

# Ion PGM<sup>™</sup> Hi-Q<sup>™</sup> OT2 Kit

## USER GUIDE

for use with:  
Ion OneTouch<sup>™</sup> 2 System

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

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**Revision history:** MAN0010902

Revision	Date	Description
B.0	11 January 2017	<ul style="list-style-type: none"> <li>• Ion PGM™ Calibration Standard removed from kit components list. Item is now ordered separately (Cat. No. A27832)</li> <li>• Appendices C ("Set up, calibrate, and maintain the Ion OneTouch™ ES") and D ("Supplemental procedures") deleted. Users are referred to the <i>Ion OneTouch™ 2 System User Guide</i> (Pub. No. MAN0014388) for this information</li> <li>• ISP quality control sections updated and moved to new user guides:               <ul style="list-style-type: none"> <li>– <i>Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide</i> (Pub. No. MAN0016387)</li> <li>– <i>Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide</i> (Pub. No. MAN0016388)</li> </ul> </li> <li>• Illustrations enhanced</li> <li>• Ion OneTouch™ ES Instrument setup clarified</li> <li>• Web links updated</li> <li>• Minor clarifications and corrections made</li> </ul>
A.0	10 March 2015	New User Guide, which includes instructions on how to use the Ion PGM™ Hi-Q™ OT2 Kit with the Ion OneTouch™ 2 System.

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# About this guide



**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.

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

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## Purpose

This user guide describes how to use the Ion OneTouch™ 2 System to prepare enriched, template-positive Ion PGM™ Hi-Q™ Ion Sphere™ Particles (ISPs) with 400 base-pair average insert libraries for sequencing on the Ion PGM™ System. The Ion OneTouch™ 2 System includes the Ion OneTouch™ 2 Instrument and the Ion OneTouch™ ES Instrument.

The user guide is organized as follows:

- Prepare template-positive ISPs containing clonally amplified DNA, using the Ion PGM™ Hi-Q™ OT2 Kit (for up to 400 base-read libraries) with the Ion OneTouch™ 2 Instrument (see Chapter 3, “Prepare template-positive Ion PGM™ Hi-Q™ ISPs”).
- Enrich the template-positive ISPs with the Ion OneTouch™ ES (see Chapter 4, “Enrich the template-positive Ion PGM™ Hi-Q™ ISPs”).

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**IMPORTANT!** Do not use the kit with the Ion OneTouch™ System. Do not mix reactions or disposables including plates, solutions, and kit reagents from other template preparation kits. Template-positive Ion PGM™ Hi-Q™ Ion Sphere™ Particles prepared with this kit should only be used in conjunction with the Ion PGM™ Hi-Q™ Sequencing Kit (Cat. No. A25592). See the latest revision of *Ion PGM™ Hi-Q™ Sequencing Kit User Guide* (Pub. No. MAN0009816).

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## Prerequisites

The manufacturer is not liable for any damage or injury that results from use of this manual by unauthorized or untrained parties. This guide uses conventions and terminology that assume a working knowledge of the Microsoft™ Windows™ operating system, the Internet, and Internet-based browsers.



# Product information

## Product description

The Ion PGM™ Hi-Q™ OT2 Kit includes the reagents required for preparing 8 reactions of template-positive Ion PGM™ Hi-Q™ Ion Sphere™ Particles (ISPs) on the Ion OneTouch™ 2 System. The Ion PGM™ Hi-Q™ OT2 Kit can be used with up to 400 base-read average insert libraries of any type prepared using any available Ion library kit.

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**IMPORTANT!** Use *only* the Ion PGM™ Hi-Q™ OT2 Kit (Cat. No. A27739) with this user guide and with the Ion OneTouch™ 2 System. Do not use the kit with the Ion OneTouch™ System. Do *not* mix reactions or disposables including plates, solutions, and kit reagents from other template preparation kits.

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## Software compatibility

The Ion PGM™ Hi-Q™ OT2 Kit is compatible with the following software and sequencing instrument systems:

- Torrent Suite™ Software v5.0 or later on the Ion PGM™ System.
- Torrent Suite™ Assay Development Software v5.0.2 or later on the Ion PGM™ Dx System<sup>[1]</sup>. To use this kit on the Ion PGM™ Dx System with Torrent Suite™ Assay Development Software, see *Ion PGM™ Hi-Q™ OT2 Kit – Assay Development Mode User Guide* (Pub. No. MAN0015793).

Be sure to update to the latest available version of Torrent Suite™ Software before using this kit.

<sup>[1]</sup> 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

**IMPORTANT!** Follow these special reagent handling instructions:

- Ion PGM™ Hi-Q™ Reagent Mix in the Ion PGM™ Hi-Q™ OT2 Kit is shipped at –30°C to –10°C and should be thawed before use. Store the thawed Reagent Mix at 2°C to 8°C.
- Use only screw caps to seal reagent tubes in the Ion PGM™ Hi-Q™ OT2 Kit. Do not seal the reagent tubes with any plastic paraffin film such as Parafilm™ M film.

### Ion PGM™ Hi-Q™ OT2 Kit summary

Box	Part No.	Quantity per kit
Ion PGM™ OT2 Supplies	A27744	1 box
Ion PGM™ Hi-Q™ OT2 Reagents	A27743	1 box
Ion PGM™ Hi-Q™ OT2 Solutions	A27742	1 box

### Kit contents and storage conditions

Component <sup>[1]</sup>	Amount	Storage
<b>Ion PGM™ OT2 Supplies (Part No. A27744)</b>		
Ion OneTouch™ Reagent Tubes	2 tubes	15°C to 30°C
Ion OneTouch™ Recovery Routers	8 routers	
Ion OneTouch™ Recovery Tubes	16 tubes	
Ion OneTouch™ Sipper Tubes	2 tubes	
Ion OneTouch™ Amplification Plate	8 plates	
Ion OneTouch™ Cleaning Adapter <sup>[2]</sup>	8 adapters	
Ion OneTouch™ Reaction Filter	8 reaction filters and tubes	
Ion OneTouch™ ES Supplies <sup>[3]</sup>	1 bag	
<b>Ion PGM™ Hi-Q™ OT2 Reagents (Part No. A27743)</b>		
Ion PGM™ Hi-Q™ Reagent Mix (violet cap)  <b>IMPORTANT!</b> The Ion PGM™ Hi-Q™ Reagent Mix is shipped at –30°C to –10°C. Immediately before use, thaw tube(s) as needed. After use, store the thawed Ion PGM™ Hi-Q™ Reagent Mix at 2°C to 8°C.	8 × 800 µL	–30°C to –10°C (2°C to 8°C after thaw)
Ion PGM™ Hi-Q™ Enzyme Mix (brown cap)	400 µL	–30°C to –10°C
Ion PGM™ Hi-Q™ ISPs (black cap)	800 µL	

Component <sup>[1]</sup>	Amount	Storage
<b>Ion PGM™ Hi-Q™ OT2 Solutions (Part No. A27742)</b>		
Ion OneTouch™ Breaking Solution (black cap)	2 × 1.2 mL	15°C to 30°C
Ion OneTouch™ Oil	450 mL	
Ion OneTouch™ Reaction Oil	25 mL	
Nuclease-free Water	30 mL	
Ion OneTouch™ Recovery Solution	350 mL	
Neutralization Solution	100 µL	
Ion OneTouch™ Wash Solution	16 mL	
MyOne™ Beads Wash Solution (green cap)	2 × 1.4 mL	
Tween™ Solution	6 mL	

<sup>[1]</sup> We have verified this protocol using this specific material. Substitution may adversely affect performance.

<sup>[2]</sup> Each Ion OneTouch™ Cleaning Adapter is used for *one* cleaning only.

<sup>[3]</sup> Ion OneTouch™ ES Supplies include 12 pipette tips and 1 box of ES 8-well strips.

## Ion PGM™ Calibration Standard

If you are performing a *de novo* sequencing experiment, add the Ion PGM™ Calibration Standard (Cat. No. A27832) to your amplification solution to enable greater accuracy of base-calling in libraries for which a reference BAM file does not exist. The Ion PGM™ Calibration Standard is ordered separately.

Description	Amount	Storage
Ion PGM™ Calibration Standard	80 µL	-30° to -10°C

## Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**.  
MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

✓	Item <sup>[1]</sup>	Source
	Ion OneTouch™ 2 System The system includes: <ul style="list-style-type: none"> <li>• Ion OneTouch™ 2 Instrument</li> <li>• Ion OneTouch™ ES Instrument</li> <li>• AC Power Supply and Cords</li> <li>• Installation Kit</li> </ul>	4474779
	Ion PGM™ Enrichment Beads (Dynabeads™ MyOne™ Streptavidin C1 Beads)	4478525
	GeneAmp™ PCR System 9700 thermal cycler or equivalent	N8050200 (Base) 4314443 (Block)
	1.5-mL Eppendorf™ DNA LoBind™ Microcentrifuge Tubes	Fisher Scientific 13-698-791
	Microcentrifuge <sup>[2]</sup>	MLS
	Pipettes (P2, P20, P200, P1000) and appropriate low-retention tips	MLS
	Vortexer with a rubber platform	MLS
	Tube rack to fit 15-mL conical tube	MLS
	Tube rack for 50-mL conical tube	MLS
	Heat block set to 50°C	MLS

<sup>[1]</sup> We have verified this protocol using this specific material. Substitution may adversely affect system performance.

<sup>[2]</sup> Must fit standard 0.2- and 1.5-mL microcentrifuge tubes; must generate  $15,500 \times g$ . To convert the RPMs of your centrifuge to RCF in units of gravity, see [tools.thermofisher.com/content/sfs/brochures/TR0040-Centrifuge-speed.pdf](https://tools.thermofisher.com/content/sfs/brochures/TR0040-Centrifuge-speed.pdf).

**Note:** We recommend using a non-interruptible power supply (UPS) for laboratories that experience frequent power outages or line voltage fluctuations. The UPS must be rated for 1500 W output or higher. The 1500 VA unit from APC provides several minutes of backup power for the Ion OneTouch™ 2 Instrument, the Ion OneTouch™ ES Instrument, the Ion PGM™ Sequencer, and the Torrent Server. Use a surge protector or line conditioner as needed (see the *Ion OneTouch™ 2 System User Guide* Pub. No. MAN0014388 for further information).

### Additional materials required for Ion OneTouch™ ES

The following additional materials are required for use of the Ion OneTouch™ ES Instrument. Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

✓	Item <sup>[1]</sup>	Source
	DynaMag™ -2 magnet	12321D
	0.2-mL PCR tubes (Axygen™ MAXYMum Recovery™ PCR Tube)	Fisher Scientific 14-222-283 or MLS
	1 M NaOH	MLS

<sup>[1]</sup> We have verified this protocol using this specific material. Substitution may adversely affect system performance.

