

Ion PGM™ Hi-Q™ OT2 Kit

Catalog Number A27739

Pub. No. MAN0010903 Rev. B.0

Note: For safety and biohazard guidelines, see the “Safety” appendix in the *Ion PGM™ Hi-Q™ OT2 Kit User Guide* (Pub. No. MAN0010902). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

IMPORTANT! If you are using the Ion OneTouch™ Dx System with Torrent Suite™ Assay Development Software^[1], see the *Ion PGM™ Hi-Q™ OT2 Kit—Assay Development Mode User Guide* (Pub. No. MAN0015793).

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Run the Ion OneTouch™ 2 Instrument

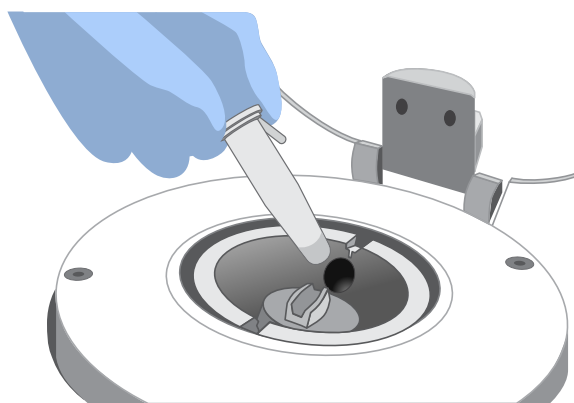
IMPORTANT! Ensure that the latest firmware is installed. To update the firmware to the current version, see the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388).

1. Close the centrifuge lid, then touch **Run** on the home screen.
2. Touch the dropdown menu, then select **PGM: Ion PGM™ Hi-Q™ OT2 Kit - 200**, or **PGM: Ion PGM™ Hi-Q™ OT2 Kit - 400**, depending on the read length of your library. Then touch **Next**.
3. Touch **Assisted**, then complete the setup tasks listed onscreen, or touch **Expert**, then set up the instrument according to the following instructions.

Set up the Ion OneTouch™ 2 Instrument

Install the Ion OneTouch™ Recovery Tubes and Ion OneTouch™ Recovery Router

1. Dispense 150 µL Ion OneTouch™ Breaking Solution into each of two Ion OneTouch™ Recovery Tubes.
2. Insert the Recovery Tubes containing Ion OneTouch™ Breaking Solution into the two centrifuge positions.



3. Install the Ion OneTouch™ Recovery Router into the center slot of the centrifuge.
4. Close the centrifuge lid.

Install the Ion OneTouch™ Amplification Plate

Remove the used Cleaning Adapter, insert the plate, and pull the handle to close the heat block. Thread the disposable tubing through the catch and pinch valve.

CAUTION! Hot Surface. Use care when working around this area to avoid being burned by hot components.

WARNING! Safety Hazard. Do not use the instrument with flammable or explosive materials. Use only the materials specified for use with the instrument to ensure safety.

CAUTION! PHYSICAL INJURY HAZARD. The pointed end of the disposable injector can puncture your skin. Keep your hand away from the point of the disposable injector.



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

Install the disposable injector

Insert the disposable injector, then confirm automatic placement of the disposable injector above the router by briefly pressing then releasing the spring-loaded top of the Injector Hub.

You should hear a click.

Install the Reagent Tubes

1. Install the Ion OneTouch™ Oil on the left front port . Invert the Ion OneTouch™ Oil bottle (450-mL size) 3 times, then fill the Reagent Tube half-full with Oil. Install the Reagent Tube. Minimize bubbles.
2. Install the Ion OneTouch™ Recovery Solution on the right front port . Invert the bottle of Recovery Solution 3 times, then fill the Reagent Tube one quarter-full with Recovery Solution. Install the Reagent Tube. Minimize bubbles.

Empty the waste container

Appropriately dispose of waste.

