**ion torrent**

by *life* technologies™

# Ion PI™ Template OT2 200 Kit v2

for use with: the Ion OneTouch™ 2 System

**Catalog Number**  4485146

**Publication Number**  MAN0007624
**Revision**  A.0

*life* technologies™

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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.

**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) for up to 200 base-pair sequencing of a library 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™ Template OT2 200 Kit v2 with the Ion OneTouch™ 2 Instrument (see "Prepare template-positive Ion PI™ Ion Sphere™ Particles" on page 19).

- Enrich the template-positive Ion PI™ Ion Sphere™ Particles with the Ion OneTouch™ ES (see "Enrich the template-positive Ion PI™ Ion Sphere™ Particles" on page 49).

**IMPORTANT!** Use *only* the Ion PI™ Template OT2 200 Kit v2 (Cat. no. 4485146) 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.

# Revision history

| Revision | Date | Description of change |
|----------|------|------------------------|
| A.0 | 15 January 2014 | • Users recommended to upgrade firmware after updating to Torrent Server Software v4.0.2<br>• Library dilution table on page 31 amended.<br>• Version numbering changed to alphanumeric format and reset to A.0 in conformance with internal document control procedures. |
| 2.0 | 01 July 2013 | • Support added for Ion AmpliSeq™ Exome libraries. Exome library users instructed to use Ion PI™ Ion Sphere™ Particles XT provided in the Ion AmpliSeq™ Exome Kit in the amplification solution in place of the standard Ion PI™ Ion Sphere™ Particles.<br>• Library dilution table added to "Prepare and install the amplification solution" on page 29. |
| 1.0 | 10 May 2013 | Provided detailed, step-by-step instructions on use of a new template preparation kit with the Ion Proton™ System: the Ion PI™ Template OT2 200 Kit v2. |

# Equipment use

The Ion OneTouch™ 2 System is an automated system used for the generation of template-positive Ion PI™ Ion Sphere™ Particles for semiconductor sequencing.

# 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.

**The Ion OneTouch™ 2 System is For Research Use Only. Not for use in diagnostic procedures.**

# Contamination

⚠ **CAUTION!** A primary source of contamination is spurious DNA fragments from previous sample processing steps. Do not introduce amplified DNA into library preparation laboratory or work area.

# Instrument clearances

**Ion OneTouch™ 2 Instrument and Ion OneTouch™ ES Space Requirements and Clearances:** Position the instrument so that the front is a minimum of 12 in. (30.5 cm) from the front of the laboratory bench. Place the instrument at least 40 in. (1 meter) away from major sources of electronic noise such as refrigerators or microwaves. For more information, refer to the *Ion Proton™ System Site Preparation Guide* (Pub. no. 4478733).

# 1 | Product information

## Kit contents and storage conditions

**Ion PI™ Template OT2 200 Kit v2**

**IMPORTANT!** Use *only* the Ion PI™ Template OT2 200 Kit v2 (Cat. no. 4485146) 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.

The Ion PI™ Template OT2 200 Kit v2 includes the reagents required for preparing 8 reactions of template-positive Ion PI™ Ion Sphere™ Particles (ISPs) on the Ion OneTouch™ 2 System.

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

- Ion PI™ Reagent Mix TL in the Ion PI™ Template OT2 200 Kit v2 is shipped at –30°C to –10°C and should be thawed before use. Store the thawed Ion PI™ Reagent Mix TL at 2°C to 8°C.
- Use only the supplied screw caps in the Ion PI™ Template OT2 200 Kit v2 to store the reagents. Do not seal the reagents with any plastic paraffin film such as Parafilm® M film.

| Box | Components† | Cap Color | Quantity | Volume | Storage |
|---|---|---|---|---|---|
| Ion PI™ Template OT2 Reactions 200 Kit v2 (Part no. 4485145) | Ion PI™ Plus Reaction Filter Assembly | — | 8 reaction filters and tubes | — | 15°C to 30°C |
| Ion PI™ Template OT2 Supplies 200 Kit v2 (Part no. 4485144) | Ion OneTouch™ Reagent Tubes | — | 2 tubes | — | 15°C to 30°C |
| | Ion OneTouch™ Recovery Routers | — | 8 routers | — | |
| | Ion PI™ Template OT2 Recovery Tubes | — | 16 tubes | — | |
| | Ion OneTouch™ Sipper Tubes | — | 2 tubes | — | |
| | Ion OneTouch™ 2 Amplification Plates | — | 8 plates | — | |
| | Ion OneTouch™ ES Supplies‡ | — | 1 bag | — | |
| | Ion OneTouch™ 2 Cleaning Adapters§ | — | 8 adapters | — | |

| Box | Components[†] | Cap Color | Quantity | Volume | Storage |
|---|---|---|---|---|---|
| Ion PI™ Template OT2 Reagents 200 Kit v2 (Part no. 4485142) | Ion PI™ Enzyme Mix TL | Brown | 1 tube | 600 µL | −30°C to −10°C |
| | Ion PI™ Ion Sphere™ Particles | Black | 1 tube | 800 µL | |
| | Ion PI™ PCR Reagent X | White | 1 tube | 480 µL | |
| | Ion PI™ Reagent Mix TL[††] | Violet | 8 tubes | 750 µL each | −30°C to −10°C (2°C to 8°C after thaw) |
| Ion PI™ Template OT2 Solutions 200 Kit v2 (Part no. 4485143) | Ion Proton™ OT2 Oil | — | 1 bottle | 450 mL | 15°C to 30°C |
| | Ion Proton™ OT2 Reaction Oil | — | 1 vial | 22 mL | |
| | Ion PI™ PCR Reagent B | Blue | 2 tubes | 1.8 mL | |
| | Nuclease-Free Water | — | 2 tubes | 15 mL each | |
| | Ion PI™ OT2 Recovery Solution | — | 1 bottle | 350 mL | |
| | Ion OneTouch™ Wash Solution | — | 2 vials | 16 mL each | |
| | Ion PI™ MyOne™ Beads Capture Solution | Green | 2 tubes | 1.5 mL each | |
| | Ion PI™ ISP Resuspension Solution | Red | 1 tube | 1.25 mL | |

† Life Technologies has validated this protocol using this specific material. Substitution may adversely affect performance.

‡ The Ion OneTouch™ ES Supplies include 12 Eppendorf® LoRetention Dualfilter, 300 µL, PCR pipette tips (Fisher Cat. no. 02-717-342), and loose 8-well strips. The ES supplies may be located at the bottom of the box.

§ Each Ion OneTouch™ 2 Cleaning Adapter is used for one cleaning *only*.

†† The Ion PI™ Reagent Mix TL is supplied in single-use tubes.

# Required materials and equipment

**Required materials for the Ion OneTouch™ 2 System**

The Ion OneTouch™ 2 System uses common molecular biology equipment, supplies, and reagents.

| ✔ | Description[†] | Supplier | Cat. number | Quantity |
|---|---|---|---|---|
| | Ion OneTouch™ 2 System<br><br>The system includes:<br>• Ion OneTouch™ 2 Instrument<br>• Ion OneTouch™ ES<br>• AC Power Supply and Cords<br>• Installation Kit<br>• Elbow fitting for Ion OneTouch™ ES | Life Technologies | 4474779 | 1 |
| | Dynabeads® MyOne™ Streptavidin C1 Magnetic Beads | Life Technologies | 65001<br>65002 | 2 mL<br>10 mL |

| ✔ | Description† | Supplier | Cat. number | Quantity |
|---|---|---|---|---|
| | PCR workstation | ISC BioExpress (or comparable) | H-6590-3B | 1 |
| | 1.5-mL Eppendorf LoBind® Tubes | Eppendorf | 022431021 | 250/box |
| | 2-mL Eppendorf LoBind® Tubes | Eppendorf | 022431048 | 250/box |
| | Microcentrifuge‡ | Major Laboratory Suppliers (MLS) | — | 1 |
| | Pipettes (P2, P20, P200, P1000) and appropriate low-retention tips | MLS | — | 1 each |
| | Vortexer with a rubber platform | MLS | — | 1 |
| | Tube rack to fit 15-mL conical tube | MLS | — | 1 |
| | Tube rack for 50-mL conical tube | MLS | — | 1 |
| | Heat block set to 50°C | MLS | — | 1 |

† Life Technologies has demonstrated this protocol using this specific material. Substitution may adversely affect system performance.

‡ Must fit standard 1.5- and 0.2-mL microcentrifuge tubes; must generate 15,500 × *g*.

**Note:** We recommend using an uninterruptable 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, the Ion Proton™ System, and the Ion Proton™ Torrent Server. Use a surge protector or line conditioner as needed (see, ).

## Additional materials required for the Ion OneTouch™ ES

| ✔ | Description† | Supplier | Cat. number | Quantity |
|---|---|---|---|---|
| | DynaMag™-2 magnet | Life Technologies | 12321D | 1 |
| | 0.2-mL PCR tubes | Axygen | PCR-02-L-C | 1000/case |
| | | BioExpress | T-3035-1 | 120 strips of 8 |
| | Xiameter® PMX-200 Silicone Fluid‡ | Neely Industries | PMX200-12500PT | 1.95 lbs. |
| | 1M NaOH | Major Laboratory Suppliers (MLS) | — | — |
| | Tween® 20 Molecular Biology Grade Detergent | MLS | — | — |

† Life Technologies has demonstrated this protocol using this specific material. Substitution may adversely affect system performance.

‡ Material required for periodic maintenance of the Ion OneTouch™ ES.

# Recommended materials and equipment for the Ion OneTouch™ 2 System

The Ion OneTouch™ 2 System uses common molecular biology equipment, supplies, and reagents.

| ✓ | Description†‡ | Supplier | Cat. number | Quantity |
|---|---|---|---|---|
| | Ion PI™ Controls Kit v2 | Life Technologies | 4482414 | 1 |
| | Benchtop absorbent paper or mat | Major Laboratory Supplier (MLS) | — | — |
| | Bleach | MLS | — | — |
| | Ethernet cable | MLS | — | 1 |
| | 1/8-inch L-wrench (hex wrench) or equivalent | MLS | — | 1 |
| | Heat block (to be set at 75°C) | MLS | — | 1 |

† Life Technologies has demonstrated this protocol using this specific material. Substitution may adversely affect system performance.

‡ For materials needed for optional quality control of ISPs by the Qubit® 2.0 Fluorometer, see Appendix A, "Quality control of Ion PI™ Ion Sphere™ Particles" on page 69.

# 2          Getting started

## Workflow

| |
|---|
| **Unpack and install the Ion OneTouch™ 2 Instrument and Ion OneTouch™ ES** (page 15) |

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|---|
| **Check the firmware** (page 16) |

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|---|
| **Initialize the Ion OneTouch™ 2 Instrument** (page 16) |

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|---|
| **Perform a verification run** (page 16) |
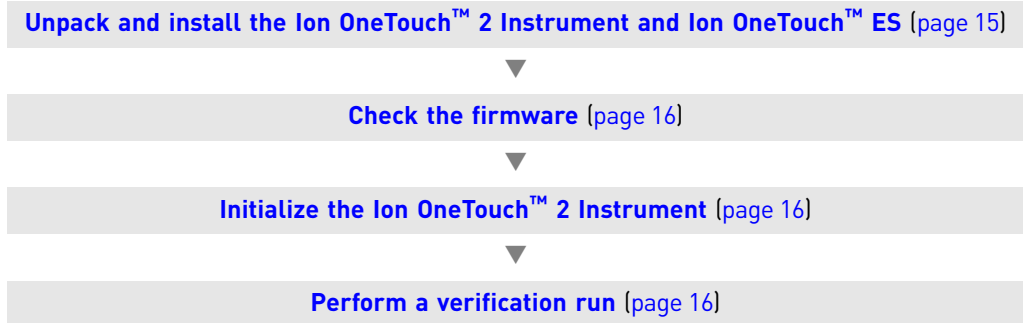
## Procedural guidelines

- Use good laboratory practices to minimize cross-contamination of products. When designing the laboratory layout, consider the need for space separation of pre- and post-PCR activities. Separate the amplicon source and post-PCR activities from pre-PCR activities. Dedicate laboratory supplies and/or equipment to the appropriate space to significantly reduce the potential for contamination.

- Unless otherwise specified, thaw reagents on ice before use.

## 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, refer to:

- Site preparation and installation requirements: Refer to the *Ion Proton™ System Site Preparation Guide* (Pub. no. 4478733).

- Unpacking and installation instructions: See "Unpack and install the Ion OneTouch™ 2 System" on page 105.

# Check the firmware

Life Technologies periodically releases *firmware updates* to the software controlling the Ion OneTouch™ 2 Instrument. The firmware updates are available through the Torrent Server. To check the firmware version, on the home screen, touch **Options**, then touch **Info**. To update the firmware, use the USB flash drive or use the Ethernet connection (see "Install a firmware update with a USB flash drive" on page 107).

IMPORTANT! Ensure that the latest firmware is installed on the Ion OneTouch™ 2 Instrument. To get the latest firmware, upgrade to Torrent Server Software v4.0.2 or later, then use a USB flash drive or an Ethernet connection to upgrade your instrument.

# Initialize the Ion OneTouch™ 2 Instrument

IMPORTANT! Before operating the Ion OneTouch™ 2 Instrument for the first time, you must perform the one-time initialization on the instrument. Initialization primes the pumps and tubing lines for reliable operation.