### Materials recommended for the Ion OneTouch™ 2 System

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

✓	Item <sup>[1,2]</sup>	Source
	Ion PGM™ Controls Kit v2	4482010
	Benchtop absorbent paper or mat	MLS
	Bleach	MLS
	Ethernet cable	MLS
	1/8-inch L-wrench (hex wrench) or equivalent tool	MLS

<sup>[1]</sup> We have verified this protocol using this specific material. Substitution may adversely affect system performance.

<sup>[2]</sup> For materials needed for optional quality control of ISPs by the Qubit™ 2.0 or Qubit™ 3.0 Fluorometer, 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).



# Before you begin

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## Ion OneTouch™ Dx Instrument users

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**IMPORTANT!** If you are using the Ion OneTouch™ Dx Instrument with Torrent Suite™ Assay Development Software<sup>[2]</sup>, see *Ion PGM™ Hi-Q™ OT2 Kit—Assay Development Mode User Guide* (Pub. No. MAN0015793).

The Ion OneTouch™ Dx Instrument workflow and steps differ from those described in this user guide.

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## Procedural guidelines

### Preventing cross-contamination:

- Use good laboratory practice to minimize cross-contamination of products and reagents.
- When designing the laboratory layout, consider the need for space separation of pre- and post-PCR activities. Dedicate laboratory supplies and/or equipment to the appropriate space to reduce the potential for contamination.
- A primary source of contamination is DNA fragments from previous sample processing steps. Do not introduce amplified DNA into library preparation laboratory or work area.

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

**Reagent thawing:**

- Unless otherwise specified, thaw reagents on ice before use. Ensure that no ice crystals are visible in the thawed reagent.

**Pipetting recommendations:**

- Vortex all reagents, *except* for enzymes, for 5 seconds. Mix enzymes by flicking the tube with your finger 4 times. Pulse centrifuge before use.
- Pipet viscous solutions slowly and ensure complete mixing.
- Change tips between pipetting steps.

## Unpack and install the Ion OneTouch™ 2 Instrument and Ion OneTouch™ ES

For detailed instructions on site preparation and installation of the Ion OneTouch™ 2 Instrument and Ion OneTouch™ ES, see:

- Site preparation and installation requirements: *Ion PGM™ System Site Preparation Guide* (Pub. No. MAN0007516).
- Unpacking and installation instructions: *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388).

## Check the firmware

Firmware updates to the software controlling the Ion OneTouch™ 2 Instrument are periodically released. To check the firmware version, touch Options on the instrument touch screen, then touch Info. To update the firmware to the appropriate version, see Chapter 2 of the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388).

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**IMPORTANT!** Ensure that the latest firmware is installed on the Ion OneTouch™ 2 Instrument

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## Initialize the Ion OneTouch™ 2 Instrument

Before operating the Ion OneTouch™ 2 Instrument for the first time, you must initialize the instrument. Initialization primes the pumps and tubing lines for reliable operation. Perform initialization at any time before the first run. In addition, re-initialization is recommended when switching between sequencing platforms, and between kits with different lots of Ion OneTouch™ Oil.

**Note:** For detailed instructions on initialization of the Ion OneTouch™ 2 Instrument, and to set up the Ion OneTouch™ 2 Instrument when switching between sequencing platforms, see the *Ion OneTouch™ 2 System User Guide* (Pub No. MAN0014388).

## Perform a verification run

To ensure optimal use of the Ion OneTouch™ 2 Instrument, we recommend first preparing and enriching template-positive Ion PGM™ Hi-Q™ Ion Sphere™ Particles (ISPs) on the system with a control library.

1. Obtain the *E. coli* DH10B Control Library bag from the Ion PGM™ Controls Kit v2 (Cat. No. 4482010) and use either the *E. coli* DH10B Control 200 Library or the *E. coli* DH10B Control 400 Library.
2. Dilute 1 µL of control library into 259 µL of Nuclease-free Water in an Eppendorf LoBind™ Tube. Use 25 µL of the dilution in the amplification solution (see “Prepare the amplification solution” on page 27).
3. Follow the operating instructions to set up and use the Ion OneTouch™ 2 Instrument to prepare template-positive ISPs and to use the Ion OneTouch™ ES to prepare enriched ISPs (see “Run the Ion OneTouch™ 2 Instrument” on page 17).
4. If you have a Qubit™ 2.0 or Qubit™ 3.0 Fluorometer, determine the percent template-positive ISPs (see Appendix B, “Quality control of Ion PGM™ Hi-Q™ ISPs”). If you do not have one of these instruments, proceed to step 5.
5. Sequence the control library on the Ion PGM™ System using the Ion 314™ Chip v2 (Cat. No. 4482261). Set the Ion PGM™ Sequencer to 500 flows if using the *E. coli* DH10B Control 200 Library, or 850 flows if using the *E. coli* DH10B Control 400 Library. Load the sample, then analyze the results.

**Note:** Use 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).

6. Review the run report from the Torrent browser and confirm successful sequencing results with the control library. The AQ20 result of the template-positive ISPs prepared from the control library must exceed the values shown in the following table using the Ion 314™ Chip v2.

Control library	AQ20 result
<i>E. coli</i> DH10B Control 200 Library	>20 Mb
<i>E. coli</i> DH10B Control 400 Library	>40 Mb

**Note:** If the AQ20 result does not meet the throughput requirement with the Ion 314™ Chip v2, sequence the control library again and measure AQ20. If the AQ20 result is still not satisfactory, contact Technical Support.

The instrument is ready for use.

# 3

## Prepare template-positive Ion PGM™ Hi-Q™ ISPs

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## Ion OneTouch™ 2 Instrument layout



- |   |   |
|---|---|
| ① Ion OneTouch™ Reaction Filter   | ⑥ Waste Container   |
| ② Clamp handle to access the Amplification Plate in the heat block  | ⑦ Oil waste tray (pull out)                                 |
| ③ Pinch valve to hold disposable tubing   | ⑧ Centrifuge to spin the Recovery Tubes and Recovery Router |
| ④ Ion OneTouch™ Recovery Solution  | ⑨ Ion OneTouch™ DL Injector Hub                             |
| ⑤ Ion OneTouch™ Oil                | ⑩ Instrument display  |

**Note:** The line drawing does not show the disposable tubing.

## Run the Ion OneTouch™ 2 Instrument

**Note:**

- If this is the first use of the instrument, perform the initialization procedure before the first run. See Chapter 2 of the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388) for the initialization procedure.
- Ensure that the latest firmware is installed in your instrument (see “Check the firmware” on page 13).

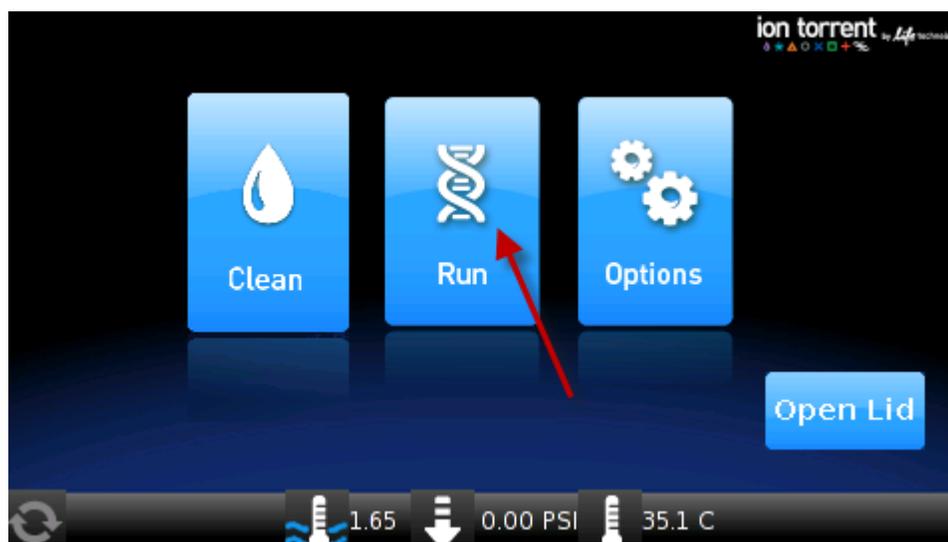
1. Ensure that the centrifuge lid of the Ion OneTouch™ 2 Instrument is closed.

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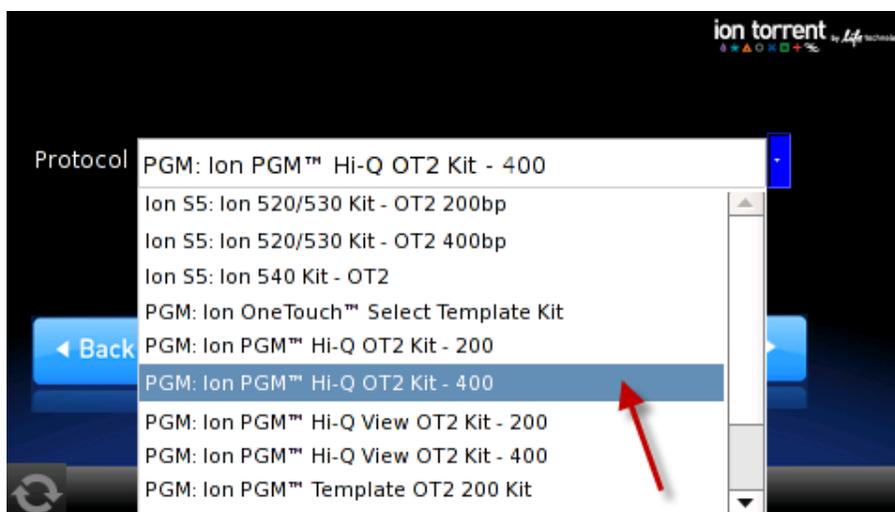
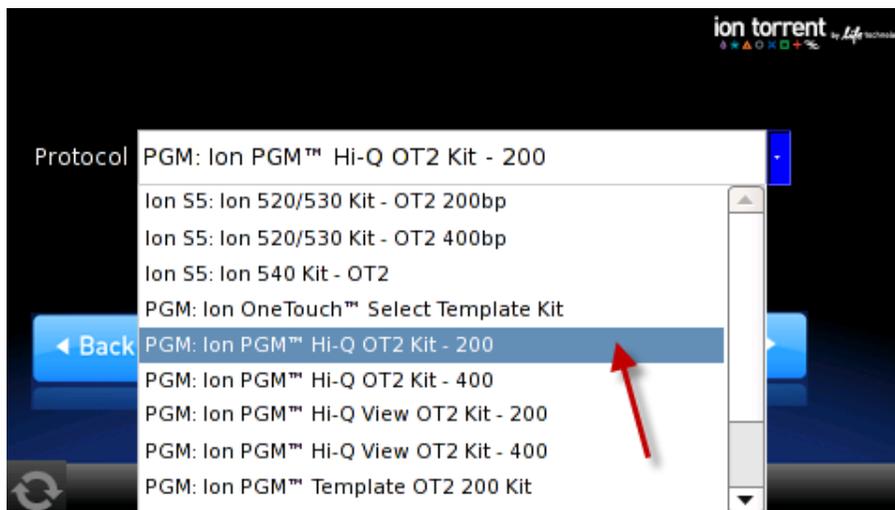
**IMPORTANT!** If you raise the centrifuge lid, do not hit the disposable injector against the instrument. You can damage the disposable injector. If you damage the disposable injector, appropriately dispose of the injector, tubing, and amplification plate. Use a new disposable injector, tubing, and Ion OneTouch™ Amplification Plate.

