iontorrent

Ion PI™ Hi-Q™ OT2 200 Kit

USER GUIDE

for use with:
Ion PI™ Hi-Q™ OT2 200 Kit
Ion OneTouch™ 2 System

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For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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Revision history: MAN0010857

Revision	Date	Description
C.0	12 January 2017	• Appendices C ("Set up, calibrate, and maintain the Ion OneTouch™ ES") and D ("Supplemental procedures") deleted. Users are referred to the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388) • ISP quality control sections updated and moved to new user guides: – *Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide* (Pub. No. MAN0016387) – *Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide* (Pub. No. MAN0016388) • Graphics enhanced • Web links updated • Ion OneTouch™ ES Instrument setup clarified • Minor clarifications and corrections made
B.0	23 March 2015	• Users advised to update Torrent Suite™ Software to v4.4.2 • Corrections and screenshot updates
A.0	19 December 2014	New user guide, which includes instructions on how to use the Ion PI™ Hi-Q™ OT2 200 Kit with the Ion OneTouch™ 2 System.

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About this guide

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

Purpose

This user guide describes how to use the Ion OneTouch™ 2 System to prepare enriched, template-positive Ion PI™ Ion Sphere™ Particles (ISPs) with 200 base-pair average insert libraries for sequencing on the Ion Proton™ 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 Ion PI™ Ion Sphere™ Particles containing clonally amplified DNA, using the Ion PI™ Hi-Q™ OT2 200 Kit with the Ion OneTouch™ 2 Instrument (see Chapter 3, "Prepare template-positive Ion PI™ ISPs").
- Enrich the template-positive ISPs with the Ion OneTouch™ ES (see Chapter 4, "Enrich the template-positive Ion PI™ ISPs").

IMPORTANT! Use *only* the Ion PI™ Hi-Q™ OT2 200 Kit (Cat. No. A26434) 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. Template-positive Ion PI™ Ion Sphere™ Particles prepared with this kit should only be used in conjunction with the Ion PI™ Hi-Q™ Sequencing 200 Kit (Cat. No. A26433 or A26772). See the *Ion PI™ Hi-Q™ Sequencing 200 Kit User Guide* (Pub. No. MAN0010947).

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.

1 Product information

Product description

The Ion PI™ Hi-Q™ OT2 200 Kit includes the reagents required for preparing 8 reactions of template-positive Ion PI™ Ion Sphere™ Particles (ISPs) on the Ion OneTouch™ 2 System. The Ion PI™ Hi-Q™ OT2 200 Kit can be used with up to 200 base-read average insert libraries of any type prepared using any available Ion library kit.

IMPORTANT! Use *only* the Ion PI™ Hi-Q™ OT2 200 Kit (Cat. No. A26434) 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.

Kit contents and storage

IMPORTANT! Follow these special reagent handling instructions:

- Ion PI™ Master Mix in the Ion PI™ Hi-Q™ OT2 200 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 PI™ Hi-Q™ OT2 200 Kit. Do not seal the reagent tubes with any plastic paraffin film such as Parafilm™ M film.

Ion PI™ Hi-Q™ OT2 200 Kit summary

Box	Part No.	Quantity per kit
Ion OneTouch™ 2 Supplies	A26367	1 box
Ion PI™ Hi-Q™ OT2 Reagents 200	A26428	1 box
Ion PI™ Hi-Q™ OT2 Solutions 200	A26429	1 box

Kit contents and storage conditions

Components[1]	Amount	Shipping and storage
Ion OneTouch™ 2 Supplies (Part No. A26367)		
Ion OneTouch™ Reagent Tubes	2 tubes	15ºC to 30ºC
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 [2]	8 adapters	
Ion OneTouch™ Reaction Filter	8 reaction filters and tubes	
Ion OneTouch™ ES Supplies[3]	1 bag	
Ion PI™ Hi-Q™ OT2 Reagents 200 (Part No. A26428)		
Ion PI™ Master Mix (violet cap) **IMPORTANT!** The Ion PI™ Master Mix is shipped at –30°C to –10°C. Immediately before use, thaw tube(s) as needed. After use, store the thawed Ion PI™ Master Mix at 2°C to 8°C.	8 × 2 mL	–30ºC to –10ºC (2ºC to 8ºC after thaw)
Ion PI™ Enzyme Mix (brown cap)	960 µL	–30ºC to –10ºC
Ion PI™ Ion Sphere™ Particles (black cap)	800 µL	
Ion PI™ Hi-Q™ OT2 Solutions 200 (Part No. A26429)		
Ion OneTouch™ Breaking Solution (black cap)	2 × 1.2 mL	15ºC to 30ºC
Ion OneTouch™ Oil	450 mL	
Ion OneTouch™ Reaction Oil	25 mL	
Nuclease-free Water	30 mL	
Ion OneTouch™ Recovery Solution	350 mL	
Ion OneTouch™ Wash Solution	16 mL	
Ion OneTouch™ ES Wash Solution	7.2 mL	
MyOne™ Beads Capture Solution (green cap)	2 × 1.5 mL	

Components[1]	Amount	Shipping and storage
ISP Resuspension Solution (red cap)	1.25 mL	15ºC to 30ºC
Tween™ Solution	6 mL	

[1] We have verified this protocol using this specific material. Substitution may adversely affect performance.
[2] Each Ion OneTouch™ Cleaning Adapter is used for *one* cleaning only.
[3] Ion OneTouch™ ES Supplies include 12 pipette tips and 1 box of ES 8-well strips.

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[1]	Source
	Ion OneTouch™ 2 System The system includes: • Ion OneTouch™ 2 Instrument • Ion OneTouch™ ES Instrument • AC Power Supply and Cords • Installation Kit	4474779
	Dynabeads™ MyOne™ Streptavidin C1 Magnetic Beads	65001 (2 mL) 65002 (10 mL)
	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[2]	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

[1] We have verified this protocol using this specific material. Substitution may adversely affect system performance.
[2] Must fit standard 0.2- and 1.5-mL microcentrifuge tubes; must generate 15,500 × *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**.

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 Proton™ 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[1]	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

[1] 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[1,2]	Source
	Ion PI™ Controls 200 Kit	4488985
	Benchtop absorbent paper or mat	MLS
	Bleach	MLS
	Ethernet cable	MLS
	1/8-inch L-wrench (hex wrench) or equivalent tool	MLS

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

[2] 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).

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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.

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 Proton™ System Site Preparation Guide* (Pub. No. 4478733).
- 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).

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 Ion PI™ Template OT2 200 v3 and Ion PI™ Hi-Q™ Template OT2 200 Kits and between Hi-Q™ 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 PI™ Ion Sphere™ Particles (ISPs) on the system with a control library.

1. Obtain the Human CEPH Control 200 Library from the Ion PI™ Controls 200 Kit (Cat. No. 4488985).

2. Dilute 1 µL of control library into 259 µL of Nuclease-free Water in an Eppendorf LoBind™ Tube. Use 100 µL of the dilution in the amplification solution (see "Prepare the amplification solution" on page 26).

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 16).

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 PI™ ISPs"). If you do not have one of these instruments, proceed to step 5.

5. Before starting a run on the Ion Proton™ System, create a Planned Run in the Torrent Browser, then select the Ion PI™ Hi-Q™ OT2 200 Kit. Load the sample, then analyze the results.

   Note: Use the Ion PI™ Hi-Q™ Sequencing 200 Kit (Cat. No. A26433). See the *Ion PI™ Hi-Q™ Sequencing 200 Kit User Guide* (Pub. No. MAN0010947).

6. Review the run report from the Torrent Browser and confirm successful sequencing results with the control library. If you have questions regarding the results, contact Technical Support.

   The instrument is ready for use.