For detailed instructions on initialization of the Ion OneTouch™ 2 Instrument, see "Initialize the Ion OneTouch™ 2 Instrument" on page 111.

# Perform a verification run

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

1. Obtain the Human CEPH Control 170 Library from the Ion PI™ Controls Kit v2 (Cat. no. 4482414).

2. In an Eppendorf LoBind® Tube, dilute 1 μL of control library into 260 μL of Nuclease-Free Water. Use 65 μL of the dilution in the amplification solution (see "Prepare and install the amplification solution" on page 29).

3. Follow the operating instructions to set up and use the Ion OneTouch™ 2 Instrument to prepare template-positive Ion PI™ Ion Sphere™ Particles and to use the Ion OneTouch™ ES to prepare enriched Ion PI™ Ion Sphere™ Particles (see "Set up the Ion OneTouch™ 2 Instrument" on page 20).

4. If you have a Qubit® 2.0 System, determine the percent template-positive Ion PI™ Ion Sphere™ Particles (see "Quality control of Ion PI™ Ion Sphere™ Particles" on page 69). If you do *not* have a Qubit® 2.0 System, proceed to step 5.

5. Before starting a run on the Ion Proton™ Sequencer, create a planned run in the Torrent Browser, and select the Ion PI™ Sequencing 200 Kit v2. Load the sample, then analyze the results.

   Note: Use the Ion PI™ Sequencing 200 Kit v2 (Cat. no. 4485149). Refer to the *Ion PI™ Sequencing 200 Kit v2 User Guide* (Pub. no. MAN0007961).

6. Review the run report from the Torrent Browser and confirm successful sequencing results with the control library.

   **Note:** If you have questions about the results, contact your Life Technologies Field Applications Specialist.

7. The instrument is ready for use.

# 3 Prepare template-positive Ion PI™ Ion Sphere™ Particles

## Workflow

| Set up the Ion OneTouch™ 2 Instrument (page 20) |
| :---: |

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| Prepare and install the amplification solution (page 29) |
| :---: |

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| Run the Ion OneTouch™ 2 Instrument (page 36) |
| :---: |

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| Recover the template-positive Ion PI™ Ion Sphere™ Particles (page 39) |
| :---: |

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| Maintain the Ion OneTouch™ 2 Instrument (page 43) |
| :---: |
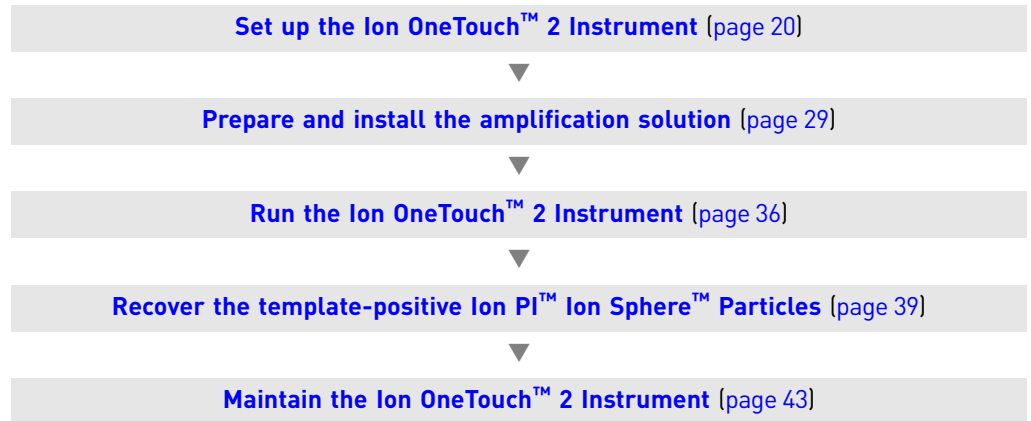
# Set up the Ion OneTouch™ 2 Instrument

**Note:** If this is the first use of the instrument, perform the one-time initialization procedure (see "Initialize the Ion OneTouch™ 2 Instrument" on page 111). Perform initialization at any time before the first run. If this is the first run after initialization, proceed to "Prepare and install the amplification solution" on page 29. The instrument consumables are already installed and ready for the run.

**Ion OneTouch™ 2 Instrument layout**
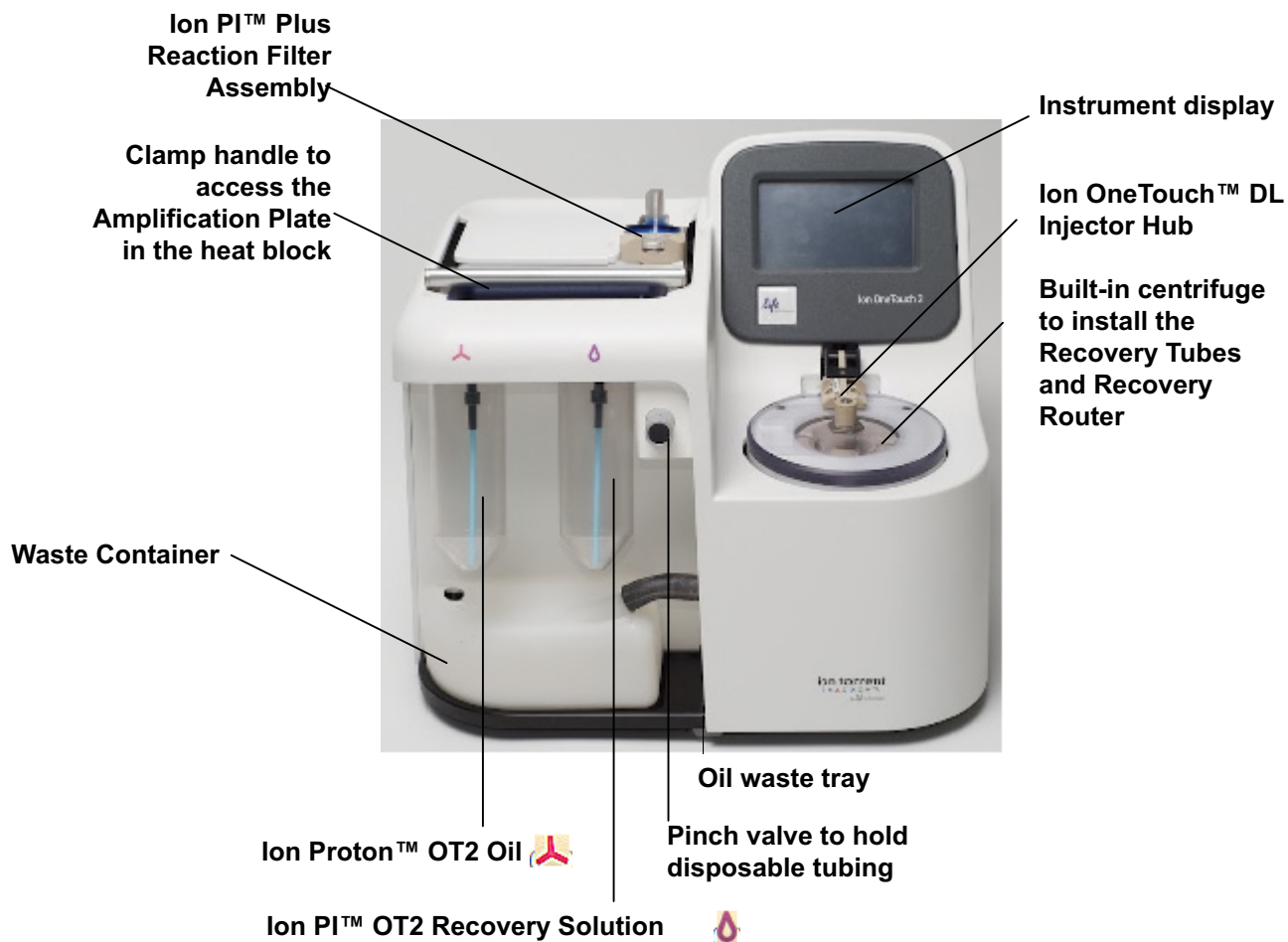


**Note:** The photograph does not show the disposable tubing.

## Materials required for this procedure

**Provided in the Ion PI™ Template OT2 Supplies 200 Kit v2 (Part no. 4485144):**

- 2 Ion OneTouch™ Reagent Tubes
- Ion OneTouch™ Recovery Router
- 2 Ion PI™ Template OT2 Recovery Tubes
- Ion OneTouch™ 2 Amplification Plate
- 2 Ion OneTouch™ Sipper Tubes

**Provided in the Ion PI™ Template OT2 Solutions 200 Kit v2 (Part no. 4485143):**

- Ion Proton™ OT2 Oil (450-mL size)
- Ion PI™ OT2 Recovery Solution

**Note:** Life Technologies has validated this protocol using only the material specified. Substitution may adversely affect performance and safety. The operations described in this chapter apply to Ion OneTouch™ 2 Instruments.

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

- Use *only* the Ion PI™ Template OT2 200 Kit v2 (Cat. no. 4485146) 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.
- Use only the supplied screw caps in the Ion PI™ Template OT2 200 Kit v2 to store the reagents. Do not seal the reagents with any plastic paraffin film such as Parafilm® M film.

## Install the Ion PI™ Template OT2 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.
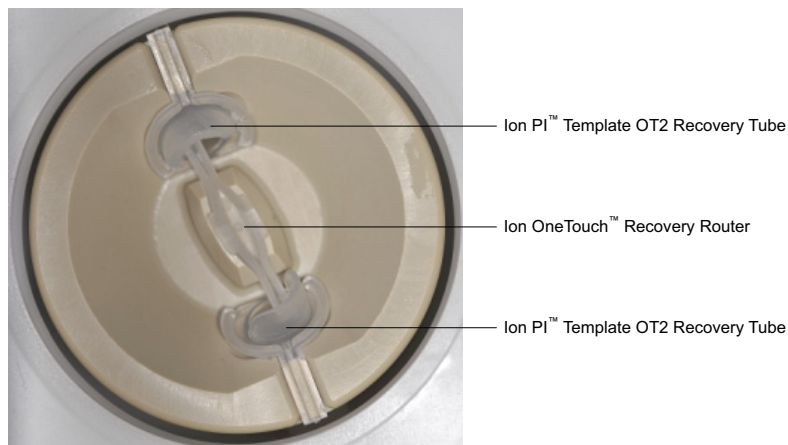
   **IMPORTANT!** Do *not* lift the lid by the tubing attached to the Ion OneTouch™ DL Injector Hub. Do *not* force the lid open.

2. Insert a Recovery Tube into each of the slots in the centrifuge. The Recovery Tubes have rounded collars at their openings (see the photograph of a Recovery Tube on page 41).

3. 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 is seated flat and secure in the center of the rotor:



Ion PI™ Template OT2 Recovery Tube

Ion OneTouch™ Recovery Router

Ion PI™ Template OT2 Recovery Tube

**Note:** The Recovery Router is intentionally offset from the Recovery Tubes.

4. Close the lid of the centrifuge.

**Install the Ion OneTouch™ 2 Amplification Plate**

1. If there is a used Ion OneTouch™ 2 Cleaning Adapter on the instrument, remove and appropriately discard it.

   **Note:** The Cleaning Adapter may be filled with Ion Proton™ OT2 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. Obtain an Ion OneTouch™ 2 Amplification Plate. The disposable tubing and disposable injector are attached to the Amplification Plate. An Amplification Plate supplied in the Ion PI™ Template OT2 200 Kit v2 has "ion" on the top tab:



**Note:** Before use, inspect the side plate port of the Amplification Plate to ensure that it is straight and perpendicular to the plate. Do *not* disconnect tubing from the top plate port. If you have questions about the plate, contact Life Technologies Technical Support.

4. Insert the Amplification Plate:

   a. 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.

   b. 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:

   

5. Pull the handle of the heat block to close the block. The disposable tubing is under the handle:

   

6. Thread the disposable tubing through the Ion OneTouch™ DL Tubing Catch:

7.  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 (see figure 9a):

    b.  Gently pull the disposable tubing upwards on the both sides of the pinch valve (see figure 9b) until the disposable tubing is in the slot and secured in the round notch on each side of the pinch valve (see figure 9c):

**9a**                    **9b**                    **9c**



    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:** Before use, inspect the long metal shaft of the disposable injector. Some disposable injectors may be slightly bent, which is normal. If you have questions about the disposable injector, contact Life Technologies Technical Support.

1. Place one hand on the centrifuge lid. Place the other hand at the top of the disposable injector, and insert the disposable injector straight into the port of the Ion OneTouch™ DL Injector Hub:
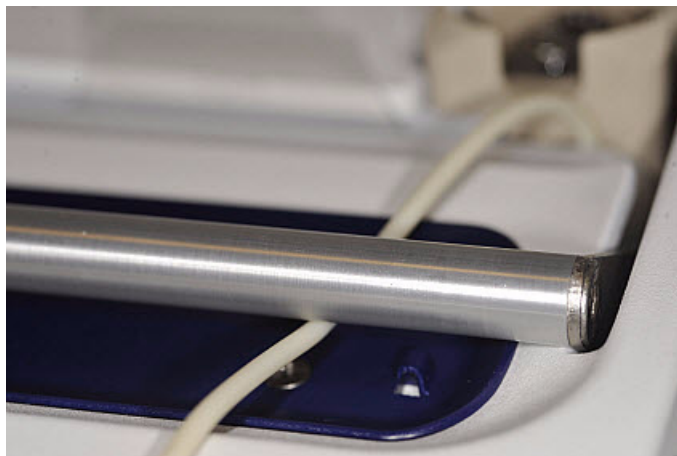
   ⚠ **CAUTION! PHYSICAL INJURY HAZARD**. The pointed end of the disposable injector can puncture your skin. Keep your hand away from the point of the disposable injector.



