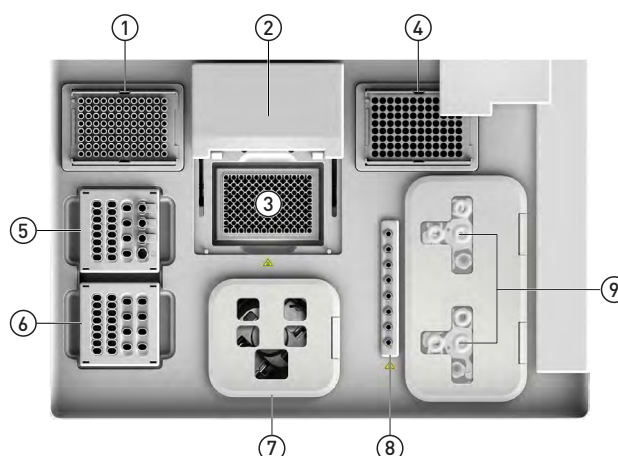
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Workflow

Create a Planned Run within the Torrent Suite™ Software.



Interior hardware and consumables



- ① New pipette tips
- ② Automated heated cover
- ③ Thermal cycler sample block
- ④ Waste pipette tips
- ⑤ Reagents station
- ⑥ Solutions station
- ⑦ Chip-loading Centrifuge (buckets not shown)
- ⑧ Enrichment station
- ⑨ Recovery Centrifuges

Create a Planned Run

1. Open the Torrent Browser for the Torrent Server connected to your Ion Chef™ System.
2. Select the **Plan** tab, click **Templates**, locate the application that you want to run (such as Whole Genome) then select either **Plan New Run** or **Plan Run** from the drop-down-list of the Settings ⚙️ tab to the right of an existing template you select from the template list.
3. In the Planned Run wizard, review each screen and edit as needed. In the **Kits** tab, select **Ion Chef**, select the **Ion PGM™ IC 200 Kit** from the **Template Kit** drop-down list, select **Ion PGM™ IC 200 Sequencing Kit** from the **Sequencing Kit** drop-down list, then enter or scan the barcodes of the Ion Chef™ Library Sample Tubes into the appropriate Sample Tube Label fields.
4. When you have completed your selections, click **Plan Run** at the bottom right of the Plan tab screen to save the run. The run is listed on the Planned Runs page under the name that you specified and is automatically used by the Ion Chef™ System when the associated sample is loaded and scanned.

Dilute the libraries

Dilute the two (2) stock libraries to a starting concentration of 50 pM with Nuclease-free Water (approximately 2.1 billion molecules per 70- μ L input volume). Then use polyclonality and low quality filter results from a sequencing run performed with ISPs templated at this starting concentration and titrate downward to achieve optimal concentrations, if necessary. Prepare a fresh dilution of each library before use with the Ion Chef™ System, and use the library dilutions within 48 hours.

Note: Library input recommendation is based on qPCR quantification. If libraries are quantified with a 2100 Bioanalyzer® instrument, a higher calculated concentration may need to be used for equivalent input.

Note: If running a control library, prepare the *E. coli* DH10B Control 200 Library, obtained from the Ion PGM™ Controls Kit v2 (Cat. no. 4482010), for use by diluting 1 μ L into 105 μ L Nuclease-free Water.

Prepare the libraries and consumables

1. At least 45 minutes prior to use, unbox the Ion PGM™ IC Reagents 200 Cartridge and allow it to thaw at room temperature.

IMPORTANT! The IC Reagents Cartridge must warm to room temperature for at least 45 minutes before use.

2. Pipet 70 μ L of each diluted library to the bottom of the appropriate Ion Chef™ Library Sample Tube (flagged tubes).

Note: If running the *E. coli* DH10B Control 200 Library, obtained from the Ion PGM™ Controls Kit v2 (Cat. no. 4482010), prepare the library for use by diluting 1 μ L of stock library into 105 μ L Nuclease-free Water.

3. Cap and store the two diluted DNA libraries on ice until you are ready to load them onto the Ion Chef™ Instrument.
4. Remove all cartridges and consumables from their wrappings and boxes, then place them on the bench next to the Ion Chef™ Instrument.