3 Prepare template-positive Ion PI™ ISPs

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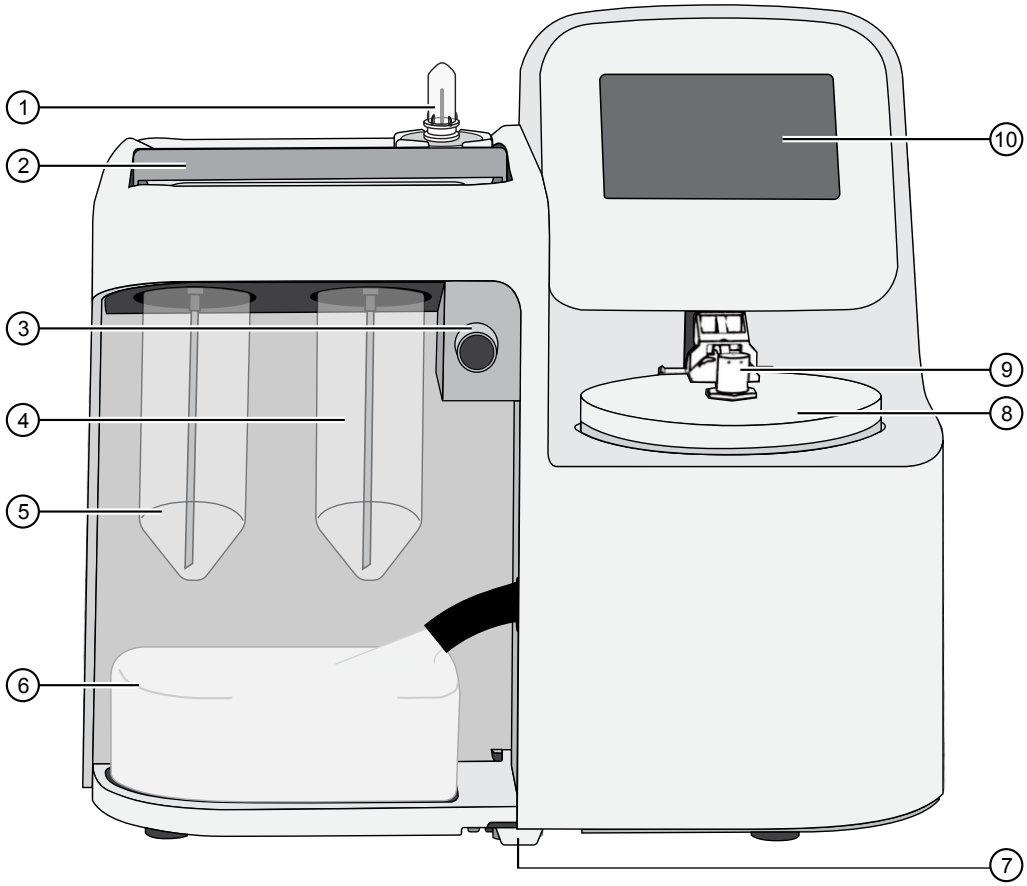
Ion OneTouch™ 2 Instrument layout



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

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

Run the Ion OneTouch™ 2 Instrument

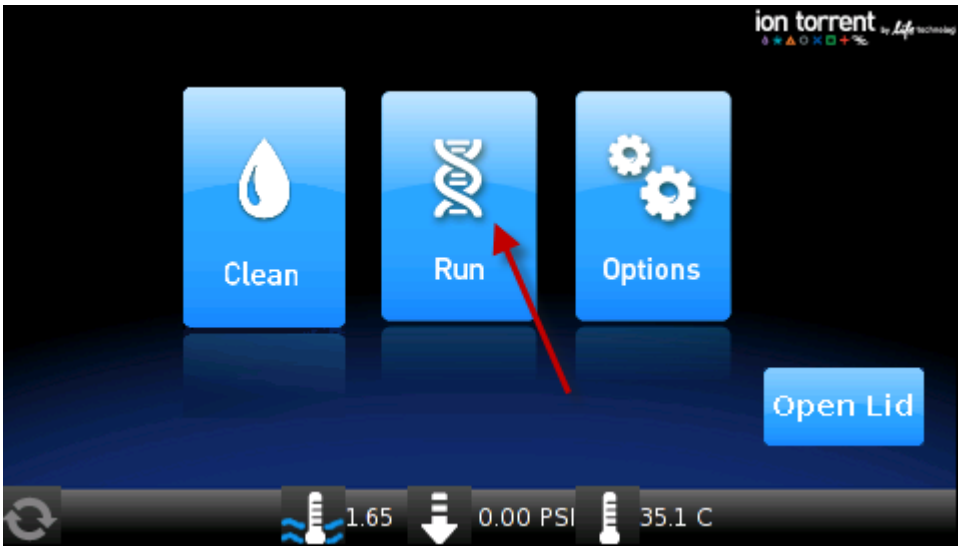
Note:

- 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 12).

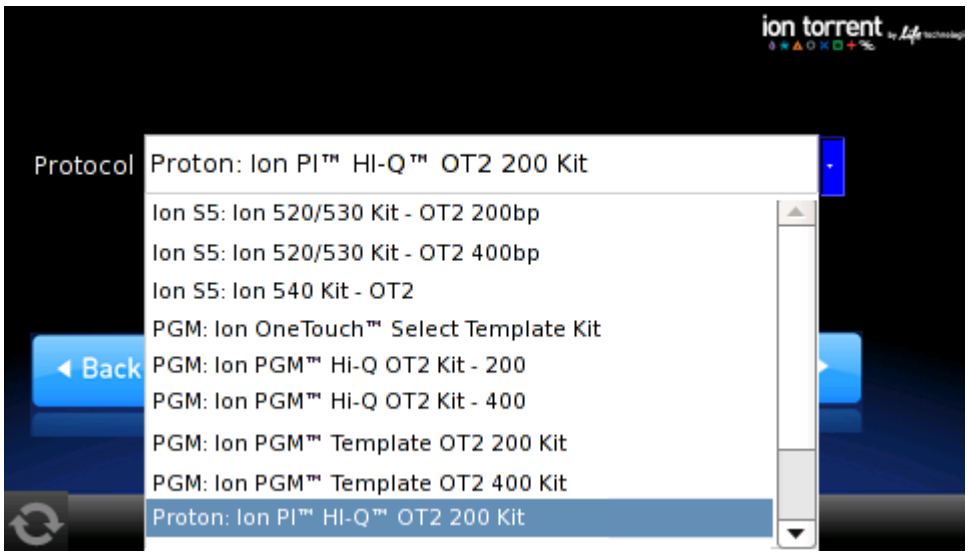
1. Ensure that the centrifuge lid of the Ion OneTouch™ 2 Instrument is closed.

   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.

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

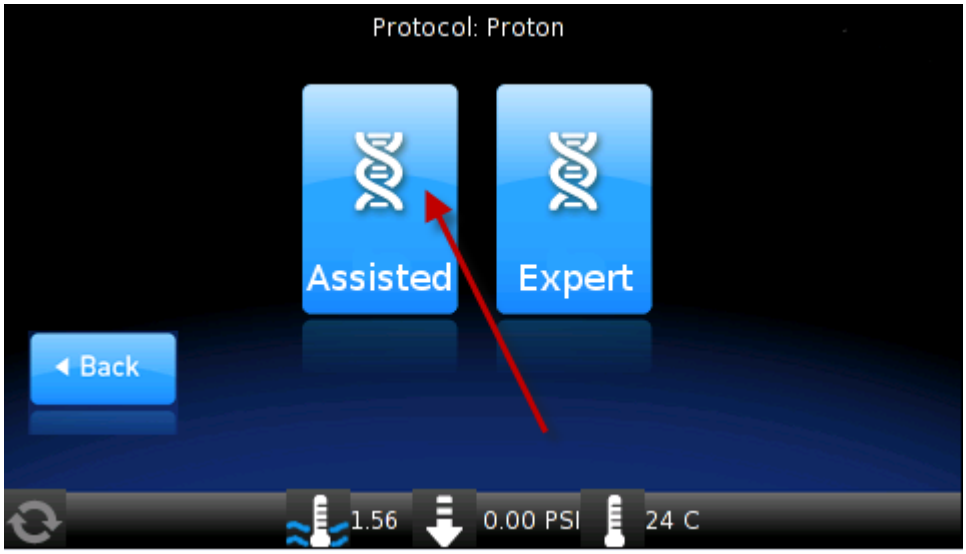


3. Touch the drop-down menu, then select **Proton: Ion PI™ Hi-Q™ OT2 200 Kit**:



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 25), 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 25. The instrument consumables are already installed and ready for the run.