Prepare the amplification solution

1. Prepare the reagents as follows:

Reagents	Preparation
Ion PGM™ Hi-Q™ Reagent Mix	<ol style="list-style-type: none">1. Allow the tube of reagent mix to come to room temperature before use.2. Vortex the solution for 30 seconds, then centrifuge the solution for 2 seconds.3. Keep the reagent mix at room temperature during use. Store unused thawed reagent mix at 2 °C to 8 °C.
Ion PGM™ Hi-Q™ Enzyme Mix	Centrifuge the enzyme for 2 seconds and place on ice.
Ion PGM™ Hi-Q™ ISPs	Place the suspension at room temperature.

2. Depending on your library type and concentration, dilute the library as shown in the following table. Use the library dilution within 48 hours of preparation.

	Ion AmpliSeq™ DNA Library	Ion AmpliSeq™ RNA Library	gDNA Fragment or Amplicon Library	Ion Total RNA-Seq Library
Library concentration	100 pM	100 pM	100 pM	100 pM
Volume of library	2 µL	4 µL	6.5 µL	5 µL
Volume of Nuclease-free Water	23 µL	21 µL	18.5 µL	20 µL
Total volume of diluted library to add to the amplification solution	25 µL	25 µL	25 µL	25 µL

- a. Vortex the diluted library for 5 seconds, then centrifuge for 2 seconds.
 - b. Place the diluted library on ice.
3. Prepare the Ion PGM™ Hi-Q™ ISPs:
 - a. Vortex the ISPs at maximum speed for 1 minute to resuspend the particles.
 - b. Centrifuge the ISPs for 2 seconds.
 - c. Pipet the ISPs up and down to mix.
 - d. *Immediately* proceed to the next step.
 4. To a 2-mL tube (violet cap) containing 800 µL of Ion PGM™ Hi-Q™ Reagent Mix, add the following components in the designated order. Add each component, then pipet the amplification solution up and down to mix:

Order	Reagent	Cap color	Volume
1	Nuclease-free Water	—	25 µL
2	Ion PGM™ Hi-Q™ Enzyme Mix	Brown	50 µL
3	Diluted library (<i>not</i> stock library)	—	25 µL
4	Ion PGM™ Hi-Q™ ISPs	Black	100 µL
—	Total	—	1,000 µL

IMPORTANT! If you are performing a *de novo* sequencing experiment that does not include a reference BAM file, add 10 µL of the Ion PGM™ Calibration Standard (Cat. No. A27832) to the amplification solution, and reduce the volume of Nuclease-free Water added to 15 µL. When creating your Planned Run in the Torrent Browser, select **Enable Calibration Standard** from the **Base Calibration Mode** dropdown list in the **Kits** tab.

5. Vortex the complete amplification solution prepared in step 4 at maximum speed for 5 seconds.

Proceed *immediately* to "Fill and install the Ion OneTouch™ Reaction Filter.

Fill and install the Ion OneTouch™ Reaction Filter

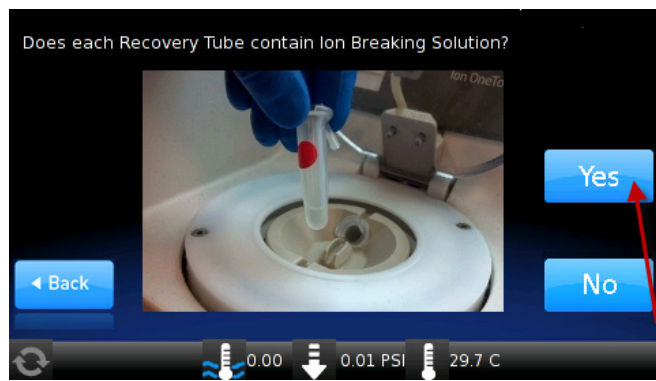
IMPORTANT! We recommend filling the Ion OneTouch™ Reaction Filter in a room dedicated to pre-PCR activities or a controlled pre-PCR hood. Do not use a reaction filter assembly from any other template preparation kit.

1. Pipet 1000 µL of the amplification solution, prepared in the previous procedure, through the sample port of the Ion OneTouch™ Reaction Filter.
2. Pipet 850 µL of Ion OneTouch™ Reaction Oil (25-mL size) through the sample port.
3. Change the tip and pipet an additional 850 µL of Ion OneTouch™ Reaction Oil through the sample port.
4. Invert then install the filled Ion OneTouch™ Reaction Filter into the three holes on the top stage of the Ion OneTouch™ 2 Instrument .