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2. On the home screen, touch **Run**:



3. Touch the drop-down menu, then select either **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:



4. Touch Next.

5. Touch **Assisted** or **Expert**:



- **Assisted** mode displays the individual task screens for setting up the run. Select this mode if you are a new user and need step-by-step onscreen guidance. Complete each task, and prepare and install the amplification solution (see page 26), then touch **Next**. After you touch **Next** on the last task, you see a progress bar, and the run begins.
- **Expert** mode bypasses the individual task screens for setting up the run. Select this mode if you are an experienced user and do not need step-by-step onscreen guidance. When you have finished the instrument setup procedure, detailed in the following sections, touch **Next**. You see a progress bar, and the run begins without the complete list of task screens.

## Set up the Ion OneTouch™ 2 Instrument

### Note:

- To set up the Ion OneTouch™ System when switching between sequencing platforms, see the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388).
- If this is the first run after instrument initialization, proceed to “Prepare and install the amplification solution” on page 26. The instrument consumables are already installed and ready for the run.

### Materials required **Provided in Ion PGM™ OT2 Supplies (Part No. A27744):**

- 2 Ion OneTouch™ Reagent Tubes
- Ion OneTouch™ Recovery Router
- 2 Ion OneTouch™ Recovery Tubes
- Ion OneTouch™ Amplification Plate
- 2 Ion OneTouch™ Sipper Tubes

### Provided in Ion PGM™ Hi-Q™ OT2 Solutions (Part No. A27742):

- Ion OneTouch™ Oil (450 mL size)
- Ion OneTouch™ Breaking Solution
- Ion OneTouch™ Recovery Solution

**Note:** We have verified this protocol using only the material specified. Substitution may adversely affect performance and safety.

---

**IMPORTANT!** Do *not* mix reactions or disposables including plates, solutions, and kit reagents from other template preparation kits with the Ion PGM™ Hi-Q™ OT2 Kit.

---

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

1. On the instrument display, touch **Open Lid**, wait until the lid clicks open, then lift and hold the side of the centrifuge lid.

---

**IMPORTANT!** Do *not* lift the lid by the tubing attached to the Ion OneTouch™ DL Injector Hub. Do *not* force the lid open.

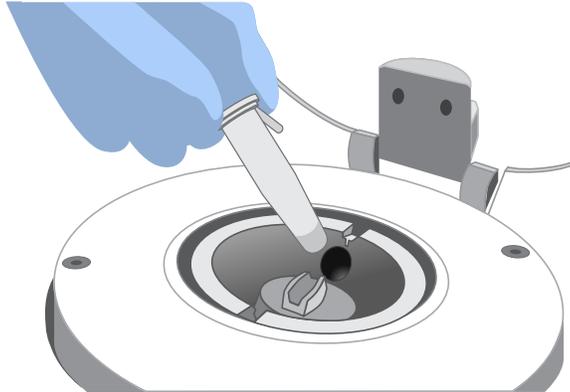
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2. Dispense 150 µL Ion OneTouch™ Breaking Solution into each of two Recovery Tubes. The Recovery Tubes have rounded collars at their openings.

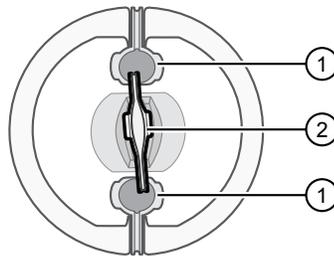
**Note:** Breaking Solution is viscous. Draw and dispense slowly.

**Note:** Ensure that the Ion OneTouch™ Breaking Solution is stored and used at room temperature. If a white precipitate is present in the tube, warm the solution at 30°C until the precipitate re-dissolves.

3. Insert a Recovery Tube containing Ion OneTouch™ Breaking Solution into each slot of the centrifuge:



4. Slide the Recovery Router in position around each Recovery Tube extension. Pinch the sides of the Recovery Router and push it down into the center slot of the centrifuge. The Recovery Router must be seated flat and secure in the center of the rotor:



- ① Ion OneTouch™ Recovery Tube
- ② Ion OneTouch™ Recovery Router

5. Close the lid of the centrifuge.

### Install the Ion OneTouch™ Amplification Plate

1. If there is a used Ion OneTouch™ Cleaning Adapter on the instrument, remove and appropriately discard it.

**Note:** The Cleaning Adapter may be filled with Ion OneTouch™ Oil.

2. Push the handle back to open the heat block.



**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.

3. Insert the Amplification Plate:
  - a. Inspect the Amplification Plate to ensure that the plate port is straight and perpendicular to the plate.

---

**IMPORTANT!** The disposable tubing and disposable injector are attached to the Amplification Plate. Do *not* disconnect tubing from the top plate port. If you have questions about the plate, contact Technical Support.

---

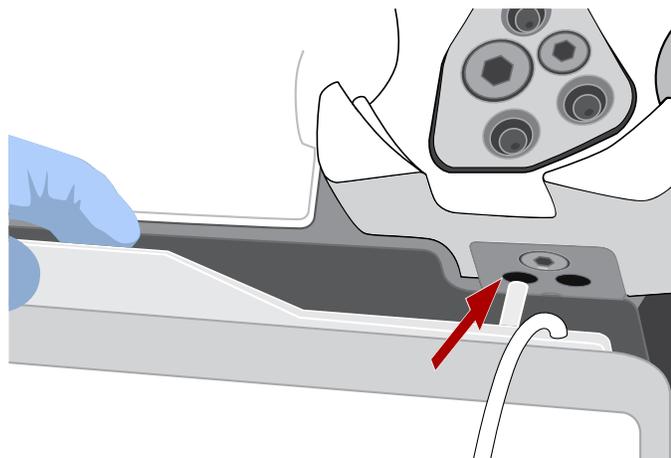
- b. Hold the disposable injector, connected to the disposable tubing, in one hand and the Amplification Plate in the other hand.



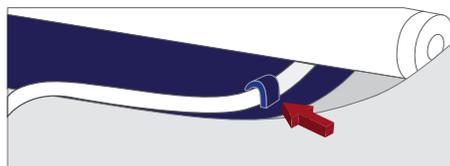
**CAUTION! PHYSICAL INJURY HAZARD.** The pointed end of the disposable injector can puncture your skin. Keep your hand away from the point of the disposable injector.

---

- c. Insert the Amplification Plate into the heat block so that the single plate port aligns with the *left* hole of the Ion OneTouch™ 2 Instrument:



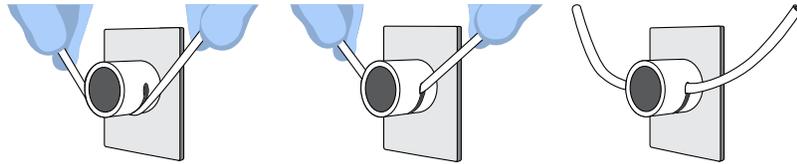
4. Pull the handle of the heat block to close the block, then thread the disposable tubing through the Ion OneTouch™ DL Tubing Catch:



**Note:** The disposable tubing is under the handle.

5. Install the disposable tubing in the pinch valve:
  - a. Align the disposable tubing with the slot that runs along the bottom of the pinch valve.

- b. Gently pull the disposable tubing upwards on the both sides of the pinch valve until the disposable tubing is in the slot and secured in the round notch on each side of the pinch valve:



- c. If necessary, adjust the disposable tubing along the notches of the open pinch valve so that there is sufficient length of disposable tubing to install the disposable injector (see “Install the disposable injector”).

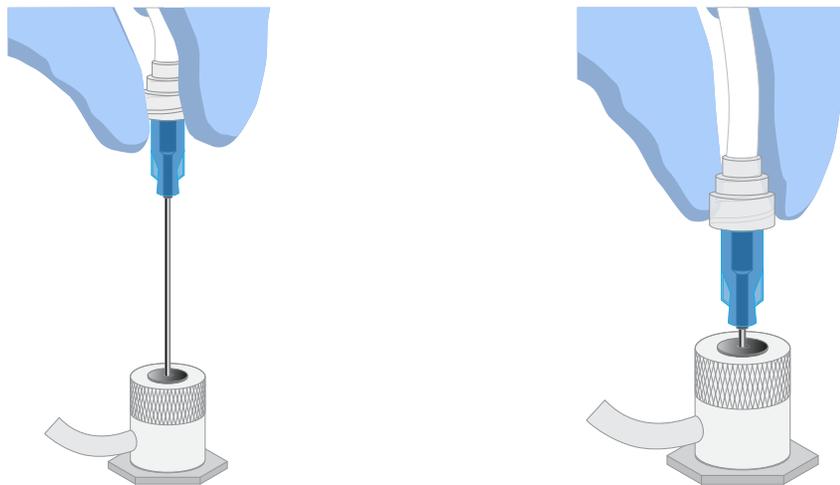
### Install the disposable injector

**Note:** The long metal shaft of the disposable injector may be slightly bent, which is normal. If you have questions about the disposable injector, contact Technical Support.

1. Ensure that the needle of the injector is screwed tightly onto the rubber tubing. Hold the centrifuge lid down with one hand, and with other hand install the disposable injector by inserting it straight down into the injector hub. Push down until it just touches the router.



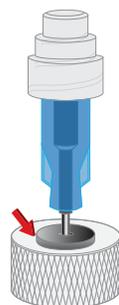
**CAUTION! PHYSICAL INJURY HAZARD.** The pointed end of the injector can puncture your skin. Keep your hand away from the point of the injector.



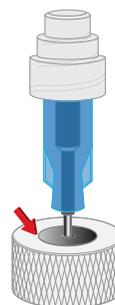
**Note:** The color of the injector may vary.

- The spring-loaded top of the injector hub will click upon release, automatically adjusting the tip to the correct distance from the router surface. You can test this by gently pushing the injector down again and releasing. You should hear a click from the hub.

Up position



Down position




---

**IMPORTANT!** If the Injector Hub remains in the down position, see Appendix A, “Troubleshooting”.

---

**IMPORTANT!** If you raise the centrifuge lid, do not hit the injector against the instrument. If you damage the disposable injector, appropriately dispose of the injector, amplification plate, and tubing. Use a new injector and Ion OneTouch™ 2 Amplification Plate.