Materials required

Provided in Ion OneTouch™ 2 Supplies (Part No. A26367):

- 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 PI™ Hi-Q™ OT2 Solutions 200 (Part No. A26429):

- 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! Follow these special reagent handling instructions:

- Use only the Ion PI™ Hi-Q™ OT2 200 Kit (Cat. No. A26434) with this user guide and with the Ion OneTouch™ 2 System. Do not mix reactions or disposables including plates, solutions, and kit reagents from other template preparation kits.
- Use only the supplied screw caps in the Ion PI™ Hi-Q™ OT2 200 Kit to store the reagents. Do not seal the reagents with any plastic paraffin film such as Parafilm™ M film.

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.

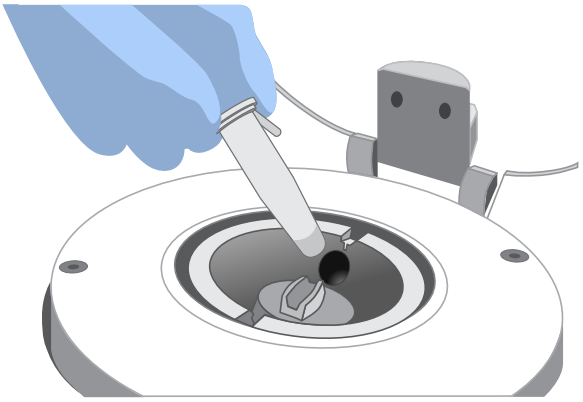
   IMPORTANT! Do *not* lift the lid by the tubing attached to the Ion OneTouch™ DL Injector Hub. Do *not* force the lid open.

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

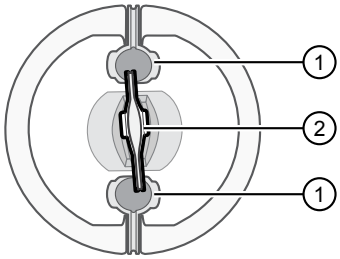
   Note: Breaking Solution is viscous. Draw and dispense slowly.

   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.

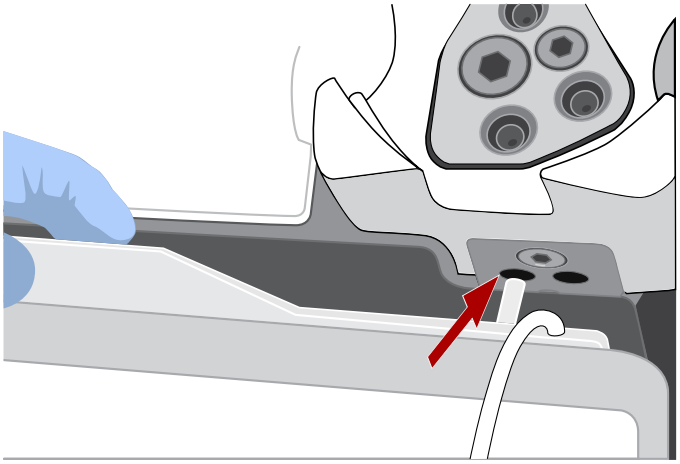
3. Insert the Amplification Plate:
   a. Inspect the Amplification Plate to ensure that the plate port is straight and perpendicular to the plate.

      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.

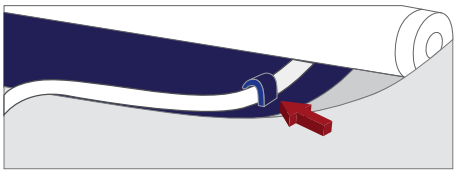
   b. Hold the disposable injector, connected to the disposable tubing, in one hand and the Amplification Plate in the other hand.

      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:



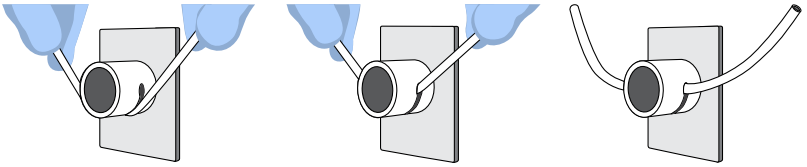
4. Pull the handle of the heat block to close the block, then thread the disposable tubing through the Ion OneTouch™ DL Tubing Catch:



   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:



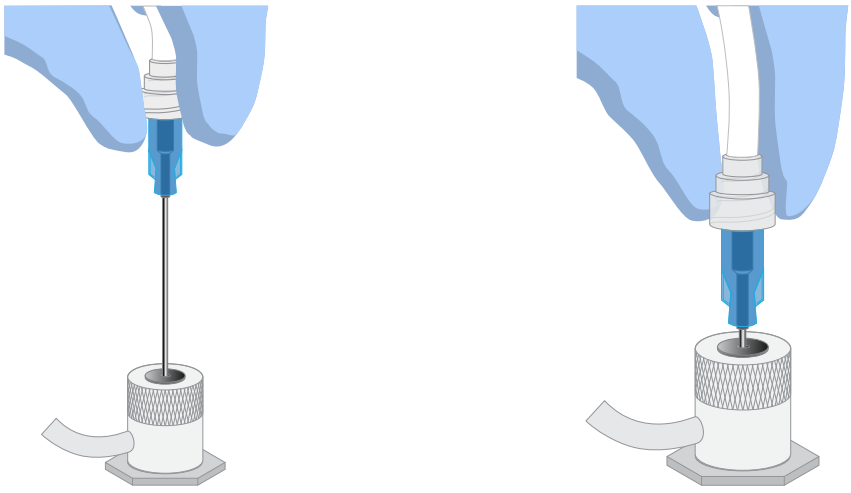
c. If necessary, adjust the disposable tubing along the notches of the open pinch valve so that there is sufficient length of disposable tubing to install the disposable injector (see "Install the disposable injector").

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.

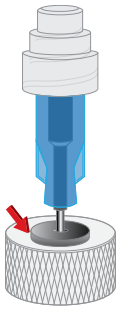
CAUTION! PHYSICAL INJURY HAZARD. The pointed end of the injector can puncture your skin. Keep your hand away from the point of the injector.



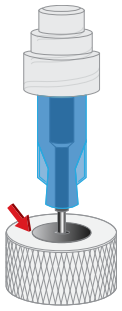
Note: The color of the injector may vary.

2. 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	1. Use a new Reagent Tube from the kit. 2. Discard the used Reagent Tube and Sipper Tube. Appropriately discard the residual Oil. 3. Use fresh gloves to attach the Luer-Lok™ end of a new Ion OneTouch™ Sipper Tube to the *left* front port. Do ***not*** let the Sipper Tube touch any surface. 4. Invert the Ion OneTouch™ Oil bottle (450-mL size) 3 times to mix, then fill the Reagent Tube *half*-full with Oil. Minimize bubbles. 5. Insert the filled Reagent Tube into the *left* front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument.
Refilling the Reagent Tube between runs	1. Remove the Reagent Tube from the instrument. 2. Invert Ion OneTouch™ Oil bottle (450-mL size) 3 times to mix. 3. Fill the Reagent Tube *half*-full with Oil. Minimize bubbles. **Note:** It is not necessary to re-mix the Oil. 4. Insert the filled Reagent Tube into the *left* front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument.

Install the Ion OneTouch™ Recovery Solution

IMPORTANT! Use only the Ion OneTouch™ Recovery Solution provided as part of the Ion PI™ Hi-Q™ OT2 200 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	1. Use a new Reagent Tube from the kit. 2. Discard the used Reagent Tube and Sipper Tube. Appropriately discard the Recovery Solution. 3. Use fresh gloves to attach the Luer-Lok™ end of a new Ion OneTouch™ Sipper Tube to the *right* front port. Do not let the Sipper Tube touch any surfaces. 4. Invert the Recovery Solution 3 times to mix, then fill the Reagent Tube *one-third*-full with Recovery Solution. Minimize bubbles. 5. Insert the filled Reagent Tube into the *right* front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument.
Refilling the Reagent Tube between runs	1. Remove the Reagent Tube from the instrument. 2. Invert the bottle of Recovery Solution 3 times. 3. Add more Recovery Solution to the solution in the Reagent Tube until the tube is *one-third*-full. Minimize bubbles. 4. Insert the filled Reagent Tube into the *right* front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument.