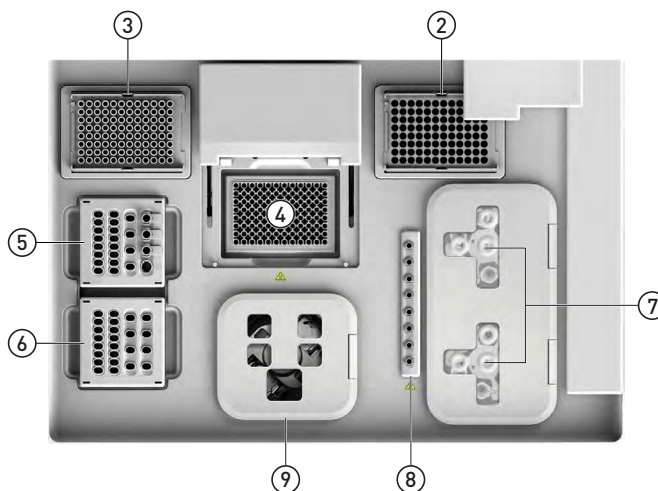
IMPORTANT! Prior to use, gently tap the IC Reagents and the IC Solutions Cartridges on the bench to force the reagents to the bottoms of the tubes.


Load the Ion Chef™ System

IMPORTANT! Rated centrifuge speeds are only intended for operation with provided buckets and approved consumable chips, tubes, and sample preparation reagents.

IMPORTANT! The Chip-loading Centrifuge is rated to operate at the listed rotational frequencies with the chip buckets, chips, and adapters. The centrifuge must be load balanced. Proper care must be taken to load the bucket properly. If excessive vibrations arise, check to ensure that items are installed properly and rotors are equally balanced on each side.

Follow the procedure below to load the Ion Chef™ Instrument, where each step corresponds to a numbered area in the following diagram.

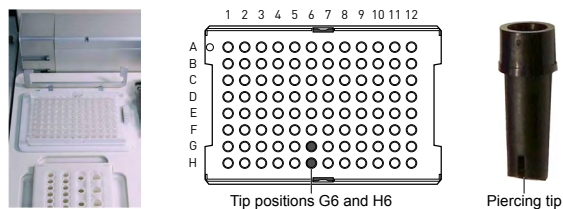


1. Open the instrument door:
 - a. In the instrument touchscreen, touch  (Open Door) then wait for the latch to open.
 - b. Lift the instrument door to the top of the travel until the latch mechanism engages.
2. Load an empty pipette tip rack to the *Used (Waste)* Pipette Tip Position, then change gloves.

IMPORTANT! To prevent contamination, change gloves immediately after moving the empty pipette tip rack to the *Used (Waste)* Pipette Tip Position.

3. Load a Ion Chef™ IC 200 Tip Cartridge to the *New Pipette Tip Position*:

- a. Unwrap the Ion Chef™ IC 200 Tip Cartridge and remove the cover to expose the pipette tips. Load two new Ion Chef™ IC Piercing Tips into tip positions G6 and H6 on the Ion Chef™ IC 200 Tip Cartridge (numbering the positions from left to right and lettering from top to bottom).

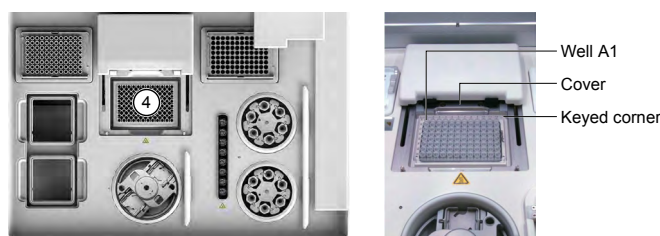


- b. Pull the catch forward, then pivot the locking bracket upwards. Load the assembled Ion Chef™ IC 200 Tip Cartridge into the *New Pipette Tip Position*, then pull the bracket downwards to lock the cartridge in place.



4. Load a new PCR plate into the thermal cycler sample block, then slide a new PCR Plate Seal underneath the automated heated cover.

IMPORTANT! When the PCR Plate Seal is positioned correctly, its tabs project upward and contact the heated cover.