Start the run

1. After installing the Ion OneTouch™ Reaction Filter, touch **Next** to start the run.

IMPORTANT! Remember to add 150 µL of Ion OneTouch™ Breaking Solution to each Recovery Tube before starting the run. On the reminder screen, confirm and touch **Yes** to advance:



Note: To cancel a run, touch **Abort**, then touch **Yes**.

2. Remove the samples ≤16 hours after starting the run. If you touched **Next** on the centrifuge screen to centrifuge samples at the end of the run, proceed *immediately* to "Recover the template-positive ISPs".

Recover the template-positive ISPs

1. At the end of the run, follow the screen prompts to centrifuge the sample.

IMPORTANT! If you removed the Reaction Tubes at the end of the run *before* the Ion OneTouch™ 2 Instrument had centrifuged the sample or have not processed the sample after 15 minutes, centrifuge the sample on the instrument. On the home screen of the instrument, touch **Open Lid**, touch **Final Spin**, then follow the screen prompts to centrifuge the sample.



CAUTION! ROTATION HAZARD. Wait until rotation stops before opening. Rotating parts can cause injury.

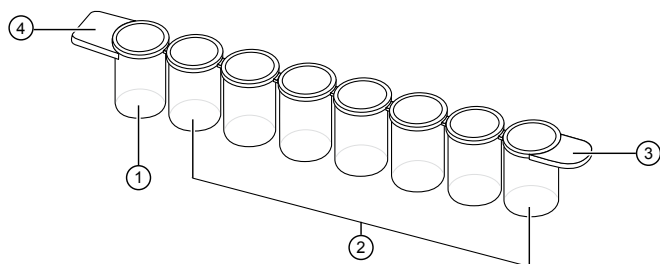
2. Immediately after the centrifuge has stopped, on the instrument display, touch **Open Lid**. Wait until the lid clicks open, then remove and discard the Ion OneTouch™ Recovery Router.
3. Remove both Ion OneTouch™ Recovery Tubes from the instrument, then put the two Recovery Tubes in a tube rack.
4. Remove all but 100 µL of the Recovery Solution from each Recovery Tube.

STOPPING POINT Add 500 µL of Ion OneTouch™ Wash Solution to each Recovery Tube and pipet up and down to disperse the ISPs. Combine the suspension from each Recovery Tube into one new labeled 1.5-mL Eppendorf LoBind™ Tube. Store the ISPs at 2°C to 8°C for up to 3 days. Before enrichment, centrifuge the ISPs at 15,500 × g for 2.5 minutes. Carefully remove all but 100 µL of supernatant. With a new tip, pipet up and down to resuspend the ISPs. Proceed to step 6.

5. Process the ISPs:
 - a. Add 500 µL of Ion OneTouch™ Wash Solution to each Recovery Tube.
 - b. Pipet the ISPs up and down to disperse the ISPs, then combine the suspension from each Recovery Tube into one new labeled 1.5-mL Eppendorf LoBind™ Tube.

Note: If a precipitate is present, incubate the tube at 50°C for 2 minutes to dissolve.
 - c. Centrifuge the ISPs for 2.5 minutes at 15,500 × g.
 - d. Use a pipette to remove all but 100 µL of the Wash Solution from the tube. Withdraw the supernatant from the surface and on the opposite side from the pellet.

- Obtain an 8-well strip from the Ion OneTouch™ ES Supplies Kit. Ensure that the square-shaped tab of an 8-well strip is on the left:



- ① Well 1
- ② Wells 2–8
- ③ Rounded tab
- ④ Square-shaped tab

- Pipet the ISPs up and down ten times to mix, then transfer the suspension into Well 1 of the 8-well strip.
- Retain an aliquot of the unenriched Ion PGM™ Hi-Q™ OT2 Kit from Well 1 for quality assessment. Assess the quality of the unenriched, template-positive ISPs using one of the following instruments:
Qubit™ 2.0 or Qubit™ 3.0 Fluorometer, Guava™ easyCyte™ 5 Flow Cytometer, or the Attune™ NxT Acoustic Focusing Cytometer.