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 PI™ Hi-Q™ OT2 Solutions 200 (Part No. A26429):

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

Provided in Ion PI™ Hi-Q™ OT2 Reagents 200 (Part No. A26428):

- Ion PI™ Master Mix
- Ion PI™ Enzyme Mix
- Ion PI™ Ion Sphere™ Particles (ISPs)

Provided in Ion OneTouch™ 2 Supplies (Part No. A26367):

- Ion OneTouch™ Reaction Filter

***(Optional)* Provided in the Ion PI™ Controls 200 Kit (Cat. No. 4488985):**

- Human CEPH Control 200 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! Use *only* the Ion PI™ Hi-Q™ OT2 200 Kit with this user guide and with the Ion OneTouch™ 2 System. Do ***not*** mix reactions or disposables including plates, solutions, and kit reagents from other template preparation kits.

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

1. Prepare the reagents as follows:

Reagents	Preparation
Ion PI™ Master Mix	1. Allow the tube of Master Mix (2 mL) to come to room temperature before use. 2. Vortex the solution for 30 seconds, then centrifuge the solution for 2 seconds. **Note:** Visually inspect the solution at the bottom of the tube and verify that there is no residual precipitate. If precipitate is visible, see Appendix A, "Troubleshooting". 3. Keep the tube of Master Mix at room temperature during use. Store thawed Master Mix at 2ºC to 8ºC.
Ion PI™ Enzyme Mix	1. Centrifuge the enzyme for 2 seconds. 2. Place on ice.
Ion PI™ Ion Sphere™ Particles	Place the suspension at room temperature.

IMPORTANT! Use only Ion PI™ Ion Sphere™ Particles (ISPs) from the Ion PI™ Hi-Q™ OT2 200 Kit with the Ion OneTouch™ 2 System. Do ***not*** use ISPs from other or previously used kits.

2. Dilute the library as shown in the table below. Use the library dilution within 48 hours of preparation.

	Ion AmpliSeq™ DNA Library	Ion AmpliSeq™ RNA Library	gDNA Fragment or Amplicon Library	Ion TargetSeq™ Exome-Enriched Library	Ion Total RNA-Seq Library
Library concentra-tion	100 pM	100 pM	100 pM	100 pM	100 pM
Volume of library	6–8 µL	6–8 µL	6–8 µL	6–8 µL	6–8 µL
Volume of Nuclease-free Water	92–94 µL	92–94 µL	92–94 µL	92–94 µL	92–94 µL
Total volume of diluted library to add to the amplifica-tion solution	**100 µL**	**100 µL**	**100 µL**	**100 µL**	**100 µL**

Note: If you are troubleshooting the amplification process, to 259 µL of Nuclease-free Water, add 1 µL of the Human CEPH Control 200 Library from the Ion PI™ Controls 200 Kit (Cat. No. 4488985). Add 100 µL of this dilution to the amplification solution in place 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 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 the tube containing 2 mL of Master Mix at 15°C to 30°C, 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	—	80 µL
2	Ion PI™ Enzyme Mix	Brown	120 µL
3	Ion PI™ ISPs	Black	100 µL
4	Diluted library (*not* stock library)	—	100 µL
—	**Total volume (including Master Mix)**	—	**2400 µL**

5. Vortex the solution prepared in step 4 at maximum speed for 5 seconds, then centrifuge the solution for 2 seconds.

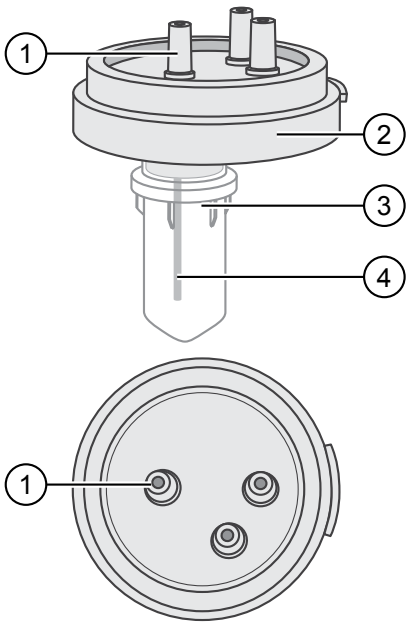
IMPORTANT! Start the run on the Ion OneTouch™ 2 Instrument ≤15 minutes after preparing the amplification solution.

6. Proceed *immediately* to "Fill 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.



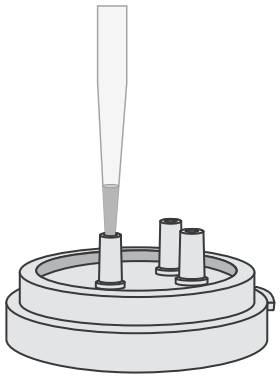
① Sample port
② Ion OneTouch™ Reaction Filter
③ Ion OneTouch™ Reaction Tube
④ Short tube from sample port into Ion OneTouch™ Reaction Tube

2. Set a P1000 pipette to 800 µL, and attach a new 1000-µL 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 800 µL 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 800 µL 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. Repeat steps 4–6 two more times, to add a total of 2.4 mL of amplification solution to the Reaction Filter.

8. If necessary, gently dab a Kimwipes™ disposable wipe around the ports to remove any liquid.

9. Set a P1000 pipette to 200 µL, and attach a new 1000-µL tip to the pipette.

10. Draw up 200 µL of Ion OneTouch™ Reaction Oil into the pipette tip.

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

12. Slowly pipet 200 µL 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.

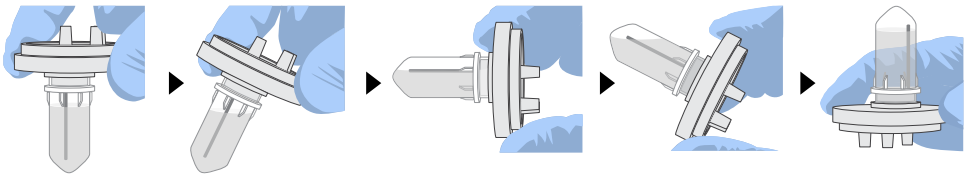
13. If necessary, gently dab a Kimwipes™ disposable wipe around the ports to remove any liquid.

Install the filled Ion OneTouch™ Reaction Filter

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

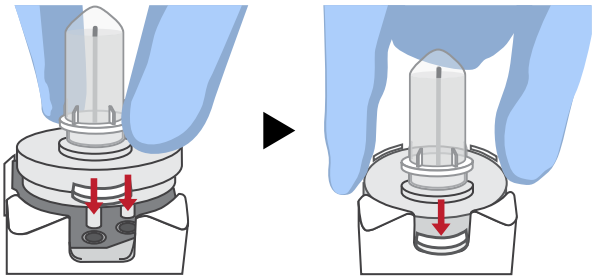
1. Position the tube rack containing the Ion OneTouch™ Reaction Filter so that the sample port on the filter is on the left.

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.



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

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:

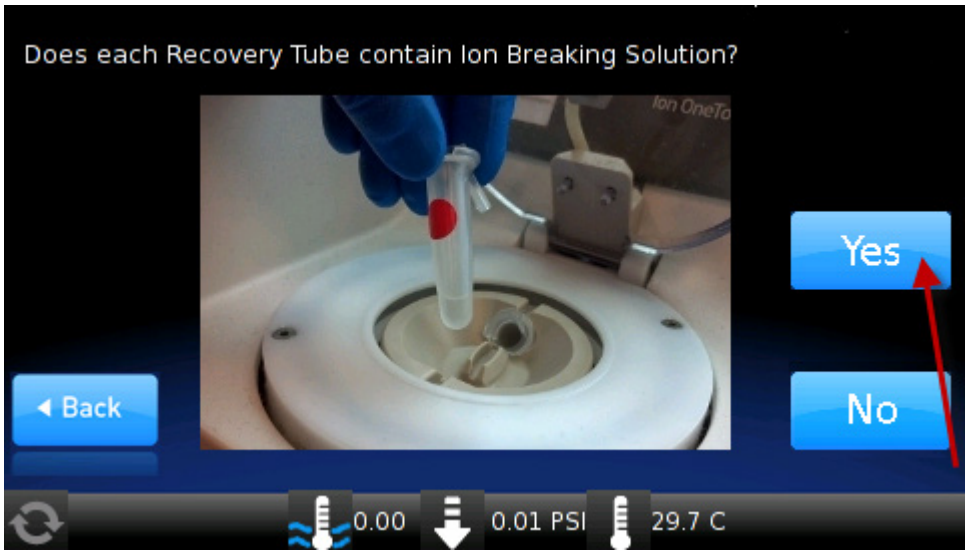


Note: After inserting the Ion OneTouch™ Reaction Filter, bubbles may shoot up into the Reaction Tube.