5. Load the Ion PGM™ IC Reagents 200 Cartridge and diluted libraries into the Reagents station:

IMPORTANT! Thaw the IC Reagents Cartridge at room temperature for 45 minutes prior to use.

- a. Gently tap the Ion PGM™ IC Reagents 200 Cartridge on the bench to force the reagents to the bottoms of the tubes.

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

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

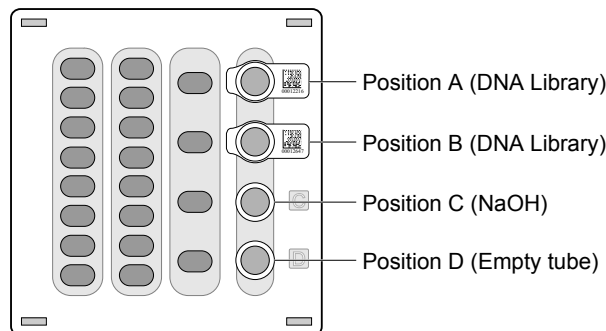


- c. Uncap and load the two Library Sample Tubes, each containing 70 µL of diluted library, into Positions A and B on the IC Reagents Cartridge.

IMPORTANT! Make sure to orient the sample tubes so that the barcodes are visible and oriented to the right (see below).

IMPORTANT! Make sure to remove the caps to the Library Sample Tubes before proceeding.

- d. Uncap both the tube of NaOH in Position C and the empty tube in Position D on the IC Reagents Cartridge.



6. Load the Ion PGM™ IC Solutions 200 Cartridge to the Solutions station:

- a. Gently tap the Ion PGM™ IC Solutions 200 Cartridge on the bench to force the reagents to the bottoms of the tubes.

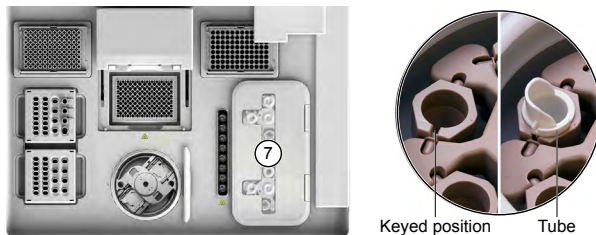
- b. Load the IC Solutions Cartridge into the Solutions station until it snaps into place and is level on the deck.



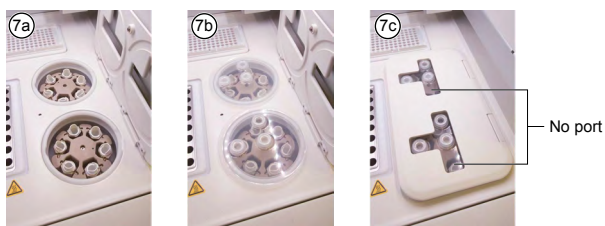
7. Load the consumables into the Recovery centrifuges:

- a. Load six Recovery Tubes into each Recovery Centrifuge.

IMPORTANT! Confirm that the Recovery Tubes are seated correctly within the centrifuge buckets. The buckets are keyed to ensure that the tubes fit in a specific orientation.



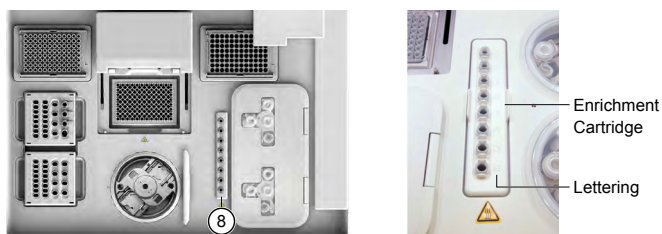
- b. Place a Recovery Station Lid over each centrifuge. Orient the lids so that the ports are located as shown in the figure below.
- c. Close the lid of the Recovery Centrifuges. Confirm that no ports are located in the positions facing the front of the instrument.