Enrich the template-positive ISPs with the Ion OneTouch™ ES". You may start the enrichment procedure while the Ion OneTouch™ 2 Instrument cleaning is in progress.

IMPORTANT! Do not store the recovered, template-positive ISPs at –30°C to –10°C. Do not store the ISPs in Ion PGM™ OT2 Recovery Solution (see step 4 of this procedure).

Clean the Ion OneTouch™ 2 Instrument

IMPORTANT! To ensure continued safe operation, visually inspect the rotor assembly and casing periodically to ensure that there are no signs of cracks or other physical damage. Follow the cleaning procedure in this section to clean the Ion OneTouch™ 2 Instrument with the Ion OneTouch™ Cleaning Adapter. *Perform the cleaning procedure after every run.*

- Determine the appropriate reagents to use for maintaining the Ion OneTouch™ 2 Instrument:

If you are	Action
Switching to the Ion PGM™ Hi-Q™ OT2 Kit from another kit?	See Chapter 5 of the <i>Ion OneTouch™ 2 System User Guide</i> (Pub. No. MAN0014388). Use the reagents from the appropriate kit to maintain the Ion OneTouch™ 2 Instrument.
Already using the Ion PGM™ Hi-Q™ OT2 Kit.	Proceed to step 2. Continue to use the reagents that are provided in the Ion PGM™ Hi-Q™ OT2 Kit.

- Check the level of Ion OneTouch™ Oil in the Reagent Tube. If the Reagent Tube has <20 mL of Oil, pour Oil into the Reagent Tube until it is half-full.

- Remove and appropriately discard the used Ion OneTouch™ Reaction Filter. Remove the assembly from the instrument by grasping the *filter*.
- Firmly insert the 3 ports of a new *single-use* Cleaning Adapter into the three holes on the top stage of the Ion OneTouch™ 2 Instrument.
- Place a 50-mL conical tube in a tube rack, then place the tube rack next to the instrument.
Note: Steps 6–9 are only necessary if you have not already removed the disposable injector before removing the Recovery Tubes from the instrument.
- Gently pull the disposable tubing downwards on both sides of the pinch valve until the disposable tubing is out of the valve.
- Remove the disposable injector from the Ion OneTouch™ DL Injector Hub.



CAUTION! PHYSICAL INJURY HAZARD. The pointed end of the disposable injector can puncture your skin. Keep your hand away from the point of the disposable injector.

- Place the used, disposable injector into the empty 50-mL conical tube in the tube rack. The conical tube is used to collect waste.
- On the home screen of the instrument, touch **Clean**.
- Complete each task that is displayed on the screen, then touch **Next**. After you touch **Next** on the last task, a progress bar appears, and the cleaning starts.
- At the end of the cleaning run, the screen displays "**Time Remaining 00:00:00, Cleaning Run Complete**". Press **Next**, then ensure that the task in bold displays: "**Remove plate, injector, conical tube, and waste**".
Note: Keep the used Cleaning Adapter on the instrument between runs.
- Appropriately discard the waste that was collected in the 50-mL conical tube.
- Remove and appropriately discard the used Amplification Plate, disposable injector, and tubing.



CAUTION! Hot Surface. Use care when working near this area to avoid injury from contact with hot components.

- On the instrument display, touch **Open Lid**, wait until the lid clicks open, then open the centrifuge lid. Wipe the residue from the centrifuge lid with dry Kimwipes™ disposable wipes, then close the centrifuge lid.
- Touch **Next** to return to the home screen on the instrument.

Enrich the template-positive Ion PGM™ Hi-Q™ ISPs

Determine if a residual volume test is necessary

Condition	Action
First use of the instrument and during monthly maintenance	Perform a residual volume test (see "Ion OneTouch™ ES Instrument installation, setup, and maintenance" in the <i>Ion OneTouch™ 2 System User Guide</i> Pub. No. MAN0014388).
Routine use and residual volume in Well 1 and Well 8 is >5.0 µL	
Routine use and residual volume in Well 1 and Well 8 is ≤5.0 µL	Operate the instrument without performing the residual volume test. Proceed to "Prepare reagents then fill the 8-well strip".