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 PI™ ISPs" on page 32.

   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 18).

7. Prepare a new amplification solution (see "Prepare and install the amplification solution" on page 25).

8. Start a new run.

Recover the template-positive Ion PI™ ISPs

Materials required

Provided in Ion PI™ Hi-Q™ OT2 Solutions 200 (Part No. A26429):

- Ion OneTouch™ Wash Solution
- ISP Resuspension Solution
- Nuclease-free Water

Other materials and equipment:

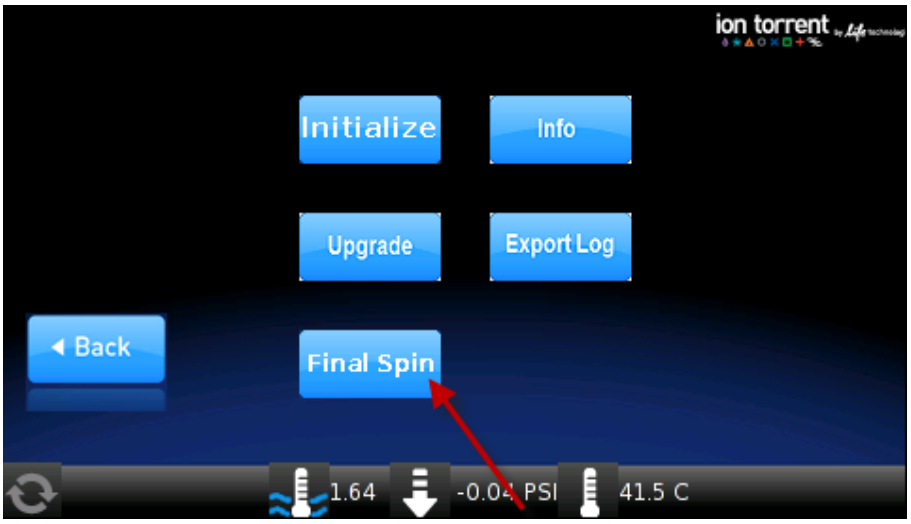
- Eppendorf™ DNA LoBind™ Microcentrifuge Tubes
- Pipettes
- Vortexer
- Microcentrifuge

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. When the centrifuge stops, *immediately* proceed to steps 3–5.

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

2. During the final centrifuge spin, place a 50-mL conical tube in a tube rack, then place the tube rack with the empty tube next to the instrument.

3. *Immediately* after the centrifuge stops:
   a. Gently pull the disposable tubing downwards on both sides of the pinch valve until the disposable tubing is out of the valve.
   b. Place one hand on the centrifuge lid, then with the other hand firmly grip the rigid plastic connector at the top of the disposable injector and steadily pull the disposable injector straight up from 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.

   c. Place the disposable injector into the empty 50-mL conical tube in the tube rack.

4. In the instrument display, touch **Open Lid**, wait until the lid clicks open, then remove and discard the Ion OneTouch™ Recovery Router.

5. *Carefully* remove both Ion OneTouch™ Recovery Tubes from the instrument, then put the two Recovery Tubes in a tube rack. You may see some cloudiness in the tube, which is normal.

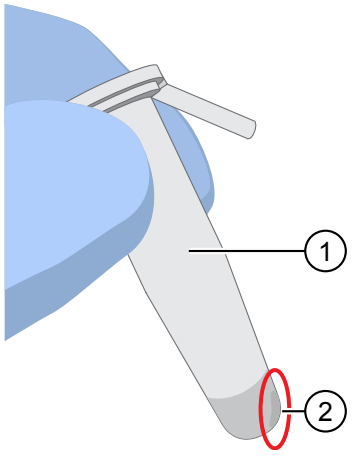
IMPORTANT! Do not store the recovered, template-positive ISPs at –30°C to –10°C. Proceed immediately to "Wash the template-positive ISPs". There is a stopping point in the next section.

Wash the template-positive ISPs

1. Use a pipette to remove all but ~100 µL of Ion OneTouch™ Recovery Solution from each Ion OneTouch™ Recovery Tube. Withdraw the supernatant from the surface and on the opposite side from the pellet. Do not disturb the pellet of template-positive ISPs:

2. Resuspend the template-positive ISPs in the remaining Recovery Solution in each tube by pipetting the suspension up and down.

3. Combine the suspension from each Recovery Tube into a new labeled 1.5-mL Eppendorf LoBind™ Tube.



① Ion OneTouch™ Recovery Tube
② Location of ISP pellet

4. Add 100 µL of Nuclease-free Water to each Recovery Tube, pipet up and down to mix, then transfer the residual beads into the labeled 1.5-mL tube.

5. Bring the combined suspensions in the labeled 1.5-mL tube to 1 mL with Nuclease-free Water.

   STOPPING POINT The ISPs can be stored at 2°C to 8°C for up to 3 days. If you stored the template-positive ISPs at 2°C to 8°C, proceed to step 8. Do not store the recovered ISPs in Ion OneTouch™ Recovery Solution.

6. Vortex for 30 seconds to completely resuspend the template-positive ISPs, then centrifuge the tube for 2 seconds.

7. (Optional) Assess the quality of the unenriched, template-positive ISPs:

If you have a	Action
Qubit™ 2.0 Fluorometer *or* Qubit™ 3.0 Fluorometer	Continue to step 8. You can perform quality control on the ISPs in step 12.
(Optional) Guava™ easyCyte™ 5 Flow Cytometer	Perform quality control on the diluted, unenriched ISPs. Transfer a 2.0-µL aliquot of the diluted,unenriched ISPs (from step 6) to a 1.5-mL Eppendorf LoBind™ Tube. See the *Ion PI™ Ion Sphere™ Particles (ISPs) Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. No. MAN0007496), available at **thermofisher.com**.

8. Centrifuge the template-positive ISP suspension for 8 minutes at 15,500 × *g*.

   Note: To convert the RPMs of your centrifuge to RCF in units of gravity, see **tools.thermofisher.com/content/sfs/brochures/TR0040-Centrifuge-speed.pdf**.

9. Remove all but 20 µL of supernatant.

10. Bring the combined washed suspensions in the new labeled tube to 100 µL in ISP Resuspension Solution.

11. Vortex the pellet for 30 seconds to completely resuspend the template-positive ISPs, then centrifuge the tube for 2 seconds.

12. (Optional) If you have not used the Guava™ easyCyte™ 5 Flow Cytometer to perform quality control on the ISPs, you can retain a sample at this point to evaluate quality of the resuspended, unenriched template-positive ISPs (step 11) using the Qubit™ 2.0 Fluorometer or Qubit™ 3.0 Fluorometer. Transfer a 2.0-µL aliquot of the resuspended, unenriched ISPs to a 0.2-mL PCR tube, then 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**, for further details.

Proceed to "Maintain the Ion OneTouch™ 2 Instrument" and Chapter 4, "Enrich the template-positive Ion PI™ ISPs". You can 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 recovered ISPs in Ion OneTouch™ Recovery Solution.

Maintain the Ion OneTouch™ 2 Instrument

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.

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 OneTouch™ 2 Supplies (Part No. A26367):

- Ion OneTouch™ Cleaning Adapter (single-use)

Provided in Ion PI™ Hi-Q™ OT2 Solutions 200 (Part No. A26429):

- 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 PI™ Hi-Q™ OT2 200 Kit from another kit?	See Chapter 5 of the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388). Use the reagents from the appropriate kit to maintain the Ion OneTouch™ 2 Instrument.
Already using the Ion PI™ Hi-Q™ OT2 200 Kit.	Proceed to step 2. Continue to use the reagents that are provided in the Ion PI™ Hi-Q™ OT2 200 Kit.

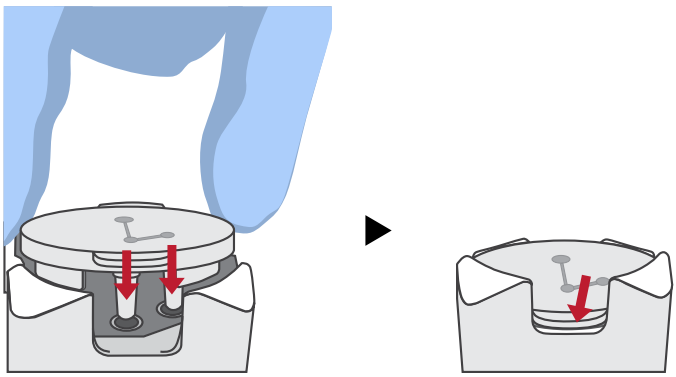
2. Check the level of Ion OneTouch™ Oil in the Reagent Tube. If the Reagent Tube has <20 mL of Oil, pour Oil into the Reagent Tube until it is half-full.