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

2. Keep your hand on the centrifuge lid, then push the disposable injector through the port until the disposable injector just stops at the base of the router:



3. Release the disposable injector.

4. Confirm automatic placement of the disposable injector above the router. Briefly press then release the spring-loaded top of the Injector Hub at the point indicated by the arrows in figures 4a–4c. You should hear a click:

| 4a (up position) | 4b (down position) | 4c (up position) |



IMPORTANT! If the Injector Hub remains in the down position, see "Troubleshooting" on page 61.

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, amplification plate, and tubing. Use a new disposable injector and Ion OneTouch™ 2 Amplification Plate.

## Install the Ion Proton™ OT2 Oil

Fill the appropriate Ion OneTouch™ Reagent Tube with Ion Proton™ OT2 Oil on the *left* front port ⚠:

**Note:** The Ion Proton™ OT2 Oil is yellow. This is normal.

| If you are... | Then... |
|---|---|
| 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 surfaces. |
| | 4. Invert the Ion Proton™ OT2 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 the Ion Proton™ OT2 Oil bottle (450-mL size) 3 times to mix. |
| | 3. Fill the Reagent Tube *half*-full with Oil. Minimize bubbles.<br><br>**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 PI™ OT2 Recovery Solution

**IMPORTANT!** Use only the Ion PI™ OT2 Recovery Solution in the Ion PI™ Template OT2 200 Kit v2 that you are currently using. Do ***not*** use a different recovery solution from another kit.

1. Ensure that the Ion PI™ OT2 Recovery Solution is clear. If it is clear, proceed to the next step. If the Recovery Solution is not clear, heat 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 PI™ OT2 Recovery Solution on the *right* front port 🔴:

| If you are... | Then... |
|---|---|
| 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. Minimize bubbles. |
| | 4. Invert the container of Recovery Solution bottle 3 times, then fill the Reagent Tube a *quarter*-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 container of Recovery Solution 3 times. |
| | 3. Add more Recovery Solution to the solution in the tube until the Reagent Tube is a *quarter*-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 and inspect the oil waste tray**

### 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. Push the tubing onto the port of 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 Life Technologies Technical Support.

# Prepare and install the amplification solution

**Materials required for this procedure**

**Provided in the Ion PI™ Template OT2 Solutions 200 Kit v2 (Part no. 4485143):**

- Ion Proton™ OT2 Reaction Oil (22-mL size)
- Ion PI™ PCR Reagent B
- Nuclease-Free Water

**Provided in the Ion PI™ Template OT2 Reagents 200 Kit v2 (Part no. 4485142):**

- Ion PI™ Reagent Mix TL
- Ion PI™ Enzyme Mix TL
- Ion PI™ PCR Reagent X
- Ion PI™ Ion Sphere™ Particles

**Provided in the Ion PI™ Template OT2 Reactions 200 Kit v2 (Part no. 4485145):**

Ion PI™ Plus Reaction Filter Assembly

**Provided in the Ion PI™ Controls Kit v2 (Cat. no. 4482414):**

Human CEPH Control 170 Library

**Other Materials and Equipment:**

- Diluted library
- Microcentrifuge
- 1.5-mL Eppendorf LoBind® Tubes
- Tube rack for15-mL conical tube
- Pipettes
- Vortexer

**Prepare the amplification solution**

IMPORTANT!  Use *only* the Ion PI™ Template OT2 200 Kit v2 (Cat. no. 4485146) 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.

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™ Reagent Mix TL (in single-use tubes) | 1. Ensure that the solution is fully thawed. Allow the reagent mix to come to room temperature before use.<br><br>2. Vortex the solution for 30 seconds, then centrifuge the solution for 2 seconds.<br><br>3. Keep the reagent mix at room temperature. After use, discard the single-use tube.<br><br>**Note:** If precipitate is visible, then see troubleshooting on page 63. |
| Ion PI™ PCR Reagent B | 1. Vortex the reagent for 1 minute, then centrifuge the solution for 2 seconds.<br><br>2. Inspect the reagent:<br>• If the solution is *clear*, then prepare the amplification solution. Keep Reagent B at room temperature.<br>• If the solution is *cloudy* or has crystals or has been accidentally stored at 2°C to 8°C, heat the reagent for 1 minute in a heat block set at 75°C. Vortex the reagent for 1 minute, then centrifuge the solution for 2 seconds.<br><br>3. Inspect the reagent again. If the reagent is:<br>• *Cloudy* or has *crystals*: repeat steps 1–2 until the reagent is clear, then equilibrate the reagent to room temperature and prepare the amplification solution.<br>• *Clear*: Equilibrate the reagent to room temperature, then prepare the amplification solution. Store Reagent B at room temperature.<br><br>**IMPORTANT!** Do not use the reagent if it is cloudy or has crystals. |
| Ion PI™ Enzyme Mix TL | 1. Centrifuge the enzyme for 2 seconds.<br><br>2. Place on ice. |
| Ion PI™ PCR Reagent X | 1. Centrifuge Reagent X for 2 seconds.<br><br>2. Place on ice. |
| Ion PI™ Ion Sphere™ Particles[†] | Place the suspension at room temperature. |

† **IMPORTANT!** For Ion AmpliSeq™ Exome libraries, use the Ion PI™ Ion Sphere™ Particles XT, included in the Ion PI™ Reagents XT Kit (Cat. no. 4487053), instead of the Ion PI™ Ion Sphere™ Particles supplied in the Ion PI™ Template OT2 200 Kit v2.

2. Depending on your library type and concentration, dilute the library in a 1.5-mL Eppendorf LoBind® Tube as shown in the table below. Use the library dilution within 48 hours of preparation.

   **Note:** If you are troubleshooting the amplification process, to 260 μL of Nuclease-Free Water, add 1 μL of the Human CEPH Control 170 Library from the Ion PI™ Controls Kit v2 (Cat. no. 4482414).

|  | Ion AmpliSeq™ DNA Library† | Ion AmpliSeq™ RNA Library | gDNA Fragment Library | Ion TargetSeq™ Exome-Enriched Library | Ion Total RNA-Seq Library |
|---|---|---|---|---|---|
| Library concentration | 100 pM | 100 pM | 100 pM | 100 pM | 100 pM |
| Volume of library | 7 μL | 7 μL | 7 μL | 7–8 μL | 7 μL |
| Volume of Nuclease-Free Water | 58 μL | 58 μL | 58 μL | 57–58 μL | 58 μL |
| **Total volume of diluted library to add to the amplification solution** | **65 μL** | **65 μL** | **65 μL** | **65 μL** | **65 μL** |

† Includes Ion AmpliSeq™ Exome libraries.

   a. Vortex the diluted library for 5 seconds, then centrifuge for 2 seconds.

   b. Place the diluted library on ice.

3. Prepare the Ion PI™ Ion Sphere™ Particles (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. In a 2-mL Eppendorf LoBind® Tube 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 | Ion PI™ Reagent Mix TL | Violet | 750 µL |
| 2 | Ion PI™ PCR Reagent B | Blue | 450 µL |
| 3 | Ion PI™ Enzyme Mix TL | Brown | 75 µL |
| 4 | Ion PI™ PCR Reagent X | White | 60 µL |
| 5 | Ion PI™ Ion Sphere™ Particles† | Black | 100 µL |
| 6 | Diluted library (*not* stock library) | — | 65 µL |
| — | **Total** | — | **1500 µL** |

† **IMPORTANT!** For Ion AmpliSeq™ Exome libraries, use the Ion PI™ Ion Sphere™ Particles XT, included in the Ion PI™ Reagents XT Kit (Cat. no. 4487053), instead of the Ion PI™ Ion Sphere™ Particles supplied in the Ion PI Template OT2 200 Kit v2.

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 min after preparing the amplification solution.

6. Proceed *immediately* to "Fill the Ion PI™ Plus Reaction Filter Assembly" on page 32.

## Fill the Ion PI™ Plus Reaction Filter Assembly

IMPORTANT! We recommend filling the Ion PI™ Plus Reaction Filter Assembly in a room dedicated to pre-PCR activities or in a controlled pre-PCR hood. Do *not* use a reaction filter assembly from an Ion PGM™ Template OT2 kit.

1. Obtain an Ion PI™ Plus Reaction Filter Assembly from the Ion PI™ Template OT2 200 Kit v2:



Sample port

Ion PI™ Plus Reaction Filter

Short tubing from sample port to Ion PI™ Plus Reaction Tube

Ion PI™ Plus Reaction Tube
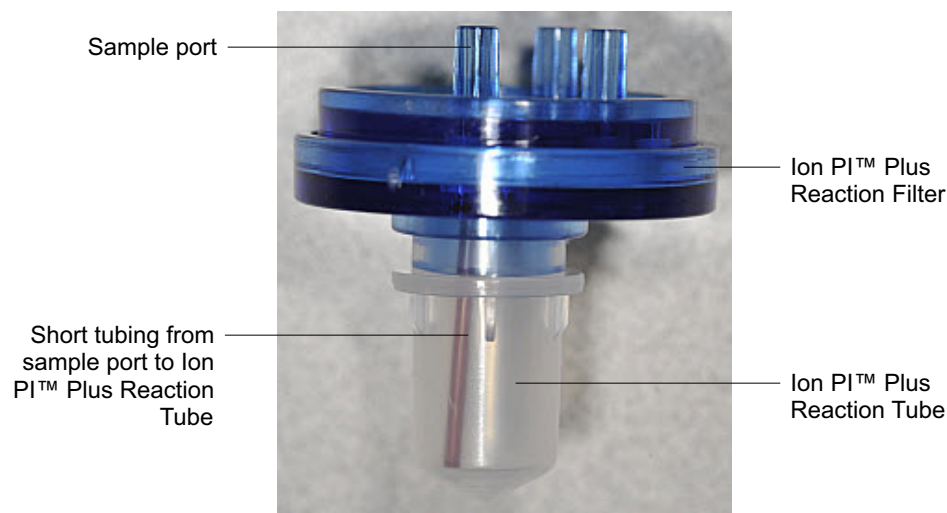
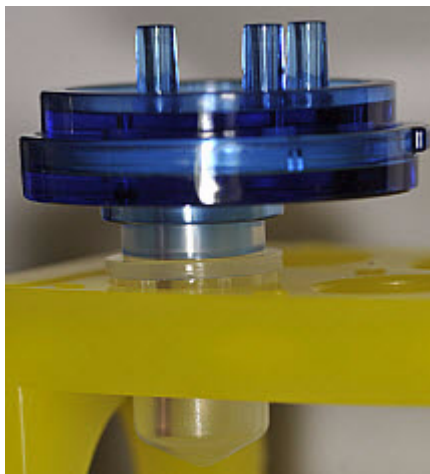2. Place the Ion PI™ Plus Reaction Filter Assembly into a tube rack so that the 3 ports of the Ion PI™ Plus Reaction Filter Assembly face *up*.



3. Locate the sample port on the Ion PI™ Plus Reaction Filter Assembly. The short tubing in the Reaction Tube is connected to the sample port:

Sample port                                    Sample port
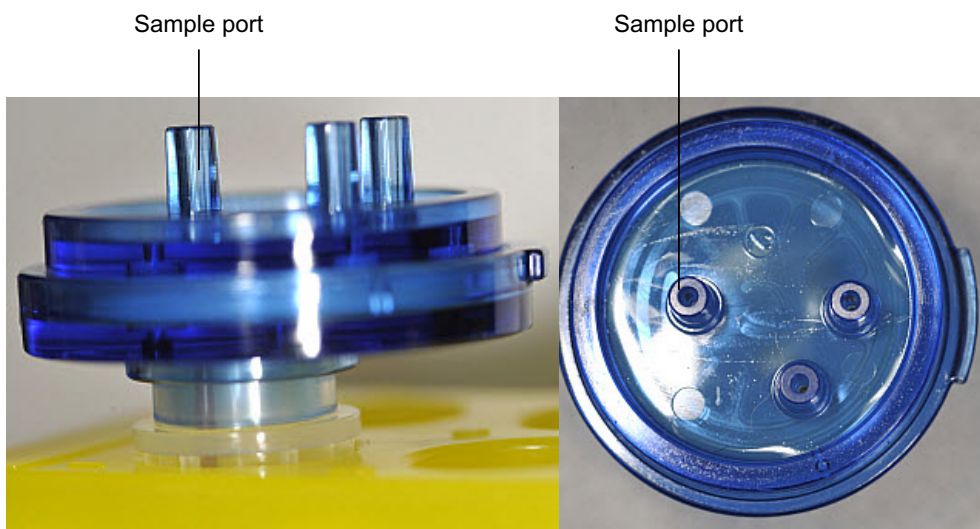


**Note:** The color of the short tubing attached to the sample port may vary.

4. Add the amplification solution through the sample port (to prepare the amplification solution, see "Prepare the amplification solution" on page 29):

   a. Set a P1000 pipette to 750 μL, and attach a new 1000-μL tip to the pipette.

   b. Vortex the amplification solution at **maximum speed for** *a full 5 seconds*, then centrifuge the solution for 2 seconds. *Immediately* proceed to the next step.

   c. Pipet the amplification solution up and down to mix, then fill the tip with 750 μL of the amplification solution.