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

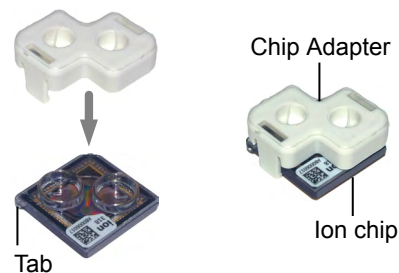
8. Load the Enrichment Cartridge, then press down on the cartridge to ensure that it is level with the instrument deck.

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

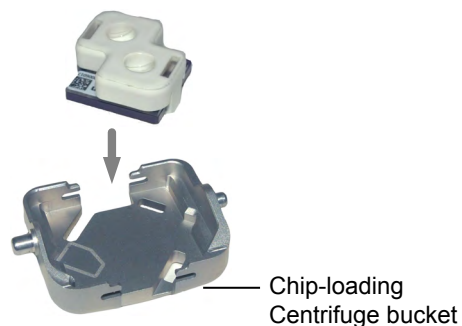


9. Load the Chip-loading Centrifuge:

- a. Attach Chip Adapters to the chips.



- b. Place the adapter/chip assemblies into centrifuge buckets so that the chip barcode aligns above the white outline imprinted on the floor of the bucket.



- c. Load the adapter/chip/bucket assemblies into the Chip-loading Centrifuge.



Note: Chip position A is 90° clockwise from the Position A marker hole. The chip loaded in this position will be loaded with ISPs prepared from the library loaded in Position A of the IC Reagents Cartridge. The chip loaded in Position B will be loaded with ISPs prepared from the library loaded in Position B of the IC Reagents Cartridge.

- d. Close the lid of the Chip-loading Centrifuge.

10. Confirm that all cartridges and reagents are installed correctly before continuing:
 - Confirm that each cartridge is at the correct location and in the correct orientation.
 - Press down on all cartridges to confirm that they are firmly locked in place.
 - Confirm that all tubes on the Ion PGM™ IC Reagents 200 Cartridge are uncapped and firmly locked in place.
 - Confirm that the centrifuge lids are installed correctly so that the ports are oriented toward the rear of the instrument.
 - Confirm that the tube and chip buckets are seated securely within the rotor arms of the Chip-loading and Recovery Centrifuges, and that the consumables they contain are correctly installed.

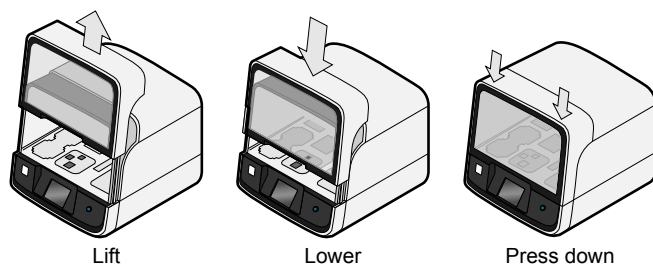


CAUTION! To ensure correct and safe instrument operation, you must confirm that all consumables are installed correctly to the deck before you start a run. The Ion Chef™ Instrument does not verify all aspects of the consumable setup prior to beginning each run.

Start the run


1. Confirm that you have loaded the instrument with all kits and consumables.
2. On the Ion Chef™ Instrument home touchscreen, touch **Set up run**.
3. Touch **Step by Step** to have the instrument lead you through the instrument setup, or touch **Quick Start** to skip the instrument setup screens.
4. Follow the on-screen instructions. When prompted, close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage. After the door closes, the instrument vision system activates.

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



5. When prompted, touch **Start check** to begin Deck Scan. Wait while the instrument scans the barcodes of all consumables and reagents to confirm their presence and compatibility.

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

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

IMPORTANT! If the kit name and chip type are incorrect, confirm that you are using the correct kit and chip. If you are using the correct kit and chip, contact Technical Support.