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.

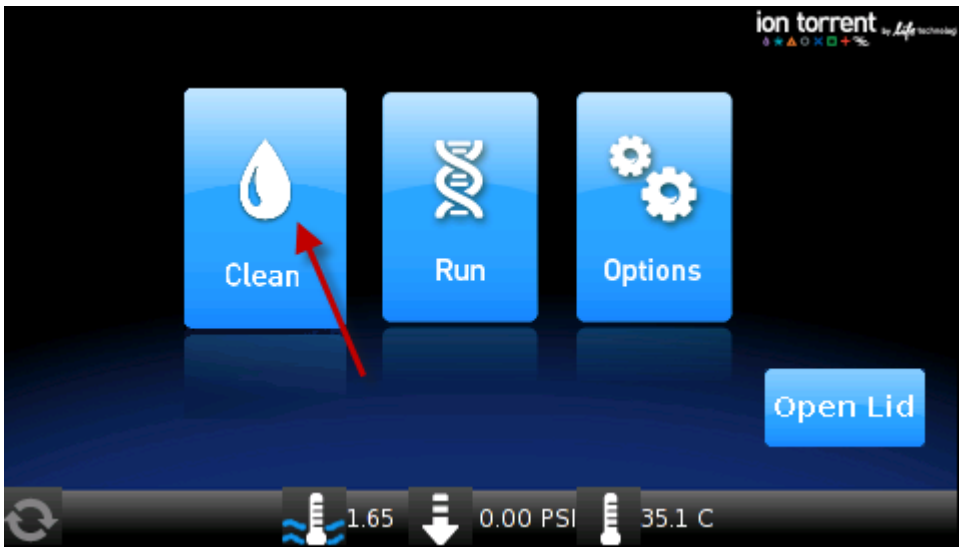
7. Gently pull the disposable tubing downwards on both sides of the pinch valve until the disposable tubing is out of the valve.

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.
    a. Push the handle to open the heat block.
    b. Remove the disposable tubing from the Ion OneTouch™ DL Catch.
    c. Gently pull back the Amplification Plate from the inlet and outlet holes of the instrument.
    d. Remove the Amplification Plate from the heat block, then appropriately discard the used Amplification Plate, injector, and tubing.
    e. 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.

4 Enrich the template-positive Ion PI™ ISPs

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- Prepare reagents then fill the 8-well strip 39
- Prepare the Ion OneTouch™ ES 41
- Perform the run 43
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- (Optional) Perform enriched ISP quality control 46

Materials required

Provided in Ion PI™ Hi-Q™ OT2 Solutions 200 (Part No. A26429):

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

Provided in Ion OneTouch™ 2 Supplies (Part No. A26367):

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

Other materials and equipment:

- 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 *Ion OneTouch™ 2 System User Guide* 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 39.

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.

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

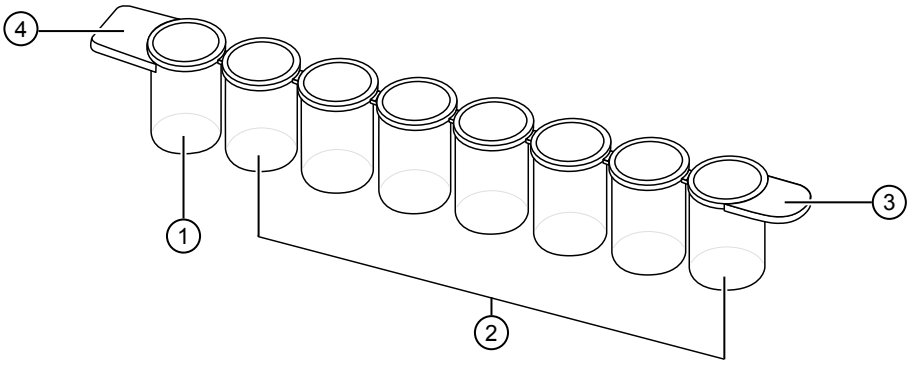
Wash and resuspend the Dynabeads™ MyOne™ Streptavidin C1 Beads

1. Vortex the tube containing the Dynabeads™ MyOne™ Streptavidin C1 Beads for 30 seconds to thoroughly resuspend the beads, then *immediately* proceed to the next step.
2. Transfer 100 µL of Dynabeads™ MyOne™ Streptavidin C1 Beads to a new 1.5-mL Eppendorf LoBind™ Tube.
3. Place the tube on a magnet such as a DynaMag™-2 magnet for 2 minutes, then remove and discard the supernatant without disturbing the pellet of Dynabeads™ MyOne™ Streptavidin C1 Beads.
4. Add 1 mL of Ion OneTouch™ Wash Solution to the aliquot of Dynabeads™ MyOne™ Streptavidin C1 Beads.
5. Remove the tube from the magnet, vortex the tube for 30 seconds, and centrifuge the tube for 2 seconds.
6. Place the tube on a magnet such as a DynaMag™-2 magnet for 2 minutes, then remove and discard the supernatant.
7. Add 130 µL of MyOne™ Beads Capture Solution to the Dynabeads™ MyOne™ Streptavidin C1 Beads.

   Note: You add the resuspended Dynabeads™ MyOne™ Streptavidin C1 Beads in the 130 µL MyOne™ Beads Capture Solution to Well 2 of the 8-well strip.
8. Remove the tube from the magnet, vortex the tube for 30 seconds, and centrifuge the tube 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 ISP Resuspension Solution. If the template-positive ISPs were stored at 2°C to 8°C, follow the washing procedure for ISPs (see "Wash the template-positive ISPs" on page 33).
2. Ensure that the square-shaped tab of the 8-well strip is on the *left*:



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

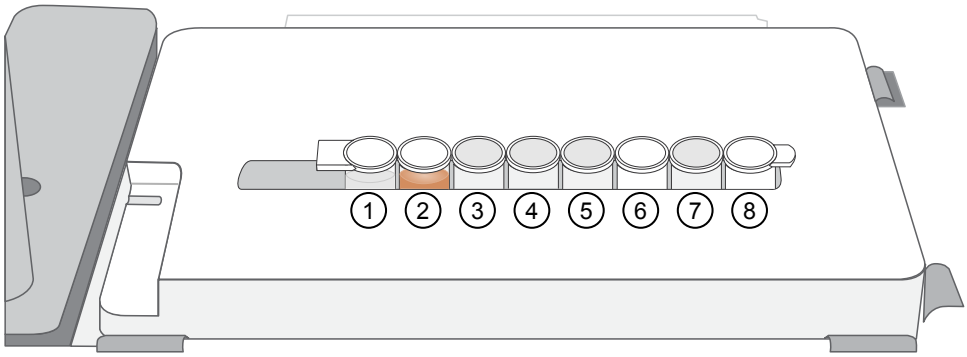
3. Pipet the ISPs up and down 10 times to mix, then transfer the entire volume (100 µL) of resuspended ISPs in ISP Resuspension Solution (step 1 of this procedure) into Well 1 of the 8-well strip.