---

## Install the Ion OneTouch™ Oil

Fill the appropriate Ion OneTouch™ Reagent Tube with Ion OneTouch™ Oil on the left front port :

If you are	Action
Using a new Template Kit	<ol style="list-style-type: none"> <li>Use a new Reagent Tube from the kit.</li> <li>Discard the used Reagent Tube and Sipper Tube. Appropriately discard the residual Oil.</li> <li>Use fresh gloves to attach the Luer-Lok™ end of a new Ion OneTouch™ Sipper Tube to the <i>left</i> front port. Do <b>not</b> let the Sipper Tube touch any surface.</li> <li>Invert the Ion OneTouch™ Oil bottle (450-mL size) 3 times to mix, then fill the Reagent Tube <i>half</i>-full with Oil. Minimize bubbles.</li> <li>Insert the filled Reagent Tube into the <i>left</i> front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument.</li> </ol>
Refilling the Reagent Tube between runs	<ol style="list-style-type: none"> <li>Remove the Reagent Tube from the instrument.</li> <li>Invert Ion OneTouch™ Oil bottle (450-mL size) 3 times to mix.</li> <li>Fill the Reagent Tube <i>half</i>-full with Oil. Minimize bubbles.</li> </ol> <p><b>Note:</b> It is not necessary to re-mix the Oil.</p> <ol style="list-style-type: none"> <li>Insert the filled Reagent Tube into the <i>left</i> front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument.</li> </ol>

## Install the Ion OneTouch™ Recovery Solution

**IMPORTANT!** Use only the Ion OneTouch™ Recovery Solution provided as part of the Ion PGM™ Hi-Q™ OT2 Kit for the Ion OneTouch™ 2 Instrument. Do *not* use a different recovery solution from another kit.

1. Ensure that the Recovery Solution is clear. If it is clear, proceed to the next step. If the Recovery Solution is not clear, warm the bottle of Recovery Solution in a 30°C bath until the Recovery Solution is clear.
2. Fill the appropriate Ion OneTouch™ Reagent Tube with Ion OneTouch™ Recovery Solution on the right front port :

If you are	Action
Using a new Template Kit	<ol style="list-style-type: none"> <li>1. Use a new Reagent Tube from the kit.</li> <li>2. Discard the used Reagent Tube and Sipper Tube. Appropriately discard the Recovery Solution.</li> <li>3. Use fresh gloves to attach the Luer-Lok™ end of a new Ion OneTouch™ Sipper Tube to the <i>right</i> front port. Do not let the Sipper Tube touch any surfaces.</li> <li>4. Invert the Recovery Solution 3 times to mix, then fill the Reagent Tube <i>a quarter</i>-full with Recovery Solution. Minimize bubbles.</li> <li>5. Insert the filled Reagent Tube into the <i>right</i> front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument.</li> </ol>
Refilling the Reagent Tube between runs	<ol style="list-style-type: none"> <li>1. Remove the Reagent Tube from the instrument.</li> <li>2. Invert the bottle of Recovery Solution 3 times.</li> <li>3. Add more Recovery Solution to the solution in the Reagent Tube until the tube is <i>a quarter</i>-full. Minimize bubbles.</li> <li>4. Insert the filled Reagent Tube into the <i>right</i> front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument.</li> </ol>

## Empty the Waste Container

1. Pull the external tubing from the port of the Waste Container.
2. Empty the Waste Container into the appropriate receptacle.
3. Reinstall the empty Waste Container.

## Inspect the oil waste tray

1. Slowly pull out the oil waste tray but do not remove it completely from the slot underneath the center of the instrument.
2. Check for oil in the oil waste tray:
  - If there is little or no oil, push the tray back fully into the instrument.
  - If there is excessive oil, remove the tray, then appropriately dispose of the oil. Reinsert the oil waste tray into the slot, then push the tray back fully into the instrument. Contact Technical Support.

## Prepare and install the amplification solution

**Materials required**    **Provided in Ion PGM™ Hi-Q™ OT2 Solutions (Part No. A27742):**

- Ion OneTouch™ Reaction Oil (25-mL size)
- Nuclease-free Water

**Provided in Ion PGM™ Hi-Q™ OT2 Reagents (Part No. A27743):**

- Ion PGM™ Hi-Q™ Reagent Mix
- Ion PGM™ Hi-Q™ Enzyme Mix
- Ion PGM™ Hi-Q™ Ion Sphere™ Particles (ISPs)

**Provided in Ion PGM™ OT2 Supplies (Part No. A27744):**

- Ion OneTouch™ Reaction Filter

**(Optional) Provided in the Ion PGM™ Controls Kit v2 (Cat No. 4482010):**

- *E. coli* DH10B Control 200 Library *or*
- *E. coli* DH10B Control 400 Library

**Other materials and equipment:**

- Diluted library
- Microcentrifuge
- Eppendorf™ DNA LoBind™ Microcentrifuge Tubes
- Tube rack for 15-mL conical tube
- Pipettes
- Vortexer

## Prepare the amplification solution

**IMPORTANT!** Do not mix components from this kit—including plates, solutions, and kit reagents—with components from other template preparation kits.

**IMPORTANT!** We recommend preparing the amplification solution in a room that is dedicated to pre-PCR activities or in a controlled pre-PCR hood.

1. Prepare the reagents as follows:

**IMPORTANT!** Use only Ion PGM™ Hi-Q™ ISPs. Do not use ISPs from other or previously used kits.

Reagents	Preparation
Ion PGM™ Hi-Q™ Reagent Mix	<ol style="list-style-type: none"> <li>1. Allow the tube of reagent mix to come to room temperature before use.</li> <li>2. Vortex the solution for 30 seconds, then centrifuge the solution for 2 seconds.</li> </ol> <p><b>Note:</b> Visually inspect the solution at the bottom of the tube and ensure that there is no precipitate remaining. If precipitate is visible, see Appendix A, “Troubleshooting”.</p> <ol style="list-style-type: none"> <li>3. Keep the reagent mix at room temperature during use. Store unused thawed reagent mix at 2 °C to 8 °C.</li> </ol>
Ion PGM™ Hi-Q™ Enzyme Mix	<ol style="list-style-type: none"> <li>1. Centrifuge the enzyme for 2 seconds.</li> <li>2. Place on ice.</li> </ol>
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
<b>Total volume of diluted library to add to the amplification solution</b>	<b>25 µL</b>	<b>25 µL</b>	<b>25 µL</b>	<b>25 µL</b>

**Note:** If you are troubleshooting the amplification process, add 1 µL of the *E. coli* DH10B Control 200 Library or *E. coli* DH10B Control 400 Library from the Ion PGM™ Controls Kit v2 (Cat. No. 4482010) to 259 µL of Nuclease-free Water. Add 25 µL of this dilution to the amplification solution instead of your library.

- 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
—	<b>Total</b>	—	<b>1,000 µL</b>

**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.

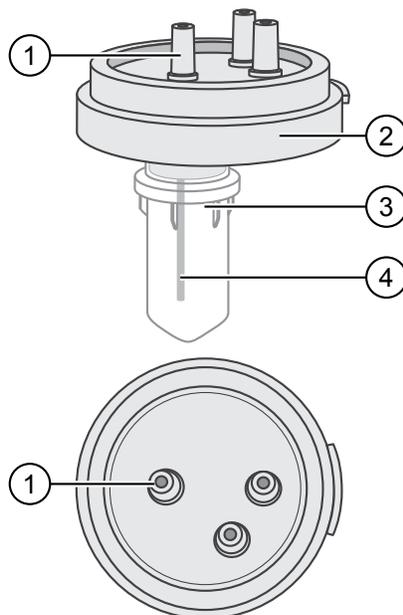
**IMPORTANT!** Start the run on the Ion OneTouch™ 2 Instrument ≤15 minutes after preparing the amplification solution.

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

## Fill the Ion OneTouch™ Reaction Filter

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

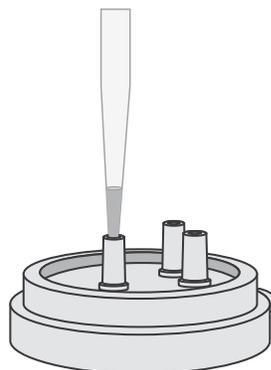
1. Place a Ion OneTouch™ Reaction Filter in a tube rack so that the three ports of the filter face up. Identify the sample port, which is connected to a short tube that extends into the Ion OneTouch™ Reaction Tube as shown below.



- ① Sample port
- ② Ion OneTouch™ Reaction Filter
- ③ Ion OneTouch™ Reaction Tube
- ④ Short tube from sample port into Ion OneTouch™ Reaction Tube

2. Set a P1000 pipette to 1000  $\mu$ L, and attach a new 1000- $\mu$ L tip to the pipette.
3. Vortex the amplification solution (prepared in the previous section) at maximum speed for 5 seconds, then centrifuge the solution for 2 seconds. Immediately proceed to the next step.
4. Pipet the amplification solution up and down to mix, then fill the pipette tip with 1000  $\mu$ L of amplification solution.

5. Insert the tip firmly into the sample port, so that the tip is perpendicular to the port and forms a tight seal.



6. Slowly pipet the 1000  $\mu\text{L}$  of amplification solution through the sample port. Keep the plunger of the pipette depressed to avoid aspirating solution. With the plunger still depressed, remove the tip from the sample port, then appropriately discard the tip.
7. If necessary, gently dab a Kimwipes™ disposable wipe around the ports to remove any liquid.
8. Set a P1000 pipette to 850  $\mu\text{L}$ , and attach a new 1000- $\mu\text{L}$  tip to the pipette.
9. Draw up 850  $\mu\text{L}$  of Ion OneTouch™ Reaction Oil into the pipette tip.
10. Insert the tip firmly into the sample port, so that the tip is perpendicular to the port and forms a tight seal.
11. Slowly pipet 850  $\mu\text{L}$  of Reaction Oil through the sample port, then keep the plunger of the pipette depressed. With the plunger depressed, remove the tip from the sample port, then appropriately discard the tip.
12. Repeat steps 9–11 one more time, to add a total of 1.7 mL of Ion OneTouch™ Reaction Oil to the Reaction Filter.
13. If necessary, gently dab a Kimwipes™ disposable wipe around the ports to remove any liquid.

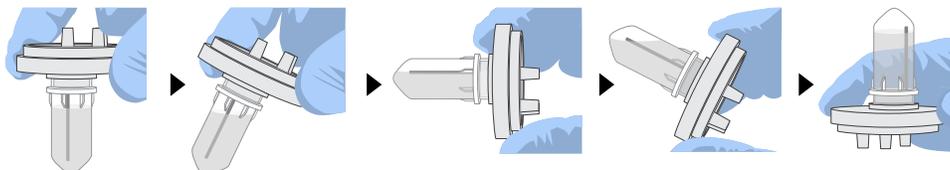
## Install the filled Ion OneTouch™ Reaction Filter

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**IMPORTANT!** Follow the steps exactly to minimize contact of the short tubing in the Reaction Tube with the amplification solution.

---

1. Position the tube rack containing the Ion OneTouch™ Reaction Filter so that the sample port on the filter is on the left.
2. Lift the Ion OneTouch™ Reaction Filter straight out of the tube rack. With the sample port still on the left, rotate the filter assembly clockwise as shown below, until the Reaction Tube is inverted and the three ports point down.