    **d.** Insert the tip firmly into the sample port so that the tip is perpendicular to the Ion PI™ Plus Reaction Filter Assembly and fully inserted into the sample port to form a tight seal:



    **e.** Slowly pipet 750 μL of the 1.5 mL of amplification solution through the sample port, Keep the plunger of the pipette depressed to avoid aspirating solution from the Ion PI™ Plus Reaction Filter Assembly. With the plunger depressed, remove the tip from the sample port.

    **f.** Slowly pipet the remaining 750 μL of amplification solution through the sample port, then keep the plunger of the pipette depressed.

    **g.** With the plunger depressed, remove the tip from the sample port, then appropriately discard the tip.

    **h.** If necessary, gently dab a Kimwipes® disposable wipe around the ports to remove any liquid.

**5.** Add Ion Proton™ OT2 Reaction Oil through the sample port:

    **a.** Set a P1000 pipette to 950 μL, and attach a new 1000-μL tip to the pipette.

    **b.** Fill the tip with 950 μL of Reaction Oil.

    **c.** Insert the tip firmly into the sample port so that the tip is perpendicular to the Ion PI™ Plus Reaction Filter Assembly and fully inserted into the sample port to form a tight seal.

    **d.** Slowly pipet 950 μL of the Reaction Oil through the sample port, then keep the plunger of the pipette depressed.

    **e.** With the plunger depressed, remove the tip from the sample port, then appropriately discard the tip.

    **f.** If necessary, gently dab a Kimwipes® disposable wipe around the ports to remove any liquid.

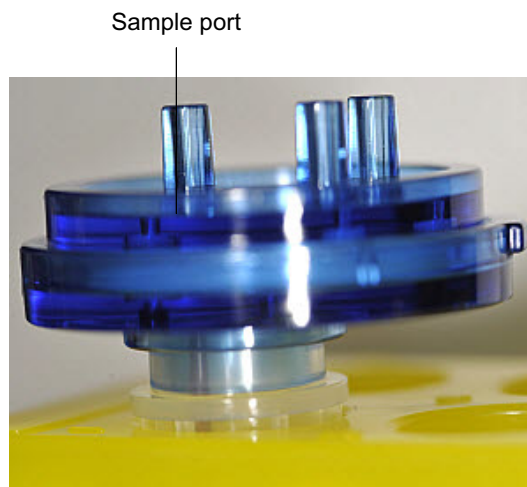**Install the filled Ion PI™ Plus Reaction Filter Assembly on the Ion OneTouch™ 2 Instrument**

1. Invert the Ion PI™ Plus Reaction Filter Assembly:

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

   a. Keep the Ion PI™ Plus Reaction Filter Assembly in the tube rack, Orient the assembly so that the sample port is on your *left*:

   Sample port

   

   b. Lift straight out the Ion PI™ Plus Reaction Filter Assembly from the tube rack. With the short tubing in the Reaction Tube on the left, rotate the assembly to your *right* until the Reaction Tube is inverted and the 3 ports of the Ion PI™ Plus Reaction Plus Filter face *down*:

   IMPORTANT!  Correct rotation of the Ion PI™ Plus Reaction Filter Assembly ensures minimal exposure of the short tubing in the Reaction Tube with the aqueous phase.
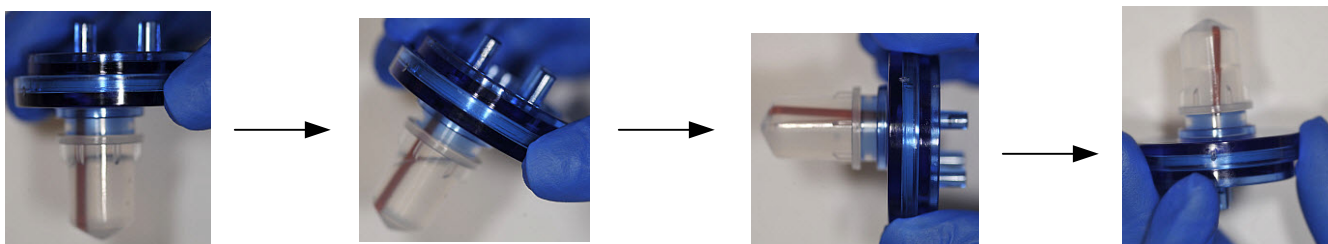
2.  Insert the 3 ports of the Ion PI™ Plus Reaction Plus Filter into the three holes (see Figure 5a) on the top stage of the Ion OneTouch™ 2 Instrument (see Figure 5b), so that the Ion PI™ Plus Reaction Filter Assembly is firmly seated on the instrument. The tab protruding on the outer edge of the Ion PI™ Plus Reaction Plus Filter fits into the front notch of the stage:



Tab

Notch
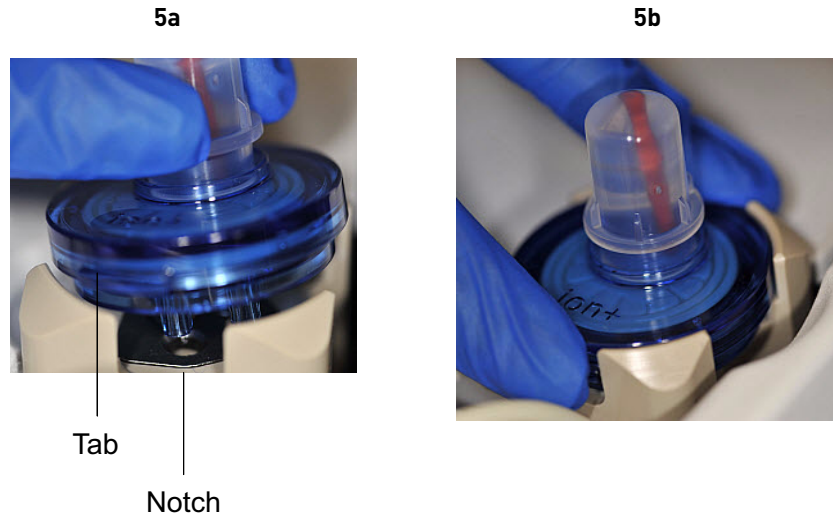
**Note:**  After inserting the Ion PI™ Plus Reaction Filter Assembly, bubbles may shoot up into the Reaction Tube.

# Run the Ion OneTouch™ 2 Instrument

**Note:**  Ensure that you have the correct firmware update for your system (see "Maintain the Ion OneTouch™ 2 Instrument" on page 43).

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, amplification plate, and tubing. Use a new disposable injector and Ion OneTouch™ 2 Amplification Plate.

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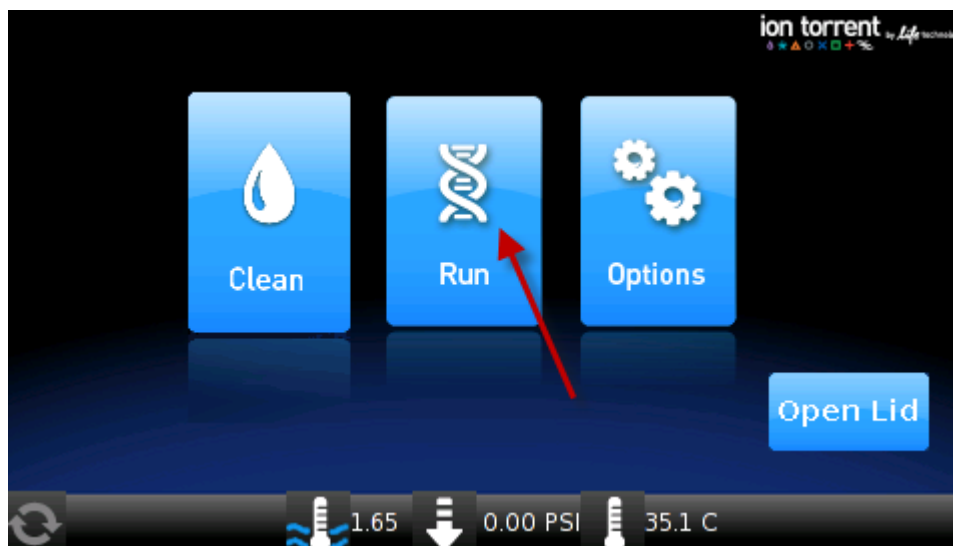


Note: For descriptions of Ion OneTouch™ 2 Instrument operations displayed during a run, see "Ion OneTouch™ 2 Instrument operations" on page 122.

3. Touch the drop-down menu, then touch **Proton: Ion PI™ Template OT2 200 Kit v2**, then touch **Next**:
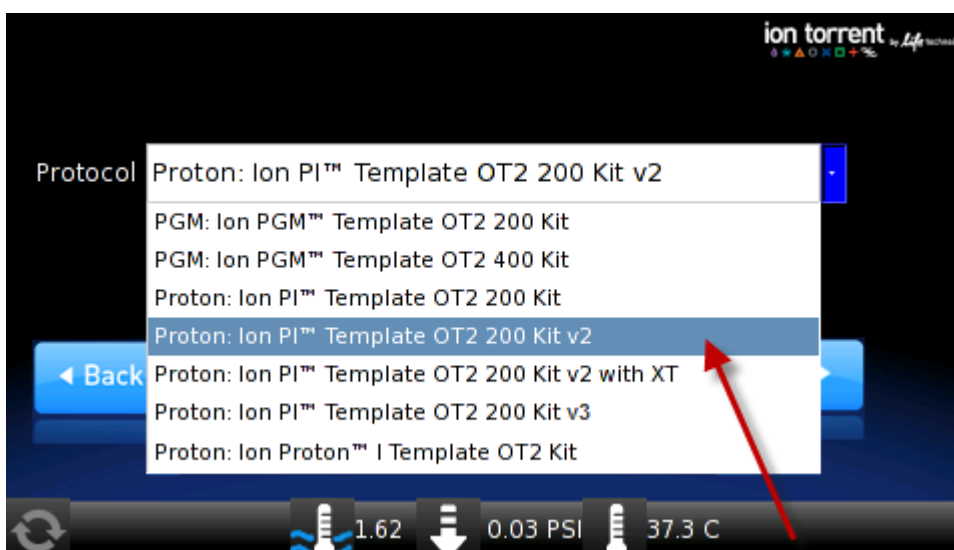
**Note:** For Ion AmpliSeq™ Exome libraries, touch **Proton: Ion PI™ Template OT2 200 Kit v2 with XT**, then touch **Next**:
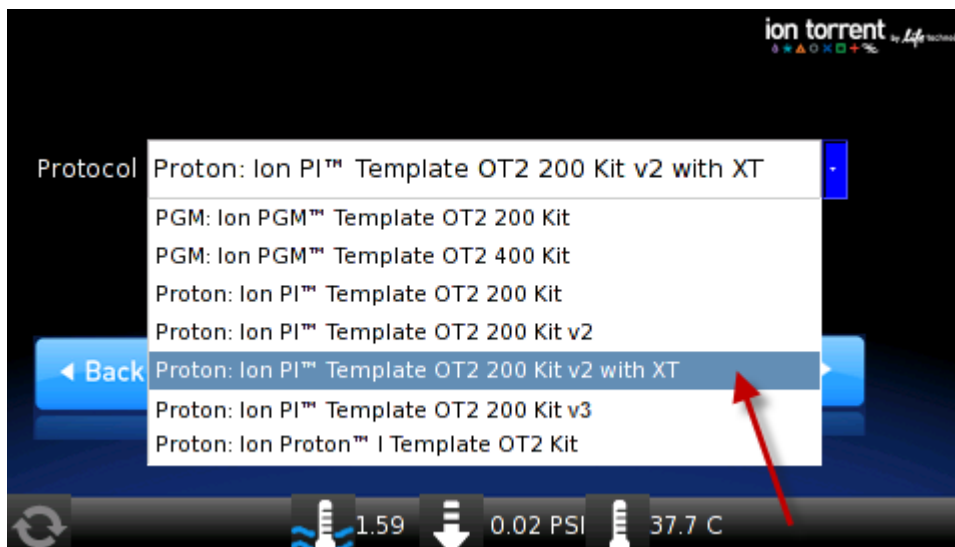


4. Touch **Assisted** or **Expert**:



- **Assisted** run. Complete each task, then touch **Next**. After you touch **Next** on the last task, you see a progress bar, and the run begins. After the runs starts, you hear clicks from the instrument. This is normal.
- **Expert** run. Empty the waste container and oil waste tray, if necessary, then touch **Next**. You see a progress bar, and the run begins without the list of task screens.

IMPORTANT!  To cancel a run, touch **Abort**, then touch **Yes** to confirm cancellation. If there is a high-pressure event on the instrument, the instrument aborts the run *automatically*, and you do *not* have to touch **Abort**.