4. Fill the remaining wells in the 8-well strip as follows (see the figure in step 5):

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 Capture Solution (prepared in "Wash and resuspend the Dynabeads™ MyOne™ Streptavidin C1 Beads" on page 40)
Well 3	300 µL of Ion OneTouch™ ES Wash Solution
Well 4	300 µL of Ion OneTouch™ ES Wash Solution
Well 5	300 µL of Ion OneTouch™ ES Wash Solution
Well 6	Empty
Well 7	300 µL of freshly-prepared Melt-Off Solution [prepared in "Prepare Melt-Off Solution" on page 39
Well 8	Empty

[1] Well closest to the square-shaped tab

5. 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:



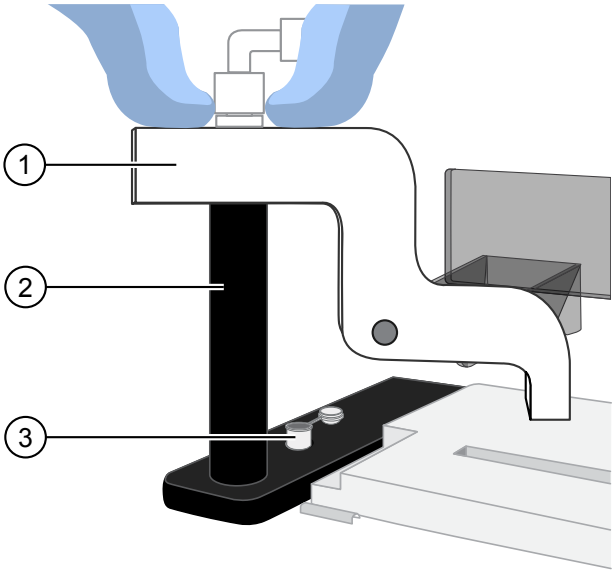
Prepare the Ion OneTouch™ ES

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. Insert an open 0.2-mL PCR tube into the hole in the base of the Tip Loader, as shown in the figure in step 3.

2. 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.

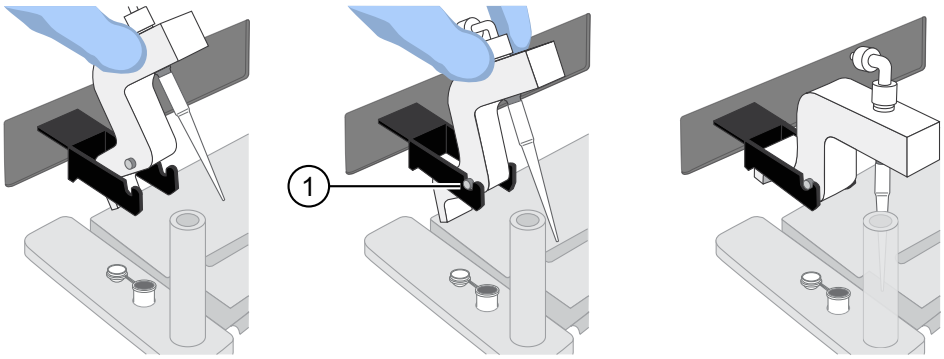
3. Keeping the fitting on the Tip Arm vertical, firmly press the Tip Arm down onto the new tip until the Tip Arm meets the Tip Loader. Hold the Tip Arm to the Tip Loader for ~1 second to ensure proper installation of the tip.



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

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

5. 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

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.

   IMPORTANT! Remove the enriched ISPs ≤15 minutes after the end of the run. Evaporation and prolonged exposure to the Melt-Off solution can cause ISP and DNA damage. Do not leave the enriched ISPs in Melt-Off solution overnight.

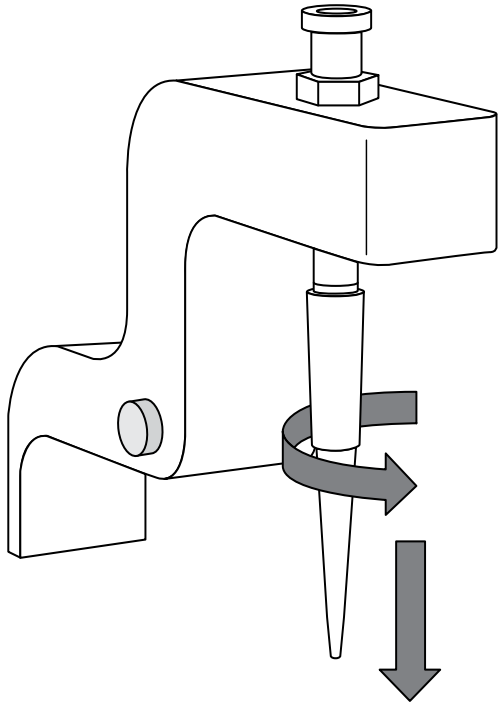
   Note: To stop a run, press **Start/Stop**. The instrument completes the current step, then stops the run and displays "End". Press **Start/Stop** again to return the Tip Arm to the home position. It is not possible to restart (where you left off) after stopping a run.

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.

   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 ~220 µL of Melt-Off Solution and Ion OneTouch™ Wash Solution. If the tube has <<200 µL of solution containing the enriched ISPs, contact Technical Support.

6. 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.



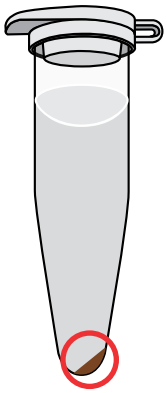
IMPORTANT! Improper removal of tips can loosen the metal tip adapter fitting on the Tip Arm and affect instrument operation.

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

Proceed immediately to "Remove and wash the enriched ISPs".

Remove and wash the enriched ISPs

1. Centrifuge the 0.2-mL PCR tube containing the enriched ISPs at 15,500 × *g* for 5 minutes.
2. Remove all but ~10 µL of supernatant without disturbing the pellet, then add 200 µL of Nuclease-free Water.
3. Pipet the solution up and down 10 times to resuspend the pellet. The pellet can be difficult to see.
4. Centrifuge the 0.2-mL PCR tube at 15,500 × *g* for 5 minutes.
5. Check for Dynabeads™ MyOne™ Streptavidin C1 Beads (a brown-tinted pellet) at the bottom of the centrifuged tube:



Are Dynabeads™ MyOne™ Streptavidin C1 Beads (brown pellet) present?	Then
No	1. Remove all but ~10 µL of supernatant without disturbing the pellet. 2. Add sufficient Nuclease-free Water for a final volume of 100 µL. 3. Pipet up and down 10 times to resuspend the pellet. 4. Sequence or store the template-positive ISPs: For the Ion PI™ Hi-Q™ OT2 200 Kit, use the Ion PI™ Hi-Q™ Sequencing 200 Kit (Cat. No. A26433 or A26772). See the *Ion PI™ Hi-Q™ Sequencing 200 Kit User Guide* (Pub. No. MAN0010947). *or* Store the enriched ISPs at 2°C to 8°C for up to 3 days.

Are Dynabeads™ MyOne™ Streptavidin C1 Beads (brown pellet) present?	Then
Yes	1. Pipet up and down 10 times to resuspend the pellet. 2. Place the 0.2-mL PCR tube against a magnet such as a DynaMag™-2 magnet for 4 minutes. 3. Transfer the supernatant to a new 0.2-mL PCR tube without disturbing the pellet. 4. Centrifuge the supernatant at 15,500 × *g* for 5 minutes. 5. Remove all but ~10 µL of supernatant without disturbing the pellet. 6. Add sufficient Nuclease-free Water for a final volume of 100 µL. 7. Pipet up and down 10 times to resuspend the pellet. 8. Sequence or store the template-positive ISPs: For the Ion PI™ Hi-Q™ OT2 200 Kit, use the Ion PI™ Hi-Q™ Sequencing 200 Kit (Cat. No. A26433 or A26772). See the *Ion PI™ Hi-Q™ Sequencing 200 Kit User Guide* (Pub. No. MAN0010947). *or* Store the enriched ISPs at 2°C to 8°C for up to 3 days.

Note: If you plan to run two Ion PI™ v3 Chips after a single initialization of the Ion Proton™ Sequencer, we recommend storing enriched, template-positive ISPs at 2°C to 8°C until sufficient ISPs are on hand to load the two chips. Then run the two chips on the same day to obtain optimal performance.

(Optional) Perform enriched ISP quality control

You can determine the appropriate library dilution and/or the enrichment efficiency by using the Guava™ easyCyte™ 5 Flow Cytometer. Transfer a 2.0-µL aliquot of the enriched ISPs to a 1.5-mL Eppendorf LoBind™ Tube. For more information, see the *Ion PI™ Ion Sphere™ Particles Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. No. MAN0007496), available at **thermofisher.com**.