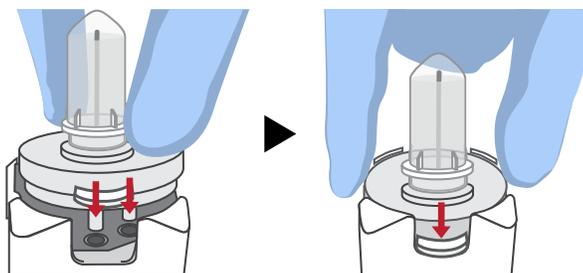


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**IMPORTANT!** Correct rotation of the Ion OneTouch™ Reaction Filter ensures minimal exposure of the short tubing in the Reaction Tube with the amplification solution.

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3. Insert the three ports into the three holes on the top stage of the Ion OneTouch™ 2 Instrument, so that the Ion OneTouch™ Reaction Filter is firmly seated (right) on the instrument. The tab protruding from the outer edge of the Reaction Filter fits into the front notch of the stage:

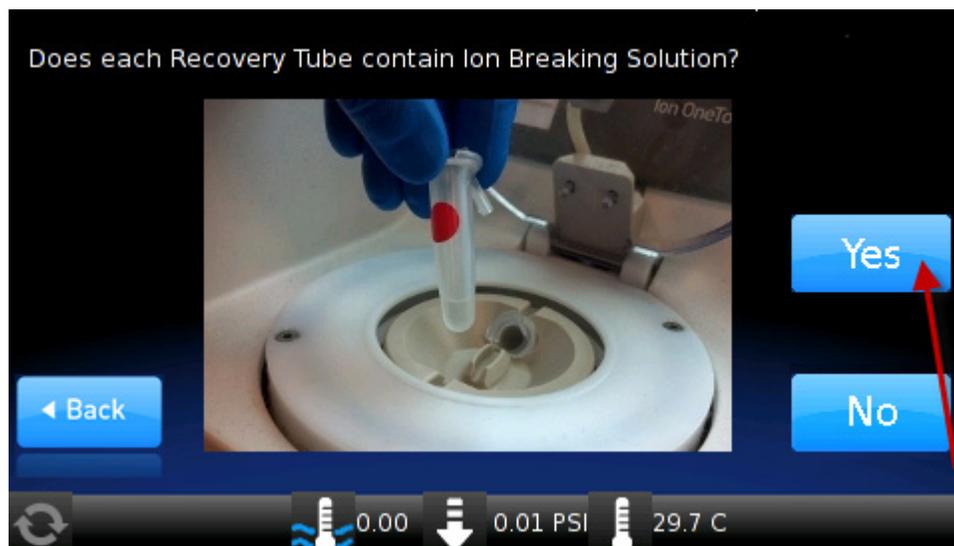


**Note:** After inserting the Ion OneTouch™ Reaction Filter, bubbles may shoot up into the Reaction Tube.

## 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:** After the run starts, the instrument makes clicking sounds. This is normal.

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 Ion PGM™ Hi-Q™ ISPs” on page 33.

**Note:** Typical instrument run time is 4.8–6.5 hours.

### Abort and restart a run

If it is necessary to abort and restart a run, follow these steps in the order listed:

1. Touch **Abort**, then touch **Yes** to confirm cancellation.

**Note:** If there is a high-pressure event on the instrument, the instrument aborts the run *automatically*, and you do *not* have to touch **Abort**.

2. After a run is aborted, download the log files for troubleshooting by Technical Support (see the *Ion OneTouch™ 2 System User Guide* [Pub. No. MAN0014388] for more information).
3. Power off the instrument.
4. If needed, retain all consumables on the instrument for troubleshooting.
5. After successful troubleshooting, appropriately discard all used consumables, then power on the instrument.
6. Set up the instrument with new kit components (see “Set up the Ion OneTouch™ 2 Instrument” on page 20).

7. Prepare a new amplification solution (see “Prepare and install the amplification solution” on page 26).
8. Start a new run.

## Recover the template-positive Ion PGM™ Hi-Q™ ISPs

**Materials required** Provided in Ion PGM™ Hi-Q™ OT2 Solutions (Part No. A27742):

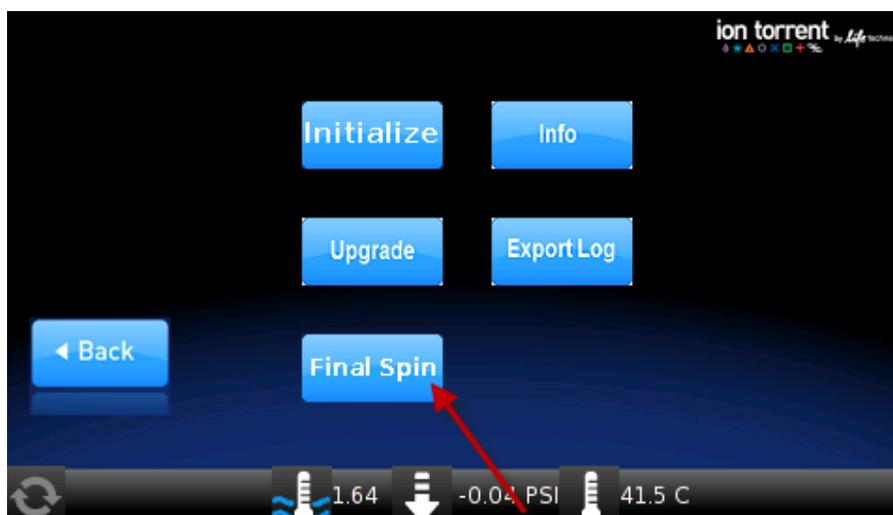
- Ion OneTouch™ Wash Solution

**Other materials and equipment:**

- Eppendorf™ DNA LoBind™ Microcentrifuge Tubes
- Pipettes
- Vortexer
- Microcentrifuge

### Recover the template-positive ISPs

1. At the end of the run, follow the screen prompts to centrifuge the sample. 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:
  - a. On the home screen of the instrument, touch **Open Lid**, wait until the lid clicks open, then insert the two filled Ion OneTouch™ Recovery Tubes from the run in the centrifuge rotor. Close the lid until it locks.
  - b. Touch **Options** ▶ **Final Spin** (see the following figure), then follow the screen prompts (touch **Next** on the next 2 screens) until the centrifugation starts. Centrifugation of the samples takes 10 minutes.



- c. *Immediately* proceed to step 2.

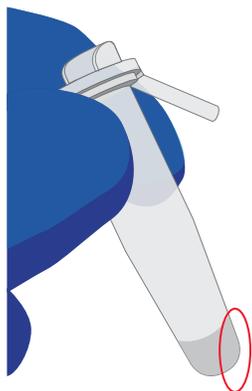


**CAUTION! ROTATION HAZARD.** Wait until rotation stops before opening. Rotating parts can cause injury.

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.

**Note:** You may see cloudiness in the tube, which is normal.

4. Use a pipette to remove all but 100 µL of the Recovery Solution from each Recovery Tube. Withdraw the supernatant from the surface and on the opposite side from the pellet. Remove any white flocculent material. Do not disturb the ISP pellet:



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**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.

Do not store the recovered ISPs in Ion PGM™ OT2 Recovery Solution.

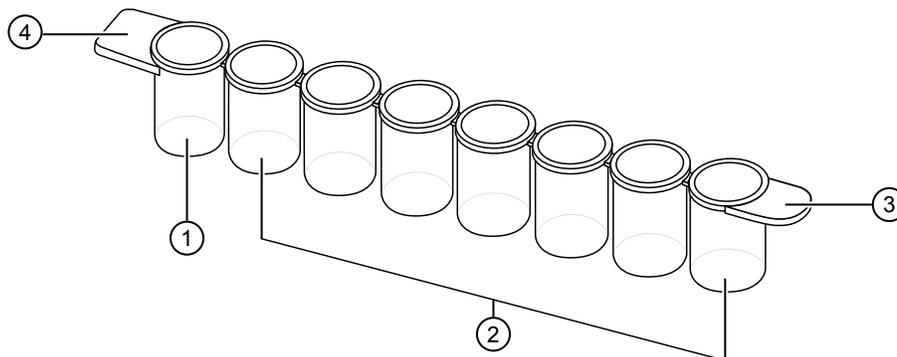
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**Note:** If the tube containing the stored ISPs has a visible precipitate, incubate the tube at 50°C for 2 minutes before centrifugation.

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.

6. 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

7. Pipet the ISPs up and down ten times to mix, then transfer the suspension into Well 1 of the 8-well strip.
8. 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:

Quality assessment by	Action
Qubit™ 2.0 or Qubit™ 3.0 Fluorometer	Transfer a 2.0-µL aliquot of the unenriched ISPs to a 0.2-mL PCR tube, then see the <i>Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide</i> (Pub. No. MAN0016387), or <i>Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide</i> (Pub. No. MAN0016388), available at <a href="http://thermofisher.com">thermofisher.com</a> .
<i>[Optional]</i> Guava™ easyCyte™ 5 Flow Cytometer	Transfer a 1.0-µL aliquot of the unenriched ISPs to a 1.5-mL Eppendorf LoBind™ Tube. See the <i>Ion Sphere™ Particles (ISPs) Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin</i> (Pub. No. MAN0015799), available at <a href="http://thermofisher.com">thermofisher.com</a> .
Attune™ NxT Acoustic Focusing Cytometer	Transfer a 1.0-µL aliquot of the unenriched ISPs to a 1.5-mL microcentrifuge tube. Put the sample on ice, then see the <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 <a href="http://thermofisher.com">thermofisher.com</a> .

Proceed to “Maintain the Ion OneTouch™ 2 Instrument” and Chapter 4, “Enrich the template-positive Ion PGM™ Hi-Q™ ISPs”. You can start the enrichment procedure while the Ion OneTouch™ 2 Instrument cleaning is in progress.

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**IMPORTANT!** Do not store the recovered, template-positive ISPs at  $-30^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$ . Do not store the ISPs in Ion PGM™ OT2 Recovery Solution (see step 4 of this procedure).

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## Maintain the Ion OneTouch™ 2 Instrument

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**IMPORTANT!** 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. Do not skip this procedure.* The cleaning procedure is performed according to the steps displayed on the instrument after removing the Recovery Tubes.

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**Note:** To set up the Ion OneTouch™ 2 Instrument when switching between sequencing platforms and/or template preparation kits, refer to Chapter 5 of the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388).

### Materials required

#### Provided in Ion PGM™ OT2 Supplies (Part No. A27744):

- Ion OneTouch™ Cleaning Adapter (single-use)

#### Provided in Ion PGM™ Hi-Q™ OT2 Solutions (Part No. A27742):

- Ion OneTouch™ Oil

#### Other materials and equipment:

- Kimwipes™ disposable wipes
- 50-mL conical tube
- Tube rack for 50-mL conical tube

**Note:** To ensure continued safe operation, visually inspect the rotor assembly and casing periodically to ensure there are no signs of cracks or other physical damage.