After a run is aborted, follow these steps in this order:

- Download the log files for troubleshooting by Life Technologies Technical Support (see "Download the log files from the Ion OneTouch™ 2 Instrument" on page 121).
- Turn OFF the instrument.
- If necessary, retain all consumables on the instrument for troubleshooting.
- After successful troubleshooting, appropriately dispose of all used consumables and turn ON the instrument (see "Unpack and install the Ion OneTouch™ 2 System" on page 105).
- Set up the instrument with new kit components (see "Set up the Ion OneTouch™ 2 Instrument" on page 20).
- Prepare a new amplification solution (see "Prepare and install the amplification solution" on page 29).
- Start a new run.

5. 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™ Ion Sphere™ Particles".

# Recover the template-positive Ion PI™ Ion Sphere™ Particles

**Materials required for this procedure**

**Provided in the Ion PI™ Template OT2 Solutions 200 Kit v2 (Part no. 4485143):**

- Ion OneTouch™ Wash Solution
- Ion PI™ ISP Resuspension Solution
- Nuclease-Free Water

**Other Materials and Equipment:**

- 1.5-mL Eppendorf LoBind® Tubes
- Pipettes
- Vortexer
- Microcentrifuge

**Recover the Ion PI™ Ion Sphere™ Particles**

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 spun 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 PI™ Template OT2 Recovery Tubes from the run in the centrifuge rotor. Close the lid until it locks.

    **b.** Touch **Options**, then touch **Final Spin** (see figure below), then follow the screen prompts (touch **Next** on the next 2 screens) until the centrifugation begins. Centrifugation of the samples takes 10 minutes.
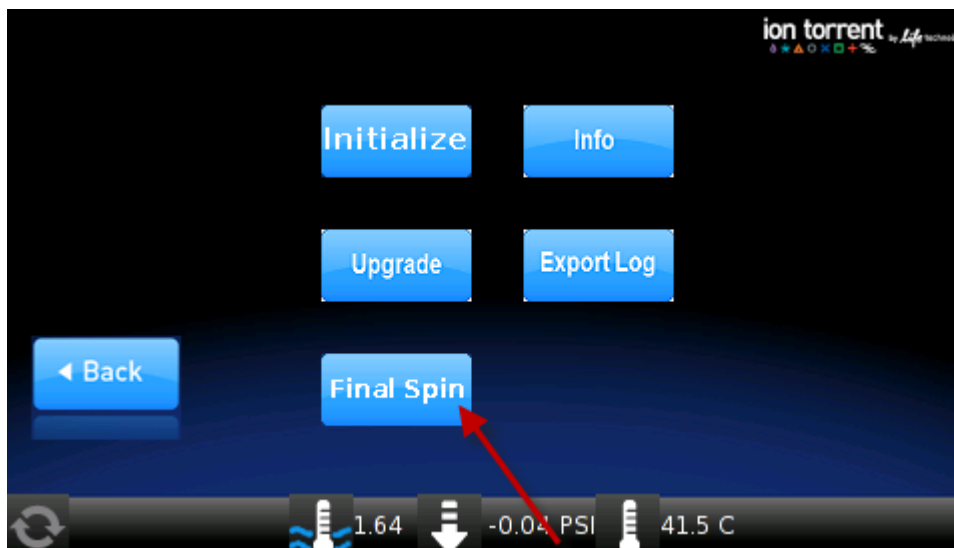


    **c.** *Immediately* proceed to step 2.

> ⚠ **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, open the lid, then remove and discard the Recovery Router.

**3.** *Carefully* remove both Ion PI™ Template OT2 Recovery Tubes from the instrument, and 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 Ion PI™ Ion Sphere™ Particles at –30°C to –10°C. Proceed *immediately* to "Wash the template-positive Ion PI™ Ion Sphere™ Particles" on page 41. There is a stopping point in the next section.

**Wash the template-positive Ion PI™ Ion Sphere™ Particles**

**Note:** If the volume of the pellet is ≥100 µL pellet in one or both Ion OneTouch™ Recovery Tubes, follow the alternative wash protocol (see the following photograph and "The volume of the pellet is ≥100 µL in one or both Ion OneTouch™ Recovery Tubes after a run on the Ion OneTouch™ 2 Instrument" on page 64):
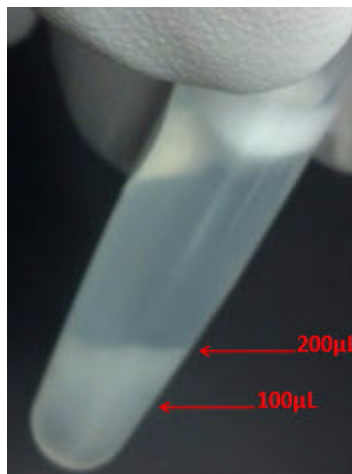


1. Label a new 1.5-mL Eppendorf LoBind® Tube for the template-positive Ion PI™ Ion Sphere™ Particles.

2. Use a pipette to remove all but ~100 µL of Ion PI™ OT2 Recovery Solution from each Ion PI™ Template OT2 Recovery Tube. Withdraw the supernatant from the surface and on the opposite side from the pellet. Do *not* disturb the pellet of template-positive Ion PI™ Ion Sphere™ Particles (ISPs):
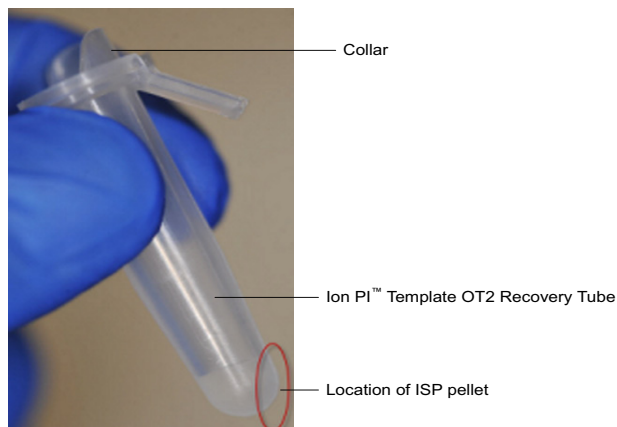


3. Resuspend the template-positive ISPs in the remaining Recovery Solution in each tube by pipetting the pellet up and down until the pellet disperses in the solution.

4. Transfer suspension from each Recovery Tube to the new labeled 1.5-mL Eppendorf LoBind® Tube.

5. Add 100 µL of Nuclease-Free Water to each of the Recovery Tubes, then pipette each aliquot in the tube up and down to mix and recover residual beads.

6. Transfer the 100-µL aliquot from each Recovery Tube to the new labeled 1.5-mL Eppendorf LoBind® Tube to combine the aliquots.

7. Bring the combined suspensions in the new labeled 1.5-mL Eppendorf LoBind® Tube to 1 mL with Nuclease-Free Water.

8. Vortex the pellet for 30 seconds to completely resuspend the template-positive Ion PI™ Ion Sphere™ Particles, then centrifuge the template-positive Ion PI™ Ion Sphere™ Particle suspension for 8 minutes at $15{,}500 \times g$.

9. Remove all but 100 µL of supernatant.

> STOPPING POINT To the 100-µL suspension, bring the volume to 1 mL with Nuclease-Free Water. Store the Ion PI™ Ion Sphere™ Particles at 2°C to 8°C for up to 3 days. If the template-positive ISPs were stored at 2°C to 8°C, centrifuge the ISPs at 15,500 × g for 8 minutes, then carefully remove all but 100 µL of supernatant. With a new tip, pipet the solution up and down to resuspend the ISPs. Proceed to step 10.
>
> Do *not* store the recovered Ion PI™ Ion Sphere™ Particles in Ion PI™ OT2 Recovery Solution.

10. Bring the combined suspensions in the new labeled 1.5-mL Eppendorf LoBind® Tube to 1 mL with Nuclease-Free Water.

11. Vortex the pellet for 30 seconds to completely resuspend the template-positive Ion PI™ Ion Sphere™ Particles, then centrifuge the tube for 2 seconds.

12. Proceed as follows:

| If you have access to a... | Then... |
|---|---|
| Qubit® 2.0 Fluorometer | Continue to step 13. You can perform quality control on the ISPs in step 17. |
| (*Optional*) Guava® easyCyte™ 5 Flow Cytometer | Perform quality control on the diluted, unenriched Ion PI™ Ion Sphere™ Particles: Transfer a 2.0-µL aliquot of the diluted, unenriched Ion PI™ Ion Sphere™ Particles (from step 11) to a 1.5-mL Eppendorf LoBind® Tube. Refer to the *Ion PI™ Ion Sphere™ Particles Quality Assessment Using the Guava® easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. no. MAN0007496), available on the Ion Community website: **ioncommunity.lifetechnologies.com**. |

13. Centrifuge the template-positive Ion PI™ Ion Sphere™ Particle suspension for 8 minutes at $15{,}500 \times g$.

14. Remove all but 20 µL of supernatant.

15. Bring the combined washed suspensions in the new labeled tube to 100 µL in Ion PI™ ISP Resuspension Solution.

16. Vortex the pellet for 30 seconds to completely resuspend the template-positive Ion PI™ Ion Sphere™ Particles, then centrifuge the tube for 2 seconds.

17. (*Optional*) If you have not used the Guava® easyCyte™ 5 flow Cytometer to perform quality control on the ISPs, you can retain the sample to assess quality of the resuspended, unenriched template-positive Ion PI™ Ion Sphere™ Particles (step 16) by the Qubit® 2.0 Fluorometer. Transfer a 2.0-µL aliquot of the resuspended, unenriched Ion PI™ Ion Sphere™ Particles to a 0.2-mL PCR tube, then see "Quality control using Qubit® 2.0 Fluorometer" on page 70.

18. Enrich the template-positive Ion PI™ Ion Sphere™ Particles (see "Enrich the template-positive Ion PI™ Ion Sphere™ Particles" on page 49).

> **IMPORTANT!** Do *not* store the recovered, template-positive ISPs at –30°C to –10°C. Do *not* store the recovered Ion PI™ Ion Sphere™ Particles in Ion PI™ OT2 Recovery Solution (see step 9 of this procedure).

# 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™ 2 Cleaning Adapter. *Always 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.

**Materials required for this procedure**

**Provided in the Ion PI™ Template OT2 Supplies 200 Kit v2 (Part no. 4485144):**

Ion OneTouch™ 2 Cleaning Adapter (*single-use*)

**Provided in the Ion PI™ Template OT2 Solutions 200 Kit v2 (Part no. 4485143):**

Ion Proton™ OT2 Oil (450-mL size)

**Other Materials and Equipment:**

- Kimwipes® disposable wipes
- 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.

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

| Are you switching from the Ion PI™ Template OT2 200 Kit v2 to another kit? | Then... |
|---|---|
| Yes | Refer to the *Demonstrated Protocol: How to Set Up the Ion OneTouch™ 2 Instrument When Switching Between the Ion PGM™ System and Ion Proton™ System* (Pub. no. MAN0007719). Use the reagents from the appropriate kit to maintain the Ion OneTouch™ 2 Instrument. |
| No | Proceed to step 2. Continue to use the reagents provided in the Ion PI™ Template OT2 200 Kit v2. |

2. Check the level of Ion Proton™ OT2 Oil in the Reagent Tube:

   a. Ensure that the *left* Reagent Tube has ≥20 mL of Oil:



   b. 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 PI™ Plus Reaction Filter Assembly. Remove the assembly from the instrument by grasping the *filter*.

   **Note:** The Reaction Tube is filled with Ion Proton™ OT2 Oil.

4. Keep the Ion OneTouch™ 2 Amplification Plate in the heat block.

5. Place a 50-mL conical tube in a tube rack, then place the tube rack with the tube to the right of the instrument (see photograph in step 10).