8. On the Run Options screen, touch the appropriate option to complete the run and enter the desired time of run completion, if necessary.
 9. On the Run Options screen, touch **Start run** to begin the run.
 10. Clean and initialize the Ion PGM™ Sequencer approximately 1.5 hours before the Ion Chef™ System finishes chip loading.
 11. If you chose to pause the run to analyze the templating efficiency, remove the samples for testing when prompted to do so by the Ion Chef™ Instrument (approximately 9.5 hours after the start of the run).
 - a. When prompted to remove the QC sample, open the instrument door.
 - b. Transfer the unenriched QC samples (entire volume) from Positions A and B of the Ion PGM™ IC Reagents 200 Cartridge on the instrument deck to two new labeled microcentrifuge tubes.
 - c. Analyze the QC samples.
 - d. Close the instrument door, then touch **Continue** to complete the run.
 12. When the run is complete, unload the Ion Chef™ Instrument and sequence the chips immediately.
- Note:** If you are performing quality assessment of enriched samples, transfer QC samples from positions A and E of the

Enrichment Cartridge to two new labeled microcentrifuge tubes. Refer to Appendix B, "Supplementary procedures", of the *Ion PGM™ IC 200 Kit User Guide* (Pub. no. MAN0007661) for more information.

Clean and initialize the Ion PGM™ Sequencer

At least one hour before the completion of the Ion Chef™ Instrument run, clean and initialize the Ion PGM™ Sequencer.

IMPORTANT! Use only the specified materials and follow the protocols found in this document. The Ion PGM™ Sequencer cleaning and initialization procedures described here are *similar* to that of other Ion sequencing kits, but the materials and protocols are not identical. Do not substitute reagents from other kits.

Before starting

- **Weekly:** Prepare a stock of 1 M NaOH by diluting 10 M NaOH with 18 MΩ water.
- **Daily:** Prepare 100 mM NaOH by diluting the 1 M stock in 18 MΩ water. You will need 500 µL of 100 mM NaOH per initialization.

Clean the Ion PGM™ System

Cleaning schedule

Clean with...	Schedule
18 MΩ water	<ul style="list-style-type: none"> • Daily, when instrument is in use • After ≤1100 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

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_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) and rinse each bottle twice with ~100 mL of 18 MΩ water.
2. Fill a glass bottle with 1 L of 18 MΩ water and add an Ion PGM™ Cleaning Tablet (chlorite tablet). Allow the tablet to completely dissolve (~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, and select the **Chlorite cleaning** checkbox. Press **Next**.
5. 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.

6. Remove all wash and reagent bottles 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. Make sure 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, and 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 begin 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 and attach the bottle in the W1 position. Make sure the cap is tight. Press **Next** to begin 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 and proceed to initialization.

Initialize the Ion PGM™ System

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 five 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.

Initialization guidelines

- For each initialization, the first run should be started within 1 hour after initialization, and the last run must be started within 27 hours after initialization.
- 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.
- After four initializations, do not use the Wash 1 or 3 Bottles for initialization or sequencing to avoid breakage or leaking. (You can reuse the bottles in the cleaning procedure.)

Note: W2 Bottles can be used up to 40 times before they must be discarded.

- Replace the Reagent Bottles and sipper tubes every time you initialize.
- Make sure that you have updated to Torrent Suite™ 4.4.2 or later.

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.
3. Note the mold line on the conditioned Wash 2 bottle. If there are two mold lines on the bottle, mark the lower line to indicate that it is the correct one.

Prepare the Wash 2 Bottle

IMPORTANT! For all the following steps, pour the 18 MΩ water directly from the purification system into the conditioned Wash 2 Bottle. Do not use water that has been collected or measured in any other containers.

1. Rinse the conditioned Wash 2 Bottle (2 L) three times with 200 mL of 18 MΩ water.
2. If your 18 MΩ water system has a spigot, extend the water spigot into **but not below** the neck of the Wash 2 Bottle.
3. Fill the bottle to the mold line. Volume of water will be ~2 liters.



4. Add the entire bottle of Ion PGM™ IC Sequencing W2 Solution to the Wash 2 Bottle.
5. Add 70 µL of freshly prepared 100 mM NaOH solution (*not* 1 M NaOH) to the Wash 2 Bottle.

6. Cap the bottle and invert five times to mix, and immediately proceed through the rest 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 three 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 and cap the bottle.
3. **Wash 3 Bottle:** Add Ion PGM™ IC Sequencing W3 Solution to the 50-mL line marked on the Wash 3 Bottle and cap the bottle.