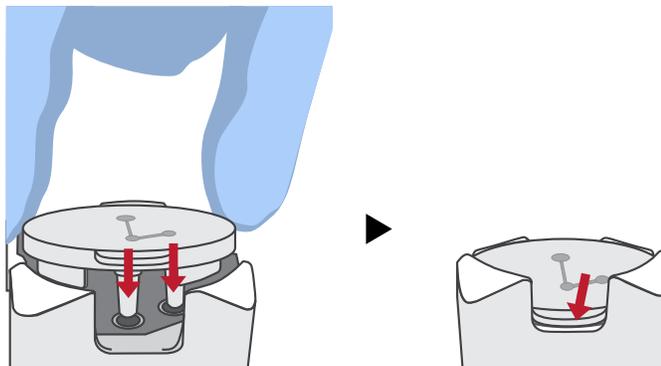
### Clean the Ion OneTouch™ 2 Instrument

1. 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.

2. 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.

3. Remove and appropriately discard the used Ion OneTouch™ Reaction Filter. Remove the assembly from the instrument by grasping the *filter*.  
**Note:** The Reaction Tube is filled with Ion OneTouch™ Oil.
4. Keep the Ion OneTouch™ Amplification Plate in the heat block.
5. 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 (see the following illustration). One of the two tabs protruding from the outer edge of the Cleaning Adapter fits into the front notch of the stage:



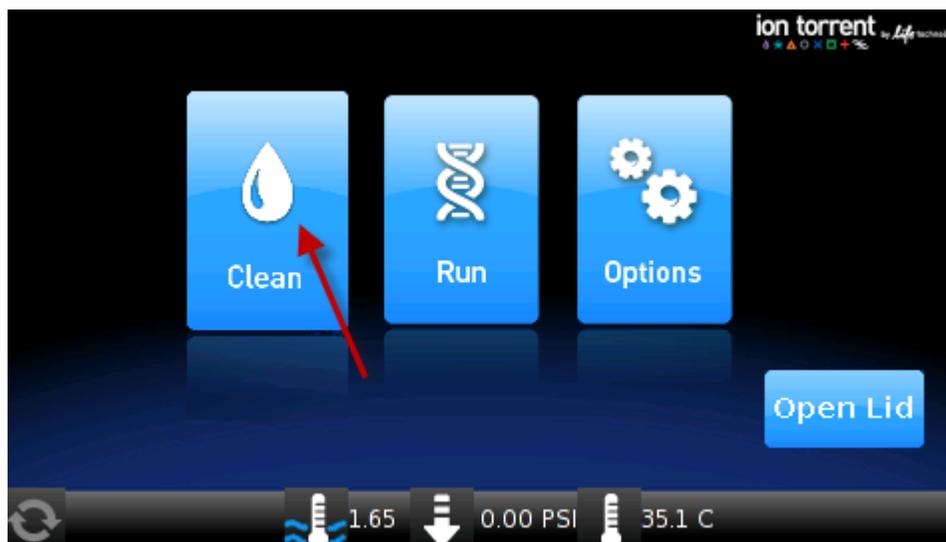
6. Place a 50-mL conical tube in a tube rack, then place the tube rack next to the instrument.  
**Note:** Steps 6–9 are only necessary if you have not already removed the disposable injector before removing the Recovery Tubes from the instrument.
7. Gently pull the disposable tubing downwards on both sides of the pinch valve until the disposable tubing is out of the valve.
8. Remove the disposable injector from the Ion OneTouch™ DL Injector Hub.
  - a. Place one hand on the centrifuge lid.
  - b. With the other hand, firmly grip the rigid plastic connector at the top of the disposable injector.
  - c. Slowly and steadily withdraw the disposable injector straight from the port of the 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.

9. Place the used, disposable injector into the empty 50-mL conical tube in the tube rack. The conical tube is used to collect waste.

10. On the home screen of the instrument, touch **Clean**.



11. 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.
12. 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.
13. Appropriately discard the waste that was collected in the 50-mL conical tube.
14. Remove and appropriately discard the used Amplification Plate, disposable injector, and tubing.
- Push the handle to open the heat block.
  - Remove the disposable tubing from the Ion OneTouch™ DL Catch.
  - Gently pull back the Amplification Plate from the inlet and outlet holes of the instrument.
  - Remove the Amplification Plate from the heat block, then appropriately discard the used Amplification Plate, injector, and tubing.
  - Leave the heat block open.



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

15. 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 wipers, then close the centrifuge lid.
16. Touch **Next** to return to the home screen on the instrument.



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

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■ Determine if a residual volume test is necessary .....	40
■ Prepare reagents then fill the 8-well strip .....	41
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## Materials required

### Provided in Ion PGM™ Hi-Q™ OT2 Solutions (Part No. A27742):

- Ion OneTouch™ Wash Solution
- MyOne™ Beads Wash Solution
- Tween™ Solution
- Neutralization Solution
- Nuclease-free Water

### Provided in Ion PGM™ OT2 Supplies (Part No. A27744):

- 8-well strip
- Eppendorf™ LoRetention Dualfilter Tips (P300)

### Other materials and equipment:

- Ion PGM™ Enrichment Beads (Cat. No. 4478525; Dynabeads™ MyOne™ Streptavidin C1 Beads)
- Eppendorf™ DNA LoBind™ Microcentrifuge Tubes
- 0.2-mL PCR tubes
- 1 M NaOH
- Pipettes
- Vortexer
- DynaMag™-2 magnet
- Microcentrifuge

## Determine if a residual volume test is necessary

---

**IMPORTANT!** Ensure that the AC line voltage module is installed correctly into the Ion OneTouch™ ES Instrument. See the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388) for information regarding instrument setup, calibration, and maintenance.

---

To determine if a residual volume test is necessary, follow these guidelines:

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" on page 41.

## 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
—	<b>Total</b>	<b>320 µL</b>

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

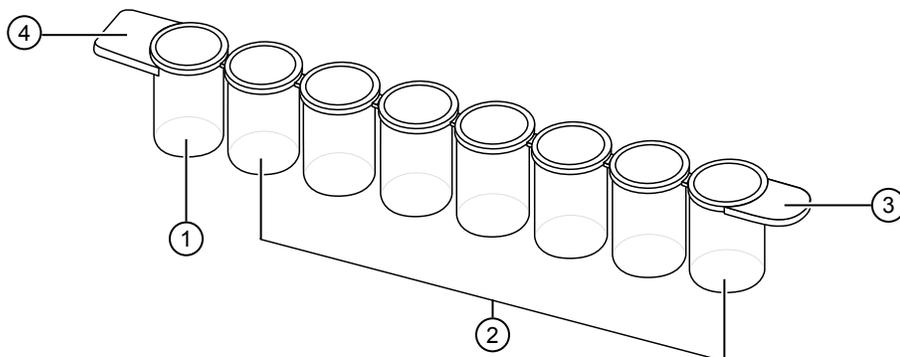
The final composition of the Melt-Off Solution is 125 mM NaOH and 0.1% Tween™ 20 detergent.

### Wash and resuspend the Dynabeads™ MyOne™ Streptavidin C1 Beads

1. Vortex the tube of Dynabeads™ MyOne™ Streptavidin C1 Beads for 30 seconds to resuspend the beads thoroughly, then centrifuge the tube for 2 seconds.
2. 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.
3. Transfer 13 µL of Dynabeads™ MyOne™ Streptavidin C1 Beads to a new 1.5-mL Eppendorf LoBind™ Tube.
4. 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.
5. Add 130 µL of MyOne™ Beads Wash Solution to the Dynabeads™ MyOne™ Streptavidin C1 Beads.
6. Remove the tube from the magnet, vortex the tube for 30 seconds, then centrifuge for 2 seconds.

## Fill the 8-well strip

1. 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 33). 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.

2. If you have not already assessed the quality of the unenriched, template-positive ISPs, use one of the following methods:

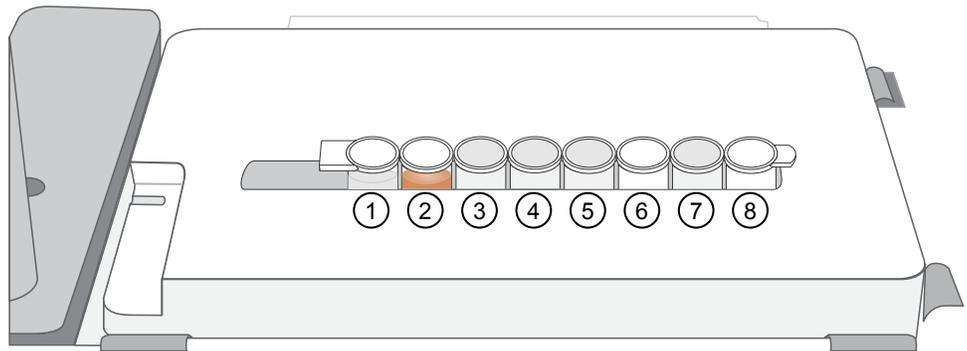
Quality assessment by	Action
Qubit™ 2.0 or Qubit™ 3.0 Fluorometer	Transfer a 2.0-µL aliquot of the unenriched ISPs to a 0.2-mL PCR tube, then see the <i>Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide</i> (Pub. No. MAN0016387), or <i>Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide</i> (Pub. No. MAN0016388), available at <a href="http://thermofisher.com">thermofisher.com</a> .
<i>[Optional]</i> Guava™ easyCyte™ 5 Flow Cytometer	Transfer a 1.0 µL aliquot of the unenriched 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 <a href="http://thermofisher.com">thermofisher.com</a> .
Attune™ NxT Acoustic Focusing Cytometer	Transfer a 1.0-µL aliquot of the unenriched ISPs to a 1.5-mL microcentrifuge 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 <a href="http://thermofisher.com">thermofisher.com</a> .

3. 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 <sup>[1]</sup>	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 41]
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 41]
Well 8	Empty

<sup>[1]</sup> 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:

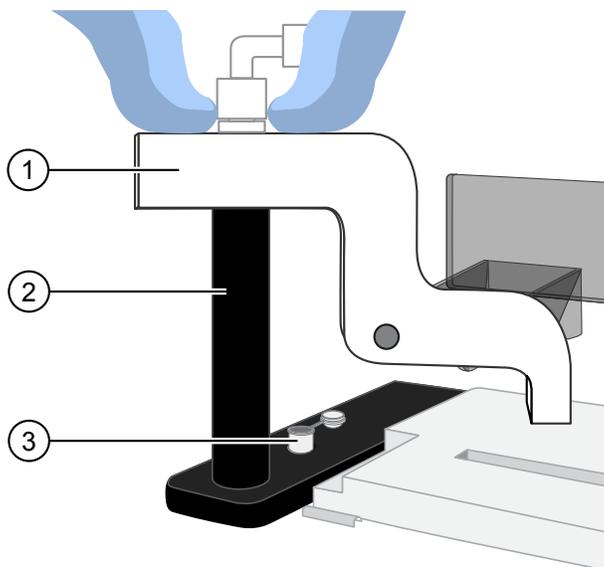


## Prepare the Ion OneTouch™ ES

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.