6. Obtain an Ion OneTouch™ 2 Cleaning Adapter:



7. Firmly insert the 3 ports of the Cleaning Adapter into the three holes (see Figure 5a) on the top stage of the Ion OneTouch™ 2 Instrument (see Figure 5b). The tab protruding from the outer edge of the Cleaning Adapter fits into the front notch of the stage:

5a                                                                                          5b



Tab

Notch
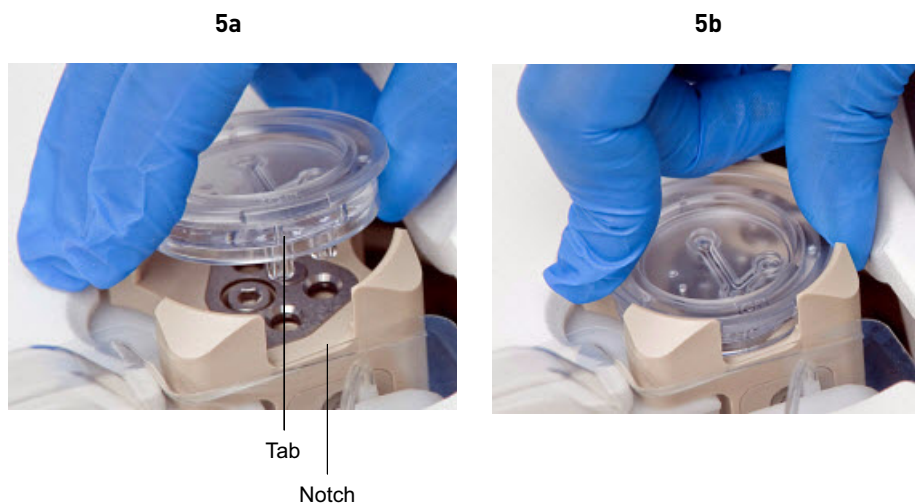
8. Remove the disposable injector from the Ion OneTouch™ DL Injector Hub:

   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.
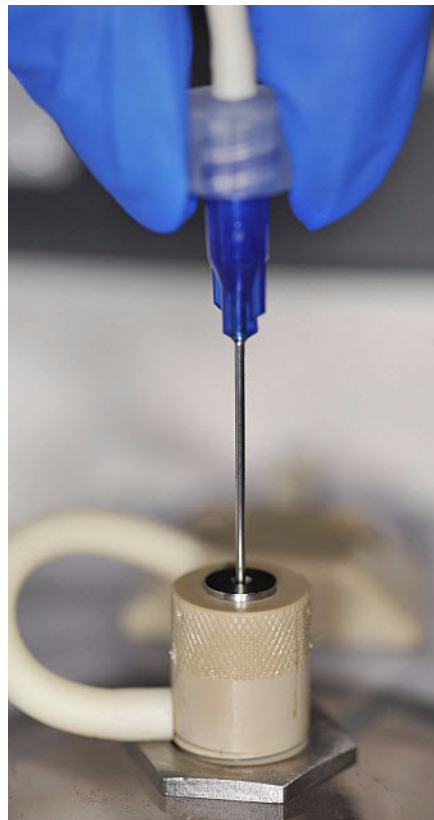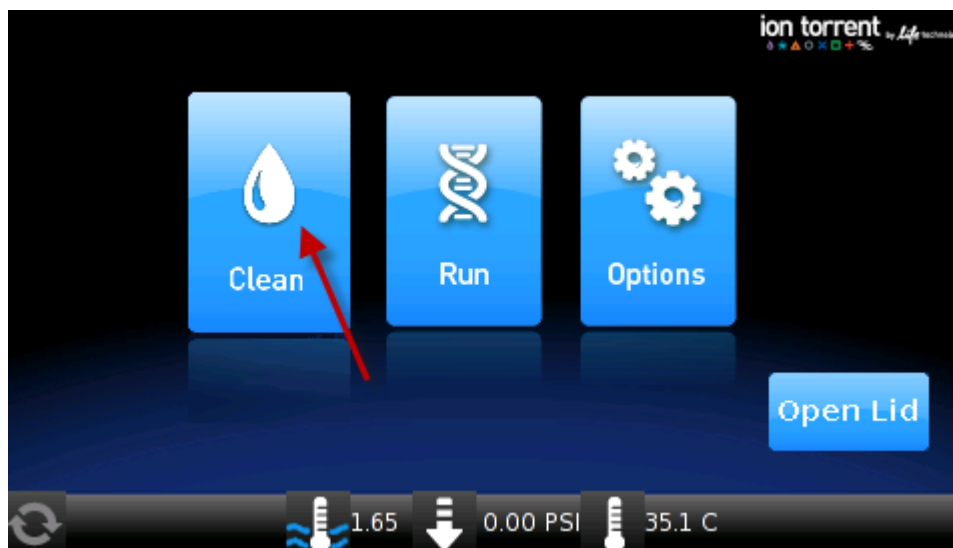


9. Gently pull the disposable tubing downwards on the both sides of the pinch valve until the disposable tubing is out of the valve.

10. Place the used, disposable injector into the empty 50-mL conical tube in the tube rack. The conical tube will be used for waste:



11. On the Ion OneTouch™ 2 Instrument home screen, touch **Clean**:



12. Complete each task displayed on the screen, then touch **Next**. After you touch **Next** on the last task, you see a progress bar, and the cleaning begins.

13. Ensure that the task in bold displays at the end of the cleaning run: "**Remove plate, injector, conical tube, and waste**".

    **Note:** Keep the used Cleaning Adapter on the instrument between runs.

14. Appropriately dispose of the waste in the 50-mL conical tube.

15. Remove and appropriately dispose of the used Amplification Plate, disposable injector, and tubing from the instrument:

    a. Push the handle to open the heat block.

    b. Remove the disposable tubing from the Ion OneTouch™ DL Tubing 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, and appropriately dispose of the used amplification plate, injector, and tubing.

  e. Leave the heat block open.

> ⚠ **CAUTION!** **Hot Surface**. Use care when working around this area to avoid being burned by hot components.
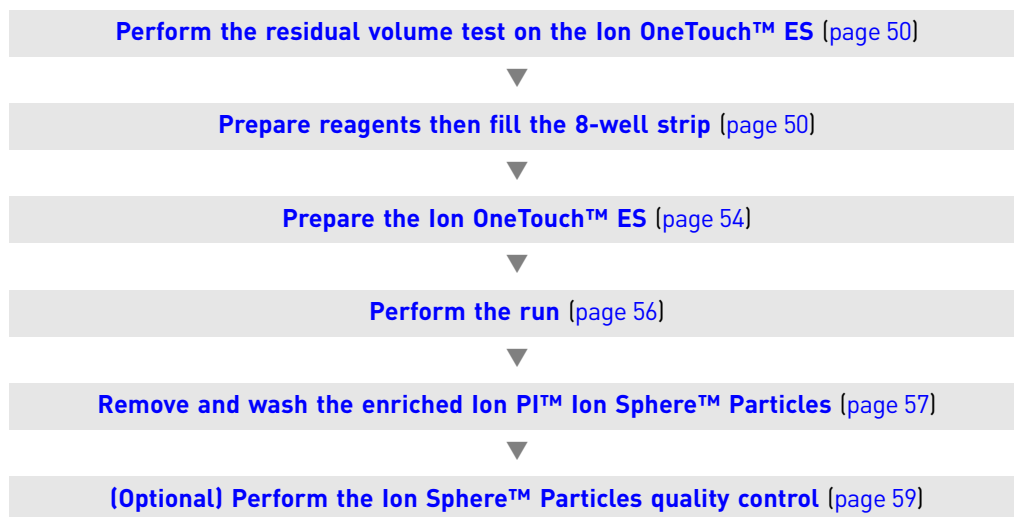
**16.** On the display, touch **Open Lid**, wait until the lid clicks open, then open the centrifuge lid. Wipe the residue from the centrifuge lid with a dry Kimwipes® disposable wipe and close the centrifuge lid.

**17.** Touch **Next** to return to the home screen on the instrument.

# 4 Enrich the template-positive Ion PI™ Ion Sphere™ Particles

## Workflow

| |
|---|
| **Perform the residual volume test on the Ion OneTouch™ ES** (page 50) |
| ▼ |
| **Prepare reagents then fill the 8-well strip** (page 50) |
| ▼ |
| **Prepare the Ion OneTouch™ ES** (page 54) |
| ▼ |
| **Perform the run** (page 56) |
| ▼ |
| **Remove and wash the enriched Ion PI™ Ion Sphere™ Particles** (page 57) |
| ▼ |
| **(Optional) Perform the Ion Sphere™ Particles quality control** (page 59) |

## Materials required

**Provided in the Ion PI™ Template OT2 Solutions 200 Kit v2 (Part no. 4485143):**

- Ion OneTouch™ Wash Solution
- Ion PI™ MyOne™ Beads Capture Solution
- Nuclease-Free Water

**Provided in the Ion PI™ Template OT2 Supplies 200 Kit v2 (Part no. 4485144):**

- 8-well strips
- Eppendorf® LoRetention Dualfilter Tips (P300)

**Other Materials and Equipment:**

- Dynabeads® MyOne™ Streptavidin C1 Magnetic Beads
- 1.5-mL Eppendorf LoBind® Tubes
- 0.2-mL PCR tubes
- 1M NaOH
- 10% Tween® 20 detergent in Nuclease-Free Water
- Pipettes
- Vortexer
- DynaMag™-2 magnet
- Microcentrifuge

# Perform the residual volume test on the Ion OneTouch™ ES

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**IMPORTANT!**  Ensure that the AC line voltage module is installed correctly into the Ion OneTouch™ ES [refer to the *Ion Proton™ System Site Preparation Guide* (Pub. no. 4478733) or the *Unpack and Install the Ion OneTouch™ 2 System for Use with the Ion Proton™ System* (Pub. no. 4478350)].

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Ensure that the Ion OneTouch™ ES is set up (see "Set up the Ion OneTouch™ ES" on page 87).

| If the condition is... | Then... |
|---|---|
| First use of the instrument and during monthly maintenance | Perform a residual volume test (see "Perform a residual volume test" on page 92). |
| Routine use and residual volume in Well 1 and Well 8 is >5.0 µL | |
| Routine use and residual volume in Well 1 and Well 8 is ≤ 5.0 µL | Operate the instrument without performing the residual volume test. Proceed to "Prepare reagents then fill the 8-well strip". |

# Prepare reagents then fill the 8-well strip

**Prepare Melt-Off Solution**

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

| Order | Component | Volume |
|---|---|---|
| 1 | Nuclease-Free Water | 277 µL |
| 2 | 1 M NaOH | 40 µL |
| 3 | 10% Tween® 20 detergent in Nuclease-Free Water | 3.0 µL |
| | **Total** | **320 µL** |

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

**Note:** 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 bottle 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 Ion PI™ 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 Ion PI™ MyOne™ Beads Capture Solution to Well 2 of the 8-well strip (see "Fill 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 Ion PI™ Ion Sphere™ Particles from the Ion OneTouch™ 2 Instrument are in 100 μL of Ion PI™ ISP Resuspension Solution. If the template-positive Ion PI™ Ion Sphere™ Particles were stored at 2°C to 8°C, follow the washing procedure for Ion PI™ Ion Sphere™ Particles (see "Wash the template-positive Ion PI™ Ion Sphere™ Particles" on page 41).

2. Ensure that the square-shaped tab of the 8-well strip is on the *left*:
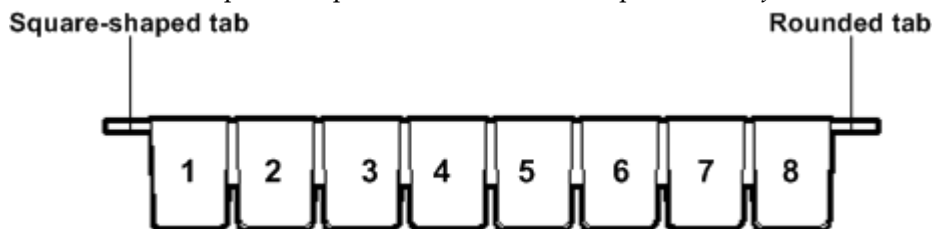
3. Pipet the Ion PI™ Ion Sphere™ Particles up and down 10 times to mix, then transfer the entire volume (100 µL) of resuspended Ion PI™ Ion Sphere™ Particles in Ion PI™ ISP Resuspension Solution (step 1 of this procedure) into Well 1 of the 8-well strip.

4. If you have not already assessed the quality of the unenriched, template-positive Ion PI™ Ion Sphere™ Particles, use one of the following methods:
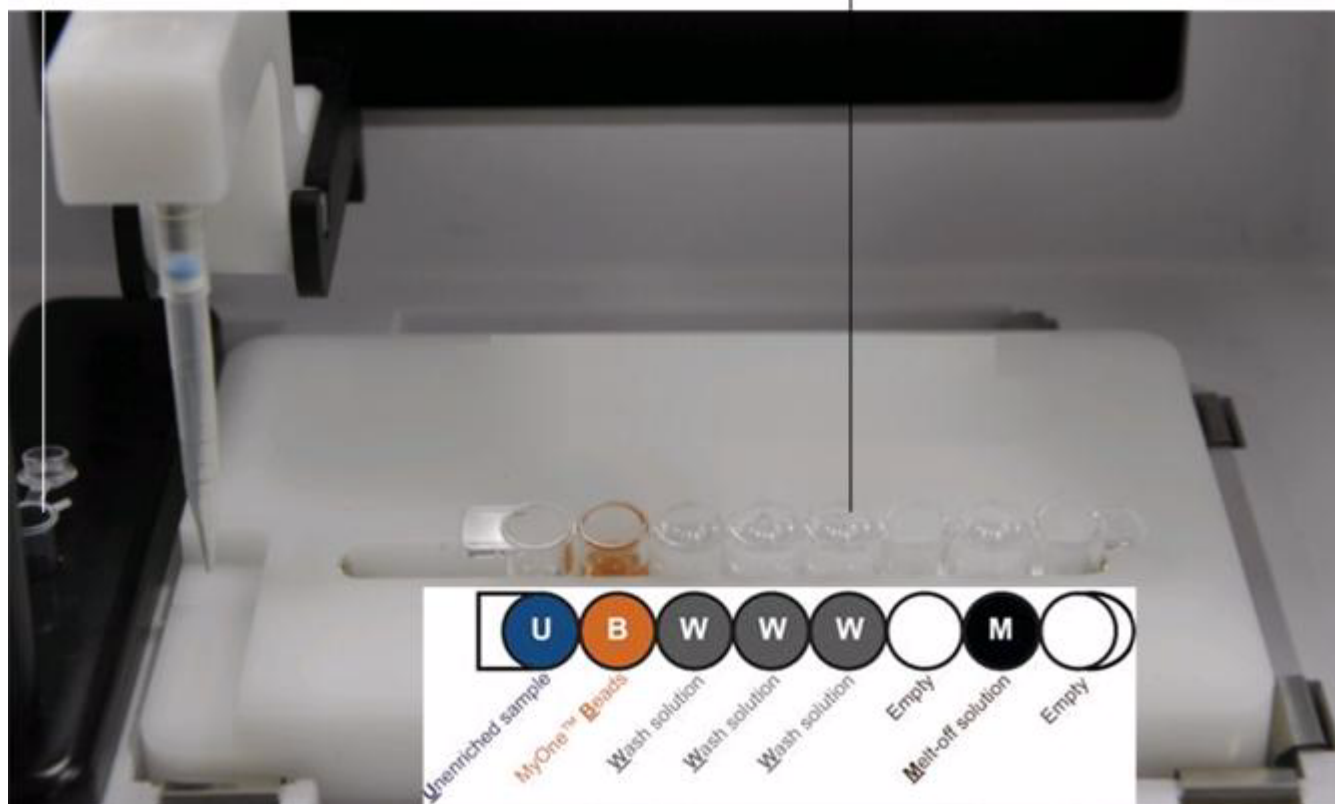
| Quality assessment by using a... | Then... |
|---|---|
| Qubit® 2.0 Fluorometer | Transfer a 2.0-µL aliquot of the unenriched Ion PI™ Ion Sphere™ Particles in Well 1 to a 0.2-mL PCR Tube, then see "Quality control using Qubit® 2.0 Fluorometer" on page 70. |
| (*Optional*) Guava® easyCyte™ 5 Flow Cytometer | 1. Add 9.0 µL of Ion OneTouch™ Wash Solution to a 1.5-mL Eppendorf LoBind® Tube.<br><br>2. Transfer a 1.0-µL aliquot of the unenriched Ion PI™ Ion Sphere™ Particles in Well 1 to the 9.0 µL of Ion OneTouch™ Wash Solution.<br><br>3. Pipet the dilution up and down to mix.<br><br>4. Put the sample on ice and use a 2.0-µL aliquot for the Guava® assay. Refer to the *Ion PI™ Ion Sphere™ Particles Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. no. MAN0007496). To access this protocol, go to the Ion Community website:<br><br>**ioncommunity.lifetechnologies.com**. |