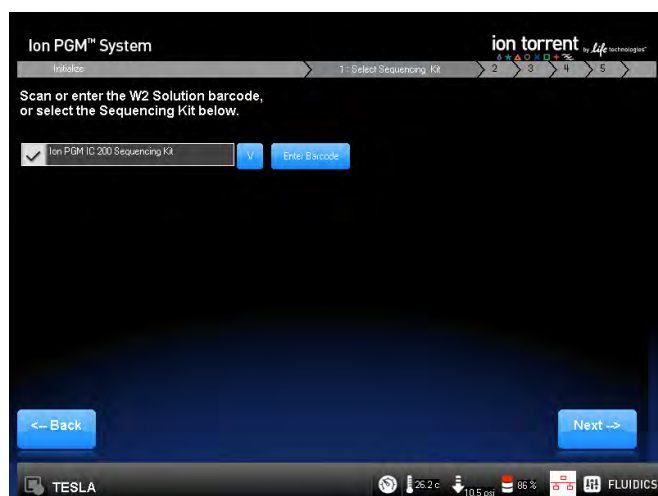
Begin the initialization

IMPORTANT! Do not remove the old sipper tubes from the dNTP ports until instructed to do so. **Do not let the new sipper tubes touch any surfaces.**

IMPORTANT! Load the bottles as quickly as possible to prevent atmospheric CO₂ from reducing the pH of the Wash 2 Bottle solution.

1. Confirm that the chip used to clean the Ion PGM™ System is still in place on the instrument.
2. On the main menu, press **Initialize**.
3. In the next screen, select **Ion PGM™ IC 200 Sequencing Kit** from the drop-down list, then press **Next**.

IMPORTANT! Do *not* select any other sequencing kit from the drop-down list. Be careful to select the correct kit type to ensure proper pH adjustment.



4. Press **Next** and confirm that the cleaning chip is on the instrument and the Reagent Bottle sipper tubes and collection trays are in place. Press **Next** again.
5. The system will verify the gas pressure. If the gas pressure is sufficient, press **Next** to begin the initialization. If the gas pressure is low, press **Yes** to retry gas-pressure verification. If the gas pressure remains low, contact Technical Support.

6. Wearing clean gloves, firmly attach a new sipper tube (long gray) to the cap in the W2 position. New sipper attachments are push-on (shown below), whereas older models may be threaded. **Do not let the sipper touch any surfaces.**

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



7. Immediately attach the prepared Wash 2 Bottle in the W2 position and tighten the cap. Press **Next**.
8. Change gloves and firmly install new sipper tubes (short gray) in the caps in the W1 and W3 positions.

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

9. Immediately attach the prepared Wash 1 and 3 Bottles and tighten the caps. Press **Next**.
10. The Ion PGM™ System will test the bottles for leaks, fill the Wash 1 Bottle, and then adjust the pH of the W2 Solution. This procedure takes ~30 minutes.

Prepare the 50-mL Reagent Bottles with dNTP solutions

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

1. After each dNTP stock solution has thawed, vortex to mix and centrifuge to collect the contents. Keep dNTP stock solutions on ice throughout this procedure.
2. Use the labels provided with the kit to label four new Reagent Bottles as dGTP, dCTP, dATP, and dTTP.
3. Using filtered pipette tips and clean gloves, carefully transfer 20 µL of each dNTP stock solution into its respective Reagent Bottle.

Attach the sipper tubes and Reagent Bottles


1. After the wash solutions have initialized (from "Begin the initialization" on page 8), 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," as shown below) and tighten firmly by hand until snug. Press **Next**.
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 the troubleshooting section of the user guide.
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.

Start the sequencing run

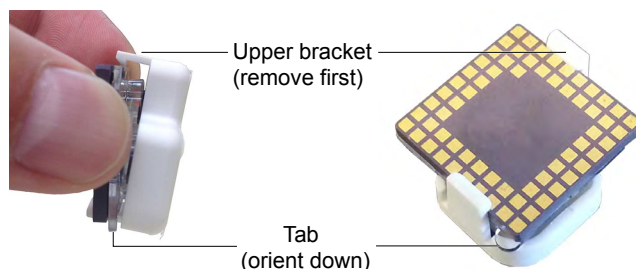
Unload and prepare the chips for sequencing

1. Open the instrument door:
 - a. In the instrument touchscreen, touch  (Open Door) then wait for the latch to open.
 - b. Lift the instrument door to the top of the travel until the latch mechanism engages.
2. Open the lid of the Chip-loading Centrifuge, then gently remove the chip/bucket assemblies from the instrument.