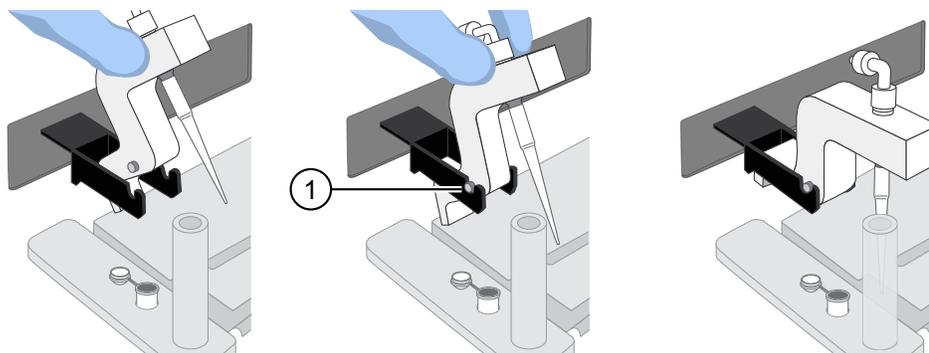
1. Add 10 µL of Neutralization Solution to a new 0.2-mL PCR tube.
2. Insert the open 0.2-mL PCR tube containing Neutralization Solution into the hole in the base of the Tip Loader, as shown in the figure in step 4.
3. Place a new tip in the Tip Loader. Remove the Tip Arm from the cradle, then align the 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.
  - a. Tilt the Tip Arm back (below left), then align the pins with the round notches in the cradle (below center).
  - b. Lower the Tip Arm into position (below center).
  - c. Move the Tip Arm forward into the working position (below right).



- ① Tip Arm pins resting in the notches in the cradle

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**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.

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## Perform the run

Before starting the run:

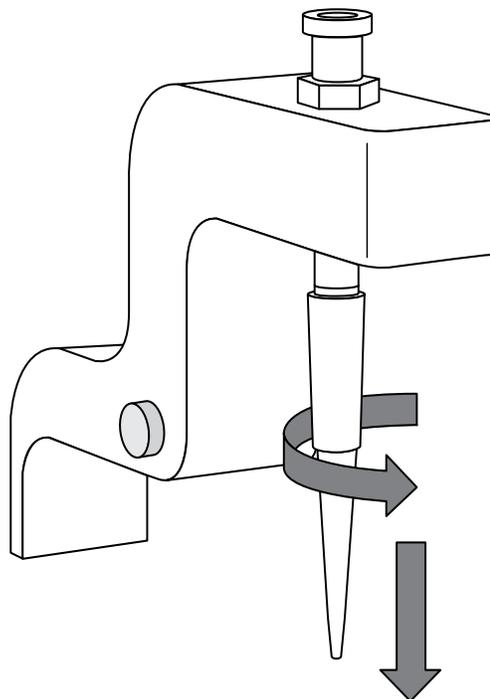
- Confirm that a new tip and open 0.2-mL PCR tube have been loaded in the Ion OneTouch™ ES Instrument, and that the 8-well strip is correctly loaded.
  - Ensure that Well 1 (ISP sample) is the left-most well and the 8-well strip is pushed to the right-most position in the slot.
1. Pipet the contents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

**Note:** To stop a run, press **Start/Stop**. The instrument completes the current step, then stops the run and displays "End". Press **Start/Stop** again to return the Tip Arm to the home position. It is not possible to restart (where you left off) after stopping a run.

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.



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

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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:

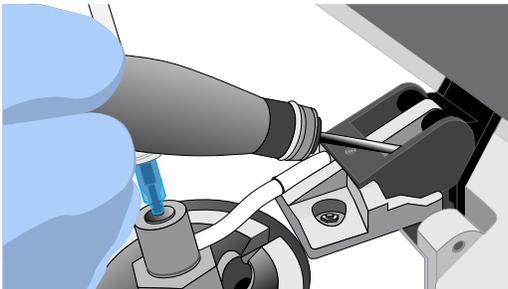
Quality assessment by	Action
Guava™ easyCyte™ 5 Flow Cytometer	Transfer a 1.0-μL aliquot of the enriched ISPs to a 1.5-mL Eppendorf LoBind™ Tube. Refer to the <i>Ion Sphere™ Particles (ISPs) Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin</i> (Pub. No. MAN0015799), available at: <a href="http://tools.thermofisher.com/content/sfs/manuals/MAN0015799.pdf">http://tools.thermofisher.com/content/sfs/manuals/MAN0015799.pdf</a>
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: <a href="http://tools.thermofisher.com/content/sfs/manuals/4477181A.pdf">http://tools.thermofisher.com/content/sfs/manuals/4477181A.pdf</a>



# Troubleshooting

## Ion OneTouch™ 2 Instrument

Observation	Possible cause	Recommended action
Firmware does not update or the status update screen does not display in ≤10 seconds	Firmware is not updating on the instrument.	<ol style="list-style-type: none"><li>1. Power the instrument OFF, then ON.</li><li>2. Ensure that the USB flash drive is FAT32-formatted and that the file is in the root directory.</li><li>3. Remove then reinsert the USB flash drive immediately after the main menu displays.</li><li>4. Repeat steps 1–3 as needed.</li></ol>
Disposable injector remains in "down" position in the Ion OneTouch™ DL Injector Hub	Reagent has built up around Injector Hub.	Clean any excess reagent from Injector Hub with moistened Kimwipes™ wipe.
	Newly installed Injector Hub does not move freely.	<ol style="list-style-type: none"><li>1. <i>Gently</i> pull from the top of the disposable injector until the disposable injector just returns to the "up" position in the Injector Hub.</li><li>2. Briefly press then release the spring-loaded top of the Injector Hub 5– 10 times at the point indicated by the arrow. You should hear a click.</li><li>3. If the Injector Hub remains in the "down" position, repeat step 2 once (up to 10 more clicks).</li></ol> <p><b>Note:</b> If the Injector Hub still does not move freely and click up into place, contact Technical Support.</p>

Observation	Possible cause	Recommended action
Centrifuge lid does not open	<ul style="list-style-type: none"> <li>• A power failure has occurred.</li> <li>• A software crash has occurred.</li> </ul>	<ol style="list-style-type: none"> <li>1. Slide a 1/8-inch L-wrench (hex wrench) or equivalent tool into the right hole and along the top edge of the centrifuge hinge:                     <div data-bbox="971 380 1479 669" style="text-align: center;">  </div> </li> <li>2. Push the tool into the hole until there is a slight compression of the tool against the instrument and the centrifuge lid unlocks and opens.</li> <li>3. Remove the tool from the hole, then open the lid.</li> <li>4. If necessary, troubleshoot the lid lock, then use the instrument normally. Do <i>not</i> force the lid open.</li> </ol>
The run fails	Various causes are possible.	<p>Retrieve the log files.</p> <p><b>IMPORTANT!</b> Do <i>not</i> turn off or power cycle the instrument until the log files are downloaded. If a run fails, contact Technical Support.</p>
Precipitate is visible in the Ion PGM™ Hi-Q™ Reagent Mix after vortexing	Thawed Reagent Mix has been stored at <2°C.	<ol style="list-style-type: none"> <li>1. Ensure that the solution is fully thawed.</li> <li>2. Vortex the solution for 30 seconds, then leave the tube at room temperature for 15 minutes.</li> <li>3. Vortex the solution again at maximum speed for 1 minute.</li> <li>4. Centrifuge the tube for 30 seconds.</li> <li>5. Visually inspect the solution at the bottom of the tube and verify that there is no residual precipitate. If precipitate is visible, then repeat steps 1–4.</li> </ol> <p><b>Note:</b> If precipitate is still visible, then contact Technical Support.</p> <ol style="list-style-type: none"> <li>6. Keep the Reagent Mix at room temperature during use.</li> <li>7. After use, store the solution at 2°C to 8°C.</li> </ol>
Excessive oil is present in the waste tray	Various causes are possible.	<ol style="list-style-type: none"> <li>1. Remove the oil waste tray, then appropriately dispose of the oil.</li> <li>2. Reinsert the tray into the slot, then push the tray back fully into the instrument.</li> <li>3. To further troubleshoot excessive oil from the instrument, contact Technical Support.</li> </ol>



## Ion OneTouch™ ES

For Ion OneTouch™ ES vertical and horizontal axis calibration and residual volume test procedures, see Chapter 3 of the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388).

Observation	Possible cause	Recommended action
Excessive foaming occurs	<ul style="list-style-type: none"><li>Instrument is improperly calibrated resulting in inadequate volume in one or more wells.</li><li>Fitting is loose.</li><li>Pipette tip is cracked.</li></ul>	<ol style="list-style-type: none"><li>Use the recommended volumes for all wells.</li><li>Ensure that fittings are tight, especially at the elbow fitting, and the pipette tip is not cracked.</li><li>If necessary, perform the residual volume test. If the residual volume test fails, then calibrate the instrument.</li></ol>
Brown pellet is present in centrifuged tube of enriched ISPs	Residual Dynabeads™ MyOne™ Streptavidin C1 Beads are present.	<ol style="list-style-type: none"><li>Pipet the suspension with the brown pellet up and down 10 times to resuspend the pellet.</li><li>Place the 0.2-mL PCR tube against a magnet such as a DynaMag™-2 magnet for 4 minutes.</li><li>Transfer the supernatant with the enriched ISPs to a new 0.2-mL PCR tube without disturbing the pellet of Dynabeads™ MyOne™ Streptavidin C1 Beads.</li><li>Sequence or store the enriched ISPs.</li></ol>

Observation	Possible cause	Recommended action
<p>E12, E22, or E23 errors display during the run or during calibration</p>	<p>Calibration values are out of range.</p>	<ol style="list-style-type: none"> <li>1. Power OFF the instrument and wait 3 seconds.</li> <li>2. While holding down <b>Vert. Adjust</b>, power ON the instrument. This step restores the factory default settings.</li> <li>3. Recalibrate the vertical axis:                             <p><b>Note:</b> The default setting for the vertical axis is 310. If the setting is &lt;310, the instrument will likely display an error, because the Tip Arm position is too high.</p> <ol style="list-style-type: none"> <li>a. Press the  (minus) button to lower the Tip Arm until the tip touches the shelf.</li> <li>b. Press the  (minus) button 8 more times. Typical vertical axis settings are ~340–370.</li> </ol> </li> <li>4. Recalibrate the horizontal axis: Press the  (plus) button to move the Tip Arm to the right until the tip touches the left tab of the strip.                             <p><b>Note:</b> The default setting for the horizontal axis is 625. Typical horizontal axis settings are ~640–670.</p> </li> </ol>
	<p>AC line voltage module is installed incorrectly.</p>	<ol style="list-style-type: none"> <li>1. Determine the voltage of the electrical outlet to plug in the Ion OneTouch™ ES.</li> <li>2. Align the arrow by the correct voltage on the AC line voltage module with the adjacent white arrow in the lower-right corner of the fuse socket.</li> </ol> <p>If the AC line voltage module is installed incorrectly:</p> <ol style="list-style-type: none"> <li>1. Gently remove the module with your fingernail or a small flathead screwdriver.</li> <li>2. Rotate the module so that the correct voltage on the module is aligned and adjacent to the white arrow in the lower right-hand corner of the fuse socket.</li> <li>3. Insert the AC line voltage module into the fuse socket.</li> </ol>