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

| Well number | Reagent to dispense in well |
|---|---|
| Well 1 (well closest to the square-shaped tab) | *Entire* template-positive ISP sample (100 µL; prepared in step 1 of this procedure) (U) |
| Well 2 | 130 µL of Dynabeads® MyOne™ Streptavidin C1 Beads resuspended in Ion PI™ MyOne™ Beads Capture Solution (prepared in "Wash and resuspend the Dynabeads® MyOne™ Streptavidin C1 Beads" on page 51) (B) |
| Well 3 | 300 µL of Ion OneTouch™ Wash Solution (W) |
| Well 4 | 300 µL of Ion OneTouch™ Wash Solution (W) |
| Well 5 | 300 µL of Ion OneTouch™ Wash Solution (W) |
| Well 6 | Empty |
| Well 7 | 300 µL of freshly-prepared Melt-Off solution (prepared in "Prepare Melt-Off Solution" on page 50) (M) |
| Well 8 | Empty |



6. 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

1. Load a new tip in the Tip Arm:

   a. Place a new tip in the Tip Loader: Remove the Tip Arm from the cradle and align the metal fitting of the Tip Arm with the tip. 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. Lift the Tip Arm *straight up* to pull the installed tip from the Tip Loader tube:
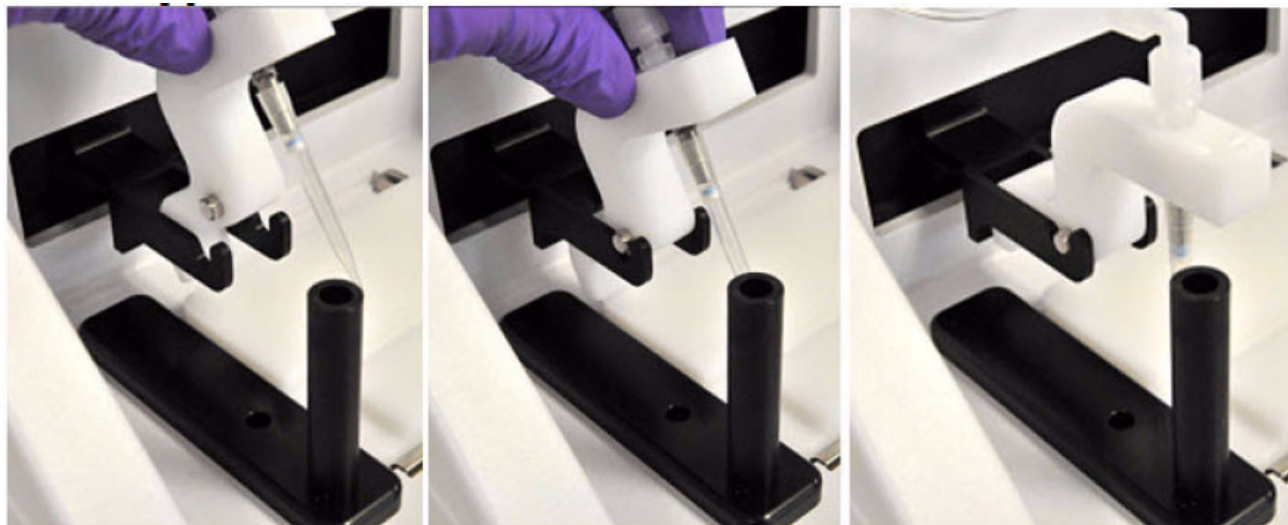
    **b.** Return the Tip Arm to the cradle: Tilt the Tip Arm back (see Figure b1). Align the pins with the round notches in the cradle (see Figure b2), then lower the Tip Arm into position (see Figure b3). Move the Tip Arm forward into the working position:

**b1**           **b2**           **b3**



**2.** 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. This is the correct position of the Tip Arm:



Install a new, opened 0.2-mL PCR tube into the hole of the Tip Loader

**3.** *Insert the opened 0.2-mL PCR tube into the hole in the base of the Tip Loader, as shown in the preceding photograph.*

# Perform the run

1. *Confirm that a new tip and opened 0.2-mL PCR tube have been loaded* and that the 8-well strip is correctly loaded. Ensure that Well 1 (ISP sample) is the left-most well and that the 8-well strip is pushed to the far-right position within the slot.

2. Pipet the contents of Well 2 up and down to resuspend the beads before starting the run. Do not introduce bubbles into the solution.

3. If necessary, turn ON the Ion OneTouch™ ES and 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).

4. Press **Start/Stop**. The screen displays "run" during the run. The run takes ~35 minutes.

   ---

   **IMPORTANT!**  Remove the enriched Ion PI™ Ion Sphere™ Particles ≤ 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 Ion PI™ Ion Sphere™ Particles in Melt-Off solution overnight.

   ---

   **Note:**  If necessary 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.

5. At the end of the run, the instrument displays "End" and beeps every 60 seconds. Press the **Start/Stop** button to silence this alarm and reset the Ion OneTouch™ ES for the next run. The instrument can be left on between runs.

6. *Immediately after the run*, securely close the tube, then remove the PCR tube containing the enriched Ion PI™ Ion Sphere™ Particles.

   **Note:**  Ensure that the 0.2-mL PCR tube has >200 µL of solution containing the enriched ISPs. If the tube has <<200 µL of solution containing the enriched ISPs, contact Life Technologies Technical Support.

7. Remove the used tip: While you are standing above the Tip Arm, and with the Tip Arm in its cradle, twist the tip *counterclockwise* and pull it downward to remove and discard the tip:
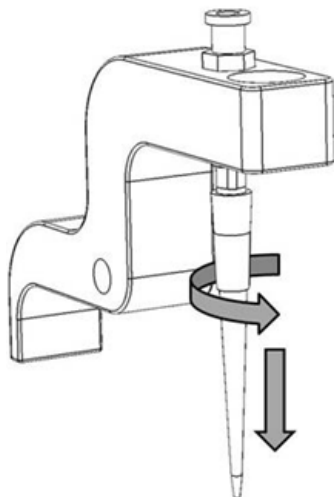


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**IMPORTANT!** Improper removal of tips can loosen the metal tip adapter fitting on the Tip Arm and affect instrument operation.
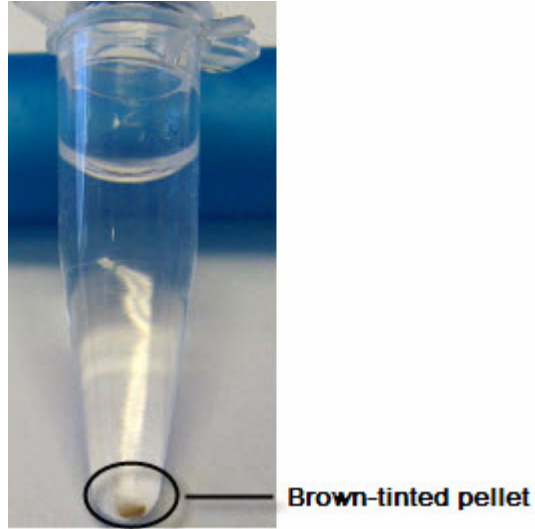
---

8. Remove and discard the used 8-well strip.

9. Proceed *immediately* to "Remove and wash the enriched Ion PI™ Ion Sphere™ Particles".

# Remove and wash the enriched Ion PI™ Ion Sphere™ Particles

1. Centrifuge the 0.2-mL PCR tube containing the enriched Ion PI™ Ion Sphere™ Particles 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 may 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:



Brown-tinted pellet

| Dynabeads® MyOne™ Streptavidin C1 Beads (brown pellet) in tube? | Then... |
|---|---|
| No | 1. Remove all but ~10 µL of supernatant without disturbing the pellet.<br><br>2. Add sufficient Nuclease-Free Water for a final volume of 100 µL.<br><br>3. Pipet up and down 10 times to resuspend the pellet.<br><br>4. Sequence or store the template-positive Ion PI™ Ion Sphere™ Particles:<br>• For the Ion PI™ Template OT2 200 Kit v2, use the Ion PI™ Sequencing 200 Kit v2 (Cat. no. 4485149). Refer to the *Ion PI™ Sequencing 200 Kit v2 User Guide* (Pub. no. MAN0007961).<br>  *or*<br>• Store the enriched ISPs at 2°C to 8°C for up to 3 days. |

| Dynabeads® MyOne™ Streptavidin C1 Beads (brown pellet) in tube? | 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 of Dynabeads® MyOne™ Streptavidin C1 Beads. |
|  | 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 Ion PI™ Ion Sphere™ Particles:<br>• For the Ion PI™ Template OT2 200 Kit v2, use the Ion PI™ Sequencing 200 Kit v2 (Cat. no. 4485149). Refer to the *Ion PI™ Sequencing 200 Kit v2 User Guide* (Pub. no. MAN0007961).<br>*or*<br>• Store the enriched ISPs at 2°C to 8°C for up to 3 days. |

# (*Optional*) Perform the Ion Sphere™ Particles 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. Refer to the *Ion PI™ Ion Sphere™ Particles Quality Assessment Using the Guava® easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. no. MAN0007496), available on the Ion Community website:

**ioncommunity.lifetechnologies.com**

# Troubleshooting

If troubleshooting does not resolve the problem, contact Life Technologies Technical Support.

| Ion OneTouch™ 2 Instrument | | |
|---|---|---|
| **Observation** | **Possible cause** | **Recommended action** |
| Firmware does not update or the status update screen does not display ≤ 10 seconds | Firmware not updating on the instrument | 1. Power the instrument OFF then ON.<br>2. Ensure that the USB flash drive is FAT32-formatted and that the file is in the root directory.<br>3. Remove then reinsert the USB flash drive *immediately* after the main menu displays.<br>4. Repeat steps 1–3 as needed. |
| Disposable injector remains in "down" position in the Ion OneTouch™ DL Injector Hub | • Reagent build-up<br>• New part | 1. *Gently* pull from the top of the disposable injector until the disposable injector just returns to the "up" position in the Injector Hub (see "Install the disposable injector" on page 25).<br>2. Briefly press then release the spring-loaded top of the Injector Hub 5-10 times at the point indicated by the arrow (see figures 4a–4c on page 26). You should hear a click.<br>3. If the Injector Hub remains in the "down" position, repeat step 2 once (up to 10 more clicks).<br><br>**Note:** If the Injector Hub still does not move freely and click up into place, then contact Life Technologies Technical Support. |

| Ion OneTouch™ 2 Instrument | | |
|---|---|---|
| **Observation** | **Possible cause** | **Recommended action** |
| Centrifuge lid does not open | • Power failure<br>• Software crash | 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:<br><br>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.<br>3. Remove the tool from the hole, then open the lid.<br>4. If necessary, troubleshoot the lid lock, then use the instrument normally. Do *not* force the lid open. |
| • Run fails<br>   *or*<br>• *Before* turning off instrument | Various | Retrieve the log files.<br><br>**IMPORTANT!** Do *not* turn off or power cycle the instrument until the log files are downloaded (see "Download the log files from the Ion OneTouch™ 2 Instrument" on page 121). If a run fails, contact Life Technologies Technical Support. |

| **Ion OneTouch™ 2 Instrument** | | |
|---|---|---|
| **Observation** | **Possible cause** | **Recommended action** |
| Precipitate in the Ion PI™ Reagent Mix TL after vortexing | Various | 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 Life Technologies Technical Support. |
| | | 6. Keep the Reagent Mix at room temperature during use. |
| | | 7. After use, store the solution at 2°C–8°C. |