IMPORTANT! When removing each chip, be careful not to disturb the residual liquid in the outer port of the Chip Adapter.



3. Remove the chip from the centrifuge bucket, then remove the Chip Adapters from the chips and discard them. To remove an adapter, hold the chip with the tab facing downward, then remove the Chip Adapter starting with the top hinge first.



4. Use an Ion Chip™ Minifuge to remove the residual liquid from the chips:
 - a. Load both chips into the Ion Chip™ Minifuge. Place each chip upside-down within a bucket so that the tab faces inwards, toward the center.

IMPORTANT! Before closing the centrifuge lid, confirm that the chips are centered within the buckets to ensure that the centrifuge is completely balanced.



- b. Close the lid and centrifuge the chips for 5 seconds.
 - c. When the centrifuge stops, remove the chips, then use a lint-free wipe to remove any liquid from the buckets.
5. Close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage.

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

6. Load one or both chips into Ion PGM™ Sequencers and promptly begin the sequencing runs.

If you cannot sequence a loaded chip immediately, place the chip into a separate chip storage container *after* centrifugation and store at +4°C until you are ready to run it (up to 4.5 hours maximum).

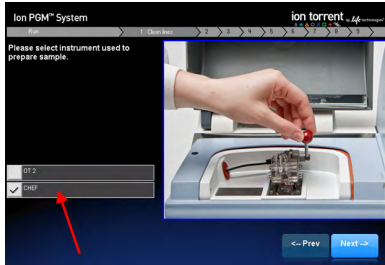
IMPORTANT! If you choose to store a loaded chip:

- Centrifuge the chip *before* you store it.
 - At least 20 minutes before you intend to run the stored chip, remove the chip from the container, and place it on a clean surface in the dark to warm to room temperature.
-

Sequence the Ion chips on the Ion PGM™ Sequencer

Sequence the loaded chips on the Ion PGM™ Sequencer as soon as possible after unloading the Ion Chef™ Instrument.

1. Touch **Run** on the main menu, then follow the on-screen instructions to empty the waste bottle, load the cleaning chip, and clean the Ion PGM™ Sequencer fluid lines.
2. When the following screen appears, touch **CHEF** to select the instrument used to prepare the sample and initiate the Chef sequencing workflow. Then touch **Next**.



3. Scan the barcode on the loaded chip, or enter the barcode manually.
4. When prompted by the instrument, ground yourself by touching the grounding plate next to the chip clamp on the instrument, replace the cleaning chip in the chip socket with the chip to be sequenced, close the chip clamp, and touch **Next**.

IMPORTANT! Do not wear gloves when transferring the chips on and off the instrument.



5. Touch **Chip Check** to perform the first chip check.
6. After the instrument successfully completes the chip check, follow the on-screen instructions to empty the waste bottle, then touch **Next**.

IMPORTANT! Ion PGM™ Sequencer – Blue sequencing requires use of the Ion PGM™ 2.5-L Waste Bottle. Be sure to completely empty and return the waste container before each run.

7. When prompted to select a Planned Run, confirm that the correct run is displayed, then touch **Next**.

8. When run information is displayed, confirm that the run details are correct, then touch **Next**. The instrument will perform a second chip check and calibration.


During the initial part of Chip Check, visually inspect the chip in the clamp for leaks. If there is a leak, press **Abort** immediately to stop the flow to the chip. When the calibration is complete (~1 minute), the touchscreen indicates the calibration status.