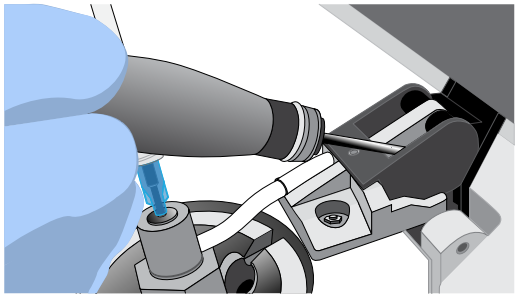
A

Troubleshooting

- Ion OneTouch™ 2 Instrument 47
- Ion OneTouch™ ES 49
- Ion Sphere™ Assay troubleshooting table 53

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.	1. Power the instrument OFF, then ON. 2. Ensure that the USB flash drive is FAT32-formatted and that the file is in the root directory. 3. Remove then reinsert the USB flash drive immediately after the main menu displays. 4. Repeat steps 1–3 as needed.
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.	1. *Gently* pull from the top of the disposable injector until the disposable injector just returns to the "up" position in the Injector Hub. 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. 3. If the Injector Hub remains in the "down" position, repeat step 2 once (up to 10 more clicks). **Note:** If the Injector Hub still does not move freely and click up into place, contact Technical Support.

Observation	Possible cause	Recommended action
Centrifuge lid does not open	• A power failure has occurred. • A software crash has occurred.	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:  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. 3. Remove the tool from the hole, then open the lid. 4. If necessary, troubleshoot the lid lock, then use the instrument normally. Do *not* force the lid open.
The run fails	Various causes are possible.	Retrieve the log files. **IMPORTANT!** Do *not* turn off or power cycle the instrument until the log files are downloaded. If a run fails, contact Technical Support.
Precipitate is visible in the Ion PI™ Master Mix after vortexing	Thawed Master Mix has been stored at <2°C.	1. Ensure that the solution is fully thawed. 2. Vortex the solution for 30 seconds, then leave the tube at room temperature for 15 minutes. 3. Vortex the solution again at maximum speed for 1 minute. 4. Centrifuge the tube for 30 seconds. 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. **Note:** If precipitate is still visible, then contact Technical Support. 6. Keep the Master Mix at room temperature during use. 7. After use, store the solution at 2°C to 8°C.

Observation	Possible cause	Recommended action
Recovery Tubes filled with gel after run.	Breaking Solution was not added to Recovery Tubes before start of run.	1. Add 150 µL Ion OneTouch™ Breaking Solution to each Recovery Tube. 2. Seal the Recovery Tubes with Parafilm™ M film and vigorously invert and vortex the tubes for 10 seconds to break the emulsion. 3. Follow the Final centrifuge procedure (see "Recover the template-positive Ion PI™ ISPs" on page 32) to recover the ISPs. Alternatively, transfer samples to two 1.5-mL tubes and centrifuge in an Eppendorf™ centrifuge for 10 minutes at 15,500 × g to pellet the ISPs. 4. Proceed to the ISP washing steps (see "Wash the template-positive ISPs" on page 33). Perform the ISP wash in 1-mL Nuclease-free Water (steps 5–9) twice.

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	• Instrument is improperly calibrated resulting in inadequate volume in one or more wells. • Fitting is loose. • Pipette tip is cracked.	1. Use the recommended volumes for all wells. 2. Ensure that fittings are tight, especially at the elbow fitting, and the pipette tip is not cracked. 3. If necessary, perform the residual volume test. If the residual volume test fails, then calibrate the instrument.

Observation	Possible cause	Recommended action
E12, E22, or E23 errors display during the run or during calibration	Calibration values are out of range.	1. Power OFF the instrument and wait 3 seconds. 2. While holding down **Vert. Adjust**, power ON the instrument. This step restores the factory default settings. 3. Recalibrate the vertical axis: **Note:** The default setting for the vertical axis is 310. If the setting is <310, the instrument will likely display an error, because the Tip Arm position is too high. a. Press the ▼ (minus) button to lower the Tip Arm until the tip touches the shelf. b. Press the ▼ (minus) button *8* more times. Typical vertical axis settings are ~340–370. 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. **Note:** The default setting for the horizontal axis is 625. Typical horizontal axis settings are ~640–670.
	AC line voltage module is installed incorrectly.	1. Determine the voltage of the electrical outlet to plug in the Ion OneTouch™ ES. 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. If the AC line voltage module is installed incorrectly: 1. Gently remove the module with your fingernail or a small flathead screwdriver. 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. 3. Insert the AC line voltage module into the fuse socket.

Observation	Possible cause	Recommended action
E12 or E22 error is displayed when the unit is initializing	• Fuse is installed incorrectly. • Unit is below operating temperature. • Program or calibration setting is bad, *or* • Tip Arm is not moving.	1. Ensure that the fuse module is installed correctly and that the unit is at its recommended operating temperature. 2. Reboot the instrument: Power OFF the instrument, wait 3 seconds, then power ON the instrument. 3. If the error persists, restore the factory defaults, then re-calibrate the instrument: a. Power OFF the instrument and wait 3 seconds. b. While holding down **Vert. Adjust**, power ON the instrument. This step restores the factory default settings. c. Repeat 3a–3b as needed to restore the factory defaults. d. Calibrate the vertical and horizontal axes.
Either of the following: • E12 or E22 errors are displayed. • Tip Arm does not move or moves slightly.	AC line voltage module is installed incorrectly.	1. Determine the voltage of the electrical outlet serving the Ion OneTouch™ ES. 2. 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. If the AC line voltage fuse module is installed incorrectly: 1. Gently remove the module with your fingernail or a small flathead screwdriver. 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. 3. Insert the AC line voltage fuse module into the fuse socket.
	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
Problems with the strip position • Strip lifts up during strip push. • Strip lifts up when tip is raised from well. • Immediately after strip push, the strip is not in contact with the magnet.	Instrument is not calibrated properly.	• Perform horizontal calibration. • Perform vertical calibration.
Tip grinds into base of instrument and Code "1999" displays	• Unit is not calibrated properly. • Vertical calibration setting is too low or out-of-range.	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. 2. Re-calibrate the instrument. 3. Perform a residual volume test.
Tip hits the top of the tray at start of run	Tray is not properly seated in the instrument.	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.
Error messages display	Various causes are possible.	1. Power the instrument OFF, then ON. 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. 3. Re-calibrate the instrument. 4. Perform a residual volume test.
Instrument does not aspirate or dispense liquids	Fitting(s) are loose.	• 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. • Ensure that the metal tip adapter fitting on the Tip Arm is finger-tight. **IMPORTANT!** After any adjustments to the metal tip adapter, recalibrate the Ion OneTouch™ ES.

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 Proton™ System observation	Possible cause	Recommended action
<10% Templated ISPs	• Lower loading • Lower % enriched • Lower key signal • Lower throughput	Too little library input into template preparation	• Increase library input to target 20–25% templated ISPs. *or* • Continue with sequencing; expect lower throughput.
>25% Templated ISPs, but <70%	Increased number of filtered reads	Too much library input into template preparation	• Decrease library input to target 20–25% templated ISPs. *or* • Continue with sequencing; expect lower throughput.
>70% Templated ISPs	• Increased % primer dimer filtered reads • Lower throughput	Adapter dimer contaminating library, more likely in short amplicon, Ion AmpliSeq™ or miRNA libraries	• Check Bioanalyzer™ traces for adapter dimer peak (Amplicon library or Ion AmpliSeq™ library peak around 70 bp; miRNA library peak around 60bp). • Re-purify library using Agencourt™ AMPure™ XP Kit clean-up steps as outlined in the appropriate user guides.
	• Low loading • Low % enriched • Lower throughput • High % filtered reads	Ion OneTouch™ 2 Instrument underperformance	Troubleshoot with Technical Support or a Field Application Scientist.

B

Quality control of Ion PI™ ISPs

- Quality control using the Ion Sphere™ Assay on the Qubit™ Fluorometer 54
- Acceptance criteria for unenriched Ion PI™ ISPs 54
- Quality control using the Guava™ easyCyte™ 5 Flow Cytometer 55

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**.

Acceptance criteria for unenriched Ion PI™ ISPs

The optimal amount of library corresponds to the library dilution point that gives Percent Templated ISPs between 10–25%.

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 53 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.
10–25%	Optimal amount of library.
>25%	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 PI™ Ion Sphere™ Particles generated for up to 200 base-read sequencing on the Ion Proton™ System. For details, see the *Ion PI™ Ion Sphere™ Particles Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. No. MAN0007496), available at **thermofisher.com**.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Instrument safety

For detailed information on instrument safety symbols and alerts, safety and electromagnetic compatibility standards, and general instrument safety, see the Safety appendix of the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388), available at **thermofisher.com**.

Chemical safety

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

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

Biological hazard safety

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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Customer and technical support

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.