| Ion OneTouch™ 2 Instrument | | |
|---|---|---|
| **Observation** | **Possible cause** | **Recommended action** |
| The volume of the pellet is ≥100 µL in one or both Ion OneTouch™ Recovery Tubes after a run on the Ion OneTouch™ 2 Instrument | Various | 1. Label 3 new 1.5-mL Eppendorf LoBind® Tubes for the template-positive Ion PI™ Ion Sphere™ Particles (ISPs).<br><br>2. Use a pipette to remove all but ~100–400 µL of supernatant above the pellet from each Recovery Tube.<br><br>3. Add 400 µL of Nuclease-Free Water to each Recovery Tube, then pipette each aliquot up and down to mix.<br><br>4. Transfer one suspension from a Recovery Tube to a new labeled 1.5-mL Eppendorf LoBind® Tube. Transfer the second suspension from a Recovery Tube to another new labeled 1.5-mL Eppendorf LoBind® Tube.<br><br>5. Add 100 µL of Nuclease-Free Water to each Recovery Tube, then pipet the suspensions up and down to mix.<br><br>6. Transfer one 100-µL suspension from a Recovery Tube to a 1.5-mL Eppendorf LoBind® Tube. Transfer the second 100-µL suspension to the second 1.5-mL Eppendorf LoBind® Tube.<br><br>7. Bring each ~500-µL suspension in the tubes to 1 mL with Nuclease-Free Water, then vortex each suspension for 30 seconds.<br><br>8. Heat both suspensions at 50°C for 2 minutes.<br><br>9. Vortex the suspensions for 30 seconds, then centrifuge the suspensions for 8 minutes at 15,500 × $g$.<br><br>10. Remove all but 100 µL of supernatant from each tube, then pipet to mix the ISPs.<br><br>11. Combine the two, 100-µL suspensions in a new labeled 1.5-mL Eppendorf LoBind® Tube for a total of 200 µL of combined suspension in the new tube.<br><br>12. Bring the suspension in the tube to 1 mL with Nuclease-Free Water.<br><br>13. Vortex the suspension for 30 seconds, then centrifuge the tube for 2 seconds. (*Optional*) Assess quality of the ISPs with the Guava® easyCyte™ 5 Flow Cytometer (see 12 on page 42).<br><br>14. Centrifuge the suspension for 8 minutes at 15,500 × $g$, then remove all but 20 µL of supernatant.<br><br>15. Bring the suspension to 100 µL in Ion PI™ ISP Resuspension Solution.<br><br>16. Vortex the pellet for 30 seconds, then centrifuge the suspension for 2 seconds. Assess quality of the ISPs with the Qubit® 2.0 Fluorometer (see step 17 on page 43).<br><br>17. Proceed to "Maintain the Ion OneTouch™ 2 Instrument" on page 43 and enrich the ISPs (see "Enrich the template-positive Ion PI™ Ion Sphere™ Particles" on page 49). |

| **Ion OneTouch™ ES** | | |
| --- | --- | --- |
| **Observation** | **Possible cause** | **Recommended action** |
| Excessive foaming | • Improperly calibrated or inadequate volume in one or more wells<br>• Loose fitting<br>• Cracked pipette tip | 1. Use the recommended volumes for all wells.<br>2. Ensure that fittings are tight, especially at the elbow fitting, and the pipette tip is not cracked.<br>3. If necessary, perform the residual volume check. If the residual volume check fails, then calibrate the instrument. |

| Ion OneTouch™ ES | | |
| --- | --- | --- |
| **Observation** | **Possible cause** | **Recommended action** |
| E12, E22, or E23 error displays during the run or during calibration | Calibration values are out of range | 1. Power OFF the instrument. and wait 3 seconds.<br><br>2. While holding down **Vert. Adjust**, power ON the instrument. This step restores the factory default settings.<br><br>3. Recalibrate the vertical axis (see page 97):<br><br>   **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.<br><br>   a. Press the ▼ (minus) button to lower the Tip Arm until the tip touches the shelf.<br>   b. Press the ▼ (minus) button *8* more times. Typical vertical axis settings are ~340–370.<br><br>4. Recalibrate the horizontal axis (see page 99): Press the ▲ (plus) button to move the Tip Arm to the right until the tip touches the left tab of the strip.<br><br>   **Note:** The default setting for the horizontal axis is 625. Typical horizontal axis settings are ~640–670. |
| | AC line voltage module installed incorrectly | 1. Determine the voltage of the electrical outlet to plug in the Ion OneTouch™ ES.<br><br>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.<br><br>If the AC line voltage module is installed incorrectly:<br><br>1. Gently remove the module with your fingernail or a small flathead screwdriver.<br><br>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.<br><br>3. Insert the AC line voltage module into the fuse socket. |

| **Ion OneTouch™ ES** | | |
|---|---|---|
| **Observation** | **Possible cause** | **Recommended action** |
| E12 or E22 error displays when the unit is initializing | • Fuse installed incorrectly<br>• Unit below operating temp<br>• Bad or calibration setting<br>  *or*<br>• Tip Arm is not moving | 1. Ensure that the fuse module is installed correctly and that the unit is at its recommended operating temperature.<br>2. Reboot the instrument: Power OFF the instrument, wait 3 seconds, then power ON the instrument.<br>3. If the error persists, restore the factory defaults, then re-calibrate the instrument:<br>  a. Power OFF the instrument and wait 3 seconds.<br>  b. While holding down **Vert. Adjust**, power ON the instrument. This step restores the factory default settings.<br>  c. Repeat steps 3a–3b as needed to restore the factory defaults.<br>  d. Calibrate the vertical and horizontal axes (see pages 97 and 99) |
| • E12 or E22 error displays<br>• Tip Arm does not move or moves slightly | AC line voltage module installed incorrectly | 1. Determine the voltage of the electrical outlet to plug in the Ion OneTouch™ ES.<br>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.<br>If the AC line voltage module is installed incorrectly:<br>1. Gently remove the module with your fingernail or a small flathead screwdriver.<br>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.<br>3. Insert the AC line voltage 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 overflowing during run | Overloaded reagent volumes | 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 vertical calibration procedure (see page 97). |
| Percent enrichment is <50% as measured by the Ion PI™ System | Multiple causes | Contact Life Technologies Technical Support. |

| Ion OneTouch™ ES | | |
| --- | --- | --- |
| **Observation** | **Possible cause** | **Recommended action** |
| Strip lifts up during strip push | Instrument is not calibrated properly | Perform horizontal position calibration (see page 99). |
| Strip lifts up when tip is raised from well | | Perform vertical calibration (see page 97). |
| *Immediately* after strip push, the strip is not in contact with the magnet | | Perform horizontal position calibration (see page 99). |
| Tip grinds into base of instrument and Code "1999" displays | • Unit not calibrated properly<br>• Vertical calibration setting too low or out-of-range | 1. Erase the memory on the instrument: Hold down the vertical adjust button while powering ON the instrument. The instrument beeps several times.<br>2. Recalibrate the instrument (see page 96).<br>3. Perform a residual volume test (see page 92). |
| Tip is hitting the top of 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 displays | Various | 1. Power the instrument OFF then ON.<br>2. If the error continues to display, erase the memory on the instrument. Hold down the vertical adjust button while powering ON the instrument. The instrument beeps several times.<br>3. Recalibrate the instrument (see page 96).<br>4. Perform residual volume test (see page 92). |
| Instrument does not aspirate or dispense liquids | Loose fitting(s) | • 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.<br>• Ensure that the metal tip adapter fitting on the Tip Arm is finger-tight.<br><br>**IMPORTANT!** After any adjustments to the metal tip adapter, recalibrate the Ion OneTouch™ ES. |

# A   Quality control of Ion PI™ Ion Sphere™ Particles

## Ion Sphere™ Quality Control assay

The Ion Sphere™ Quality Control assay on the Qubit® 2.0 Fluorometer labels the Ion Proton™ I Ion Sphere™ Particles (ISPs) with two different fluorophores: Alexa Fluor® 488 and Alexa Fluor® 647.

- The probe labeled Alexa Fluor® 488 anneals to primer B sites, or all of the ISPs present.
- The probe labeled Alexa Fluor® 647 anneals to primer A sites, or only the ISPs with extended templates.

The ratio of the Alexa Fluor® 647 fluorescence (templated ISPs) to the Alexa Fluor® 488 fluorescence (all ISPs present) yields the % templated ISPs.

The following is the schematic of the probes labeled Alexa Fluor® 488 and Alexa Fluor® 647 annealed to an ISP:

**Note:** The drawing is not to scale.
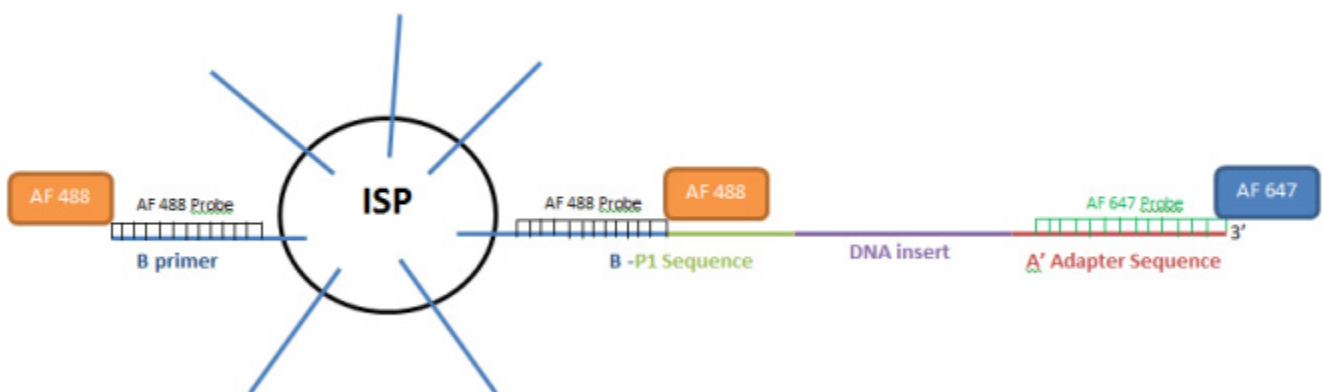
# Quality control using Qubit® 2.0 Fluorometer

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**IMPORTANT!**  Effective 15 February 2012, the Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standards replace the spectrally similar FAM™ and Cy®5 dyes, respectively, previously used in the kit. To update the existing plugin on the Qubit® 2.0 Fluorometer with the new dye names, install the new plugin file, Ion_PluginV310_AF.qbt.
For details, see the .

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Both sets of dye-labeled calibration standards can be used in conjunction with firmware version 3.00 and previous versions of plugin files (ion_plugin.qbt or Ion_plugin_AF.qbt).

We highly recommend that you install the new firmware version 3.10 and the new Ion plugin file (Ion_PluginV310_AF) for ease of use as these new versions allow you to operate the Ion plugin on the Qubit® 2.0 Fluorometer without requiring the USB drive to be connected into the device at all times.

The selection options on the user interface of the instrument matches the new Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standards once the new plugin file is installed.

## Equipment/ supplies required

| | Description† | Supplier | Cat. number | Quantity |
|---|---|---|---|---|
| | Qubit®2.0 Fluorometer, including USB drive | Life Technologies | Q32866 | 1 |
| | Qubit® Assay Tubes | Life Technologies | Q32856 | 1 |
| | Qubit® 2.0 Easy Calculator Microsoft® Excel® Spreadsheet‡ | — | — | 1 |
| | Ion PI™ Template OT2 200 Kit v2 | Life Technologies | 4485146 | 1 |
| | Ion Sphere™ Quality Control Kit | Life Technologies | 4468656 | 1 |
| | Unenriched Ion PI™ Ion Sphere™ Particles | — | — | — |
| | 0.2-mL PCR tubes | Axygen | PCR-02-L-C | 1000/case |
| | | BioExpress | T-3035-1 | 120 strips of 8 |
| | • GeneAmp® 96-well PCR System 9700 *or* • DNA Engine Tetrad® 2 or DNA Engine Dyad® Thermal Cyclers | Applied Biosystems  Bio-Rad | N8050200 (Base)  4314443 (Block)  Major Laboratory Supplier | 1 |

† Life Technologies has validated this protocol using this specific material. Substitution may adversely affect system performance.

‡ To download the spreadsheet, go to the Ion Community website at **ioncommunity.lifetechnologies.com/community/products/pgm/ user_guides_and_bulletins** (log-in required).

The Quality Control of Ion Proton™ I Ion Sphere™ Particles (ISPs) using a Qubit® 2.0 Fluorometer protocol consists of three sections:

- "Section 1: Upgrade the Qubit® 2.0 Fluorometer firmware and software".
- "Section 2: Calculate the Qubit® 2.0 Fluorometer calibration factor" on page 78.
- "Section 3: Measure the unenriched, templated Ion PI™ Ion Sphere™ Particles" on page 80.

---

**IMPORTANT!** Before using the Qubit® 2.0 Fluorometer for the first time, sections 1 and 2 must be completed.

---

**Note:** Sections 1 and 2 only need to be completed one time for each Qubit® 2.0 Fluorometer.