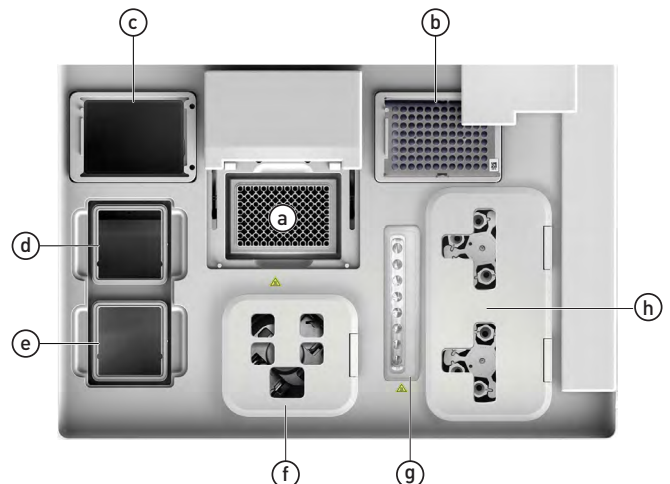
- If the chip *passes* calibration, touch **Next** to begin the run.
- If the chip *fails* calibration, touch **Abort**, reseal the chip, then touch **Calibrate** to recalibrate. If the chip fails calibration again, proceed with the run and contact Technical Support after the run is complete.

9. Twenty minutes before the end of the first run, remove the remaining chip from the chip container in the refrigerator, and place it on a clean surface to warm to room temperature.
10. When first run is complete, sequence the remaining chip as soon as possible. Perform a cleaning and/or initialization if required.

Clean the instrument

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

1. Open the instrument door:
 - a. In the instrument touchscreen, touch  (Open Door) then wait for the latch to open.
 - b. Lift the instrument door to the top of the travel until the latch mechanism engages.
2. Remove and dispose of any used consumables from the instrument.



- a. Remove and discard the PCR plate from the PCR sample block.

- b. Remove and discard the box of used pipette tips from the waste tip position.

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

- c. Move the empty Tip Cartridge to the waste tip position.

IMPORTANT! Do not discard the empty Tip Cartridge.

- d. Remove and discard the Ion PGM™ IC Reagents 200 Cartridge.

IMPORTANT! Make sure to transfer the QC samples before you remove and discard the IC Reagents Cartridge.

- e. Remove and discard the Ion PGM™ IC Solutions 200 Cartridge.
- f. Close the lid of the Chip-loading Centrifuge.
- g. Remove and discard the Enrichment Cartridge.
- h. Remove and discard the consumables from the Recovery Centrifuges, including the:
- Recovery Station Lids
 - Recovery Tubes
3. Inspect the Recovery Centrifuge for residue. If excessive liquid is present, clean the centrifuge bowl and buckets as described in the *Ion PGM™ IC 200 Kit User Guide* (Pub. no. MAN0007661). Close the Recovery Centrifuge lid.

IMPORTANT! Clean the Recovery Centrifuge occasionally, only when excess liquid is noticeable in the bowl and/or buckets. You do not need to clean the centrifuge after every run.

4. Close the instrument door by first lifting it slightly to disengage the locking mechanism, then pushing down on the door until the locks engage.
5. From the Ion Chef™ Instrument touchscreen, begin the cleaning:
- a. On the screen that appears after run completion, touch **Next**.
- Note:** You may also clean the instrument at any time starting from the home touchscreen. Touch **Settings**, then touch **Clean Ion Chef**.
- b. Confirm that you have removed all consumables from the Ion Chef™ Instrument, except for the empty Tip Cartridge in the waste tip position, then touch **Next**.

- c. With the door closed, touch **Start**. The instrument performs a load check before starting the cleaning routine. The Ion Chef™ Instrument stops ventilation and illuminates the ultraviolet (UV) light within the instrument.



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

Kits, consumables, and accessories

Catalog or Part no.	Description
4484080	Ion PGM™ IC 200 Kit, 8-reactions
4488812	Ion PGM™ IC 200 Starter Kit, 8-reactions ^[1]
4488374	Ion Chef™ Installation Kit
4488144	Ion 314™ Chip Kit v2 BC, 8-pack
4488145	Ion 316™ Chip Kit v2 BC, 4-pack
4488149	Ion 316™ Chip Kit v2 BC, 8-pack
4488146	Ion 318™ Chip Kit v2 BC, 4-pack
4488150	Ion 318™ Chip Kit v2 BC, 8-pack
A25043	Wash 2 Bottle Conditioning Solution

^[1] Includes the Ion PGM™ Controls Kit.

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

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