Observation	Possible cause	Recommended action
E12 or E22 error is displayed when the unit is initializing	<ul style="list-style-type: none"> <li>Fuse is installed incorrectly.</li> <li>Unit is below operating temperature.</li> <li>Program or calibration setting is bad, <i>or</i></li> <li>Tip Arm is not moving.</li> </ul>	<ol style="list-style-type: none"> <li>Ensure that the fuse module is installed correctly and that the unit is at its recommended operating temperature.</li> <li>Reboot the instrument: Power OFF the instrument, wait 3 seconds, then power ON the instrument.</li> <li>If the error persists, restore the factory defaults, then re-calibrate the instrument: <ol style="list-style-type: none"> <li>Power OFF the instrument and wait 3 seconds.</li> <li>While holding down <b>Vert. Adjust</b>, power ON the instrument. This step restores the factory default settings.</li> <li>Repeat 3a–3b as needed to restore the factory defaults.</li> <li>Calibrate the vertical and horizontal axes.</li> </ol> </li> </ol>
Either of the following: <ul style="list-style-type: none"> <li>E12 or E22 errors are displayed.</li> <li>Tip Arm does not move or moves slightly.</li> </ul>	AC line voltage module is installed incorrectly.	<ol style="list-style-type: none"> <li>Determine the voltage of the electrical outlet serving the Ion OneTouch™ ES.</li> <li>Align the arrow by the correct voltage on the AC line voltage fuse module with the adjacent white arrow in the lower-right corner of the fuse socket.</li> </ol> <p>If the AC line voltage fuse module is installed incorrectly:</p> <ol style="list-style-type: none"> <li>Gently remove the module with your fingernail or a small flathead screwdriver.</li> <li>Rotate the module so that the correct voltage on the module is aligned and adjacent to the white arrow in the lower right-hand corner of the fuse socket.</li> <li>Insert the AC line voltage fuse module into the fuse socket.</li> </ol>
	Instrument is not at the recommended operating temperature	Ensure that the Ion OneTouch™ ES is at an operating temperature of 60°F to 77°F (15°C to 25°C).
Solution overflows during run	Reagent volumes are overloaded.	Repeat with reagent volumes described in enrichment procedure.
Tip is causing 8-well strip to lift out of tray slot during run	Tip is not aligned vertically.	Perform the vertical calibration procedure.
Percent template-positive ISPs after enrichment is <50% as measured by flow cytometry	Multiple causes are possible.	Contact Technical Support.



Observation	Possible cause	Recommended action
<p>Problems with the strip position</p> <ul style="list-style-type: none"> <li>• Strip lifts up during strip push.</li> <li>• Strip lifts up when tip is raised from well.</li> <li>• Immediately after strip push, the strip is not in contact with the magnet.</li> </ul>	<p>Instrument is not calibrated properly.</p>	<ul style="list-style-type: none"> <li>• Perform horizontal calibration.</li> <li>• Perform vertical calibration.</li> </ul>
<p>Tip grinds into base of instrument and Code "1999" displays</p>	<ul style="list-style-type: none"> <li>• Unit is not calibrated properly.</li> <li>• Vertical calibration setting is too low or out-of-range.</li> </ul>	<ol style="list-style-type: none"> <li>1. Restore the factory default settings on the instrument: Hold down the vertical adjust button while powering ON the instrument. The instrument beeps several times.</li> <li>2. Re-calibrate the instrument.</li> <li>3. Perform a residual volume test.</li> </ol>
<p>Tip hits the top of the tray at start of run</p>	<p>Tray is not properly seated in the instrument.</p>	<p>Check for debris between the tray and the instrument, then reinstall the tray. Press down firmly to ensure that tray is fully seated in the instrument.</p>
<p>Error messages display</p>	<p>Various causes are possible.</p>	<ol style="list-style-type: none"> <li>1. Power the instrument OFF, then ON.</li> <li>2. If the error continues to display, restore the factory default settings on the instrument. Hold down the vertical adjust button while powering ON the instrument. The instrument beeps several times.</li> <li>3. Re-calibrate the instrument.</li> <li>4. Perform a residual volume test.</li> </ol>
<p>Instrument does not aspirate or dispense liquids</p>	<p>Fitting(s) are loose.</p>	<ul style="list-style-type: none"> <li>• Ensure that the Luer-Lok™ connections at the elbow on the Tip Arm and at the tubing on the rear syringe pump are finger-tight.</li> <li>• Ensure that the metal tip adapter fitting on the Tip Arm is finger-tight.</li> </ul> <p><b>IMPORTANT!</b> After any adjustments to the metal tip adapter, recalibrate the Ion OneTouch™ ES.</p>



## Ion Sphere™ Assay troubleshooting table

The following table contains troubleshooting information for unenriched ISPs tested with the Ion Sphere™ Assay on the Qubit™ 2.0 or Qubit™ 3.0 Fluorometer.

Qubit™ Fluorometer observation	Ion PGM™ System observation	Possible cause	Recommended action
<10% Templated ISPs	<ul style="list-style-type: none"> <li>• Lower loading</li> <li>• Lower % enriched</li> <li>• Lower key signal</li> <li>• Lower throughput</li> </ul>	Too little library input into template preparation	<ul style="list-style-type: none"> <li>• Increase library input to target 20–25% templated ISPs.</li> </ul> <p><i>or</i></p> <ul style="list-style-type: none"> <li>• Continue with sequencing; expect lower throughput.</li> </ul>
>30% Templated ISPs, but <70%	Increased number of filtered reads	Too much library input into template preparation	<ul style="list-style-type: none"> <li>• Decrease library input to target 20–25% templated ISPs.</li> </ul> <p><i>or</i></p> <ul style="list-style-type: none"> <li>• Continue with sequencing; expect lower throughput.</li> </ul>
>70% Templated ISPs	<ul style="list-style-type: none"> <li>• Increased % primer dimer filtered reads</li> <li>• Lower throughput</li> </ul>	Adapter dimer contaminating library, more likely in short amplicon, Ion AmpliSeq™ or miRNA libraries	<ul style="list-style-type: none"> <li>• Check Bioanalyzer™ traces for adapter dimer peak (Amplicon library or Ion AmpliSeq™ library peak around 70 bp; miRNA library peak around 60bp).</li> <li>• Re-purify library using Agencourt™ AMPure™ XP Kit clean-up steps as outlined in the appropriate user guides.</li> </ul>
	<ul style="list-style-type: none"> <li>• Low loading</li> <li>• Low % enriched</li> <li>• Lower throughput</li> <li>• High % filtered reads</li> </ul>	Ion OneTouch™ 2 Instrument underperformance	Troubleshoot with Technical Support or a Field Application Scientist.



# Quality control of Ion PGM™ Hi-Q™ ISPs

- Quality control using the Ion Sphere™ Assay on the Qubit™ Fluorometer . . . . 55
- Acceptance criteria for unenriched Ion PGM™ Hi-Q™ ISPs . . . . . 56
- Quality control using the Guava™ easyCyte™ 5 Flow Cytometer . . . . . 57
- Quality control using the Attune™ Acoustic Focusing Cytometer . . . . . 57

## Quality control using the Ion Sphere™ Assay on the Qubit™ Fluorometer

You can assess the percentage of templated ISPs in unenriched samples with the Ion Sphere™ Assay using the Ion Sphere™ Quality Control Kit (Cat. No. 4468656) and the Qubit™ 2.0 or Qubit™ 3.0 Fluorometer. For detailed protocols, see the

- *Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide* (Pub. No. MAN0016387) or
- *Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide* (Pub. No. MAN0016388)

available at [thermofisher.com/order/catalog/product/4468656](https://www.thermofisher.com/order/catalog/product/4468656).

## Acceptance criteria for unenriched Ion PGM™ Hi-Q™ ISPs

The optimal amount of library corresponds to the library dilution point that gives Percent Templated ISPs between 10–30%.

Samples that fall within the recommended range generally produce the most data; however, samples that fall outside of the recommended range can still meet the throughput specifications on the Ion chips.

The recommended optimal range is not intended to be a pass/fail criteria. The range provides guidance for the quality of the sample.

**Note:** If the results are outside the desired Percent Templated ISPs range, then increase or decrease the library input appropriately. See the “Ion Sphere™ Assay troubleshooting table” on page 54 for more information.

Percent Templated ISPs	Description
<10%	Sample contains an insufficient number of templated ISPs to achieve optimal loading density on the Ion Chip.
10–30%	Optimal amount of library.
>30%	Sample will yield multi-templated ISPs (mixed reads).

## Quality control using the Guava™ easyCyte™ 5 Flow Cytometer

The Guava™ easyCyte™ 5 Flow Cytometer can be used for quality assessment of unenriched and enriched Ion PGM™ Hi-Q™ Ion Sphere™ Particles generated for up to 400 base-read sequencing on the Ion OneTouch™ 2 System. For details, see the *Ion Sphere™ Particles Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. No. MAN0015799), available at [thermofisher.com](http://thermofisher.com).

## Quality control using the Attune™ Acoustic Focusing Cytometer

The Applied Biosystems™ Attune™ Acoustic Focusing Cytometer can be used for quality assessment of unenriched and enriched Ion PGM™ Hi-Q™ Ion Sphere™ Particles generated for up to 400 base-read sequencing on the Ion PGM™ Sequencer. For details, see the *Demonstrated Protocol: Ion Sphere™ Particles (ISPs) Quality Assessment using the Applied Biosystems™ Attune™ Acoustic Focusing Cytometer User Bulletin* (Pub. No. 4477181), available at [thermofisher.com](http://thermofisher.com).

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**IMPORTANT!** Thermo Fisher Scientific Demonstrated Protocols have been successfully demonstrated by research and development but not verified. There are no technical specifications for demonstrated protocols. Users assume all risk when using these protocols, and recognize that support for Thermo Fisher Scientific Demonstrated Protocols occurs through community discussion.

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# 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.
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## 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 OneTouch™ 2 System User Guide* (Pub. No. MAN0014388), available at [thermofisher.com](http://thermofisher.com).

## Chemical safety



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**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.
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## Biological hazard safety



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**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](http://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](http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf)
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# Documentation and support

## Customer and technical support

Visit [thermofisher.com/support](http://thermofisher.com/support) for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
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  - Software, patches, and updates
  - Training for many applications and instruments
- 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

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