Prepare reagents then fill the 8-well strip

Prepare Melt-Off Solution

Prepare fresh Melt-Off Solution by combining the components in the following order:

Order	Component	Volume
1	Tween™ Solution	280 µL
2	1 M NaOH	40 µL
—	Total	320 µL

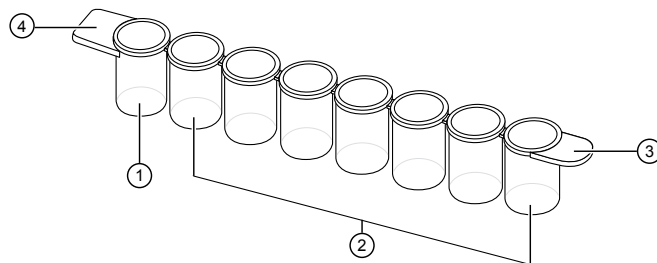
IMPORTANT! Prepare Melt-Off Solution as needed, but appropriately dispose of the solution after 1 day.

Wash and resuspend the Dynabeads™ MyOne™ Streptavidin C1 Beads

- Vortex the tube of Dynabeads™ MyOne™ Streptavidin C1 Beads for 30 seconds to resuspend the beads thoroughly, then centrifuge the tube for 2 seconds.
- Open the tube, then use a new tip to pipet the dark pellet of beads up and down until the pellet disperses. Immediately proceed to the next step.
- Transfer 13 µL of Dynabeads™ MyOne™ Streptavidin C1 Beads to a new 1.5-mL Eppendorf LoBind™ Tube.
- Place the tube on a magnet such as a DynaMag™-2 magnet for 2 minutes, then carefully remove and discard the supernatant without disturbing the pellet of Dynabeads™ MyOne™ Streptavidin C1 Beads.
- Add 130 µL of MyOne™ Beads Wash Solution to the Dynabeads™ MyOne™ Streptavidin C1 Beads.
- Remove the tube from the magnet, vortex the tube for 30 seconds, then centrifuge for 2 seconds.

Fill the 8-well strip

- Ensure that the template-positive ISPs from the Ion OneTouch™ 2 Instrument are in 100 µL of Ion OneTouch™ Wash Solution and are loaded in Well 1 of the 8-well strip (see "Recover the template-positive ISPs" on page 3). Well 1 with the ISPs is on the *left*:



- ① Well 1
- ② Wells 2–8
- ③ Rounded tab
- ④ Square-shaped tab

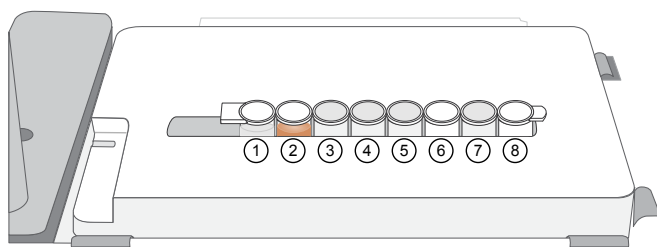
Note: If the template-positive ISPs were stored at 2°C to 8°C, centrifuge the ISPs at 15,500 × g for 2.5 minutes, then carefully remove all but 100 µL of supernatant. With a new tip, pipet up and down to resuspend the ISPs. Transfer the suspension from the tube into Well 1 of the 8-well strip.

- If you have not done so already, assess the quality of the unenriched, template-positive ISPs using the Qubit™ 2.0 or Qubit™ 3.0 Fluorometer, the Guava™ easyCyte™ 5 Flow Cytometer, or the Attune™ NxT Acoustic Focusing Cytometer.
- Note:** See the *Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide* (Pub. No. MAN0016387), or the *Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide* (Pub. No. MAN0016388) for detailed procedures for using the Ion Sphere™ Assay for determining the percentage of template-positive ISPs in unenriched samples.
- Fill the remaining wells in the 8-well strip as follows (see the figure in step 4):

Well number	Reagent to dispense in well
Well 1 ^[1]	Entire template-positive ISP sample [100 µL; prepared in step 1 of this procedure]
Well 2	130 µL of Dynabeads™ MyOne™ Streptavidin C1 Beads resuspended in MyOne™ Beads Wash Solution] prepared in "Wash and resuspend the Dynabeads™ MyOne™ Streptavidin C1 Beads" on page 5]
Well 3	300 µL of Ion OneTouch™ Wash Solution
Well 4	300 µL of Ion OneTouch™ Wash Solution
Well 5	300 µL of Ion OneTouch™ Wash Solution
Well 6	Empty
Well 7	300 µL of freshly-prepared Melt-Off Solution [prepared in "Prepare Melt-Off Solution" on page 5]
Well 8	Empty

^[1] Well closest to the square-shaped tab

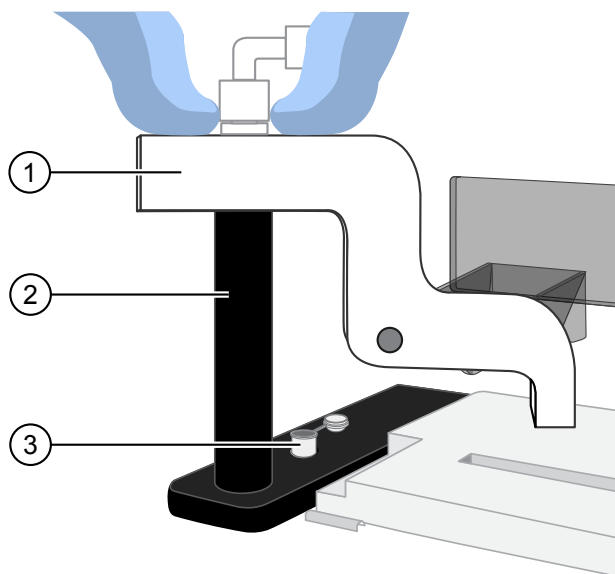
4. Confirm that the square-shaped tab is on the left, then insert the filled 8-well strip with the 8-well strip pushed all the way to the right end of the slot of the Tray:



Prepare the Ion OneTouch™ ES

Before every enrichment performed on the Ion OneTouch™ ES Instrument, install a new PCR collection tube and a new Eppendorf™ LoRetention Dualfilter P300 pipette tip.

1. Add 10 μL of Neutralization Solution to a new 0.2-mL PCR tube.
2. Insert the open 0.2-mL PCR tube containing Neutralization Solution into the hole in the base of the Tip Loader, as shown in the figure in step 4.
3. Place a new tip in the Tip Loader. Remove the Tip Arm from the cradle, then align the metal fitting of the Tip Arm with the tip.
4. Keeping the fitting on the Tip Arm vertical, firmly press the Tip Arm down onto the new tip until the Tip Arm meets the Tip Loader. Hold the Tip Arm to the Tip Loader for ~1 second to ensure proper installation of the tip.



- ① Tip Arm
- ② Tip Loader
- ③ 0.2-mL PCR collection tube containing Neutralization Solution

5. Lift the Tip Arm *straight* up to pull the installed tip from the Tip Loader tube.

6. Return the Tip Arm to the cradle.

IMPORTANT! Ensure that the back/bottom end of the Tip Arm is not resting on top of the thumb screw, causing the Tip Arm to tilt forward.

Perform the run

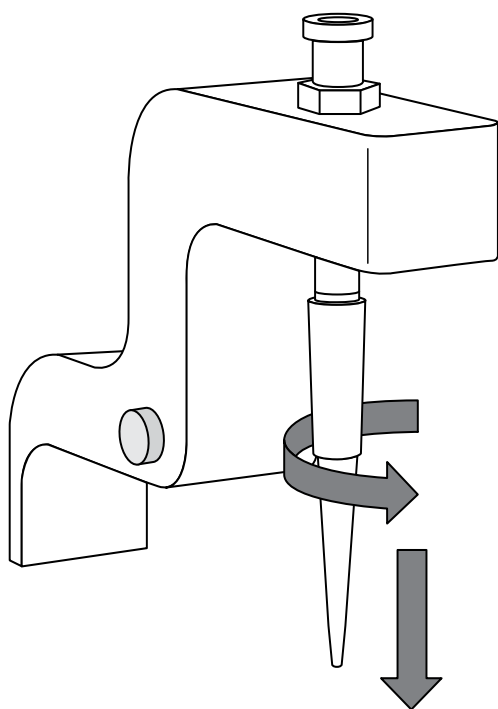
Before starting the run:

- Confirm that a new tip and open 0.2-mL PCR tube have been loaded in the Ion OneTouch™ ES Instrument, and that the 8-well strip is correctly loaded.
- Ensure that Well 1 (ISP sample) is the left-most well and the 8-well strip is pushed to the right-most position in the slot.

1. Pipet the contents of Well 2 up and down to resuspend the beads before starting the run. Do not introduce bubbles into the solution.
2. If needed, power on the Ion OneTouch™ ES Instrument, then wait for the instrument to initialize. The screen displays "rdy". The Tip Arm performs a series of initialization movements and returns to the home position (~5 seconds).
3. Press **Start/Stop**. The screen displays "run" during the run. The run takes ~35 minutes.
4. At the end of the run, the instrument displays "End" and beeps every 60 seconds. Press the **Start/Stop** button to silence this alarm, then reset the Ion OneTouch™ ES Instrument for the next run. The instrument can be left on between runs.
5. Immediately after the run, securely close, then remove the PCR tube containing the enriched ISPs.
6. Mix the contents of the PCR tube by gently inverting the tube 5 times.

Note: Ensure that the 0.2-mL PCR tube has >200 μL of solution containing the enriched ISPs. After a successful run on the instrument, the sample is in ~230 μL of Melt-Off Solution, Ion OneTouch™ Wash Solution, and Neutralization Solution. If the tube has <<200 μL of solution containing the enriched ISPs, contact Technical Support.

7. Remove the used tip: with the Tip Arm in its cradle, twist the tip counterclockwise (as viewed from above), then pull it downward to remove and discard the tip.



IMPORTANT! Improper removal of tips can loosen the metal tip adapter fitting on the Tip Arm and affect instrument operation.

8. Remove, then discard the used 8-well strip.

Sequence or store the template-positive ISPs

- Proceed to sequencing using the Ion PGM™ Hi-Q™ Sequencing Kit (Cat. No. A25592). See the *Ion PGM™ Hi-Q™ Sequencing Kit User Guide* (Pub. No. MAN0009816, Rev. C.0 or later).

or

- Store the enriched ISPs at 2°C to 8°C for up to 3 days.

Perform quality control on enriched ISPs

You can determine the enrichment efficiency using one of the following methods:

Quality assessment by	Action
Guava™ easyCyte™ 5 Flow Cytometer	Transfer a 1.0-μL aliquot of the enriched ISPs to a 1.5-mL Eppendorf LoBind™ Tube. Refer to the <i>Ion Sphere™ Particles (ISPs) Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin</i> (Pub. No. MAN0015799), available at: http://tools.thermofisher.com/content/sfs/manuals/MAN0015799.pdf
Attune™ NxT Acoustic Focusing Cytometer	Transfer a 1.0-μL aliquot of the enriched ISPs to a 1.5-mL Eppendorf LoBind™ Tube. Put the sample on ice, then refer to <i>Demonstrated Protocol: Ion Sphere™ Particles (ISPs) Quality Assessment using the Applied Biosystems™ Attune™ Acoustic Focusing Cytometer User Bulletin</i> (Pub. No. 4477181), available at: http://tools.thermofisher.com/content/sfs/manuals/4477181A.pdf

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

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Revision history: MAN0010903

Revision	Date	Description
B.0	11 January 2017	<ul style="list-style-type: none"> • Illustrations enhanced • Ion OneTouch™ ES Instrument setup clarified • Web links updated • Minor clarifications and corrections made
A.0	10 March 2015	New Quick Reference

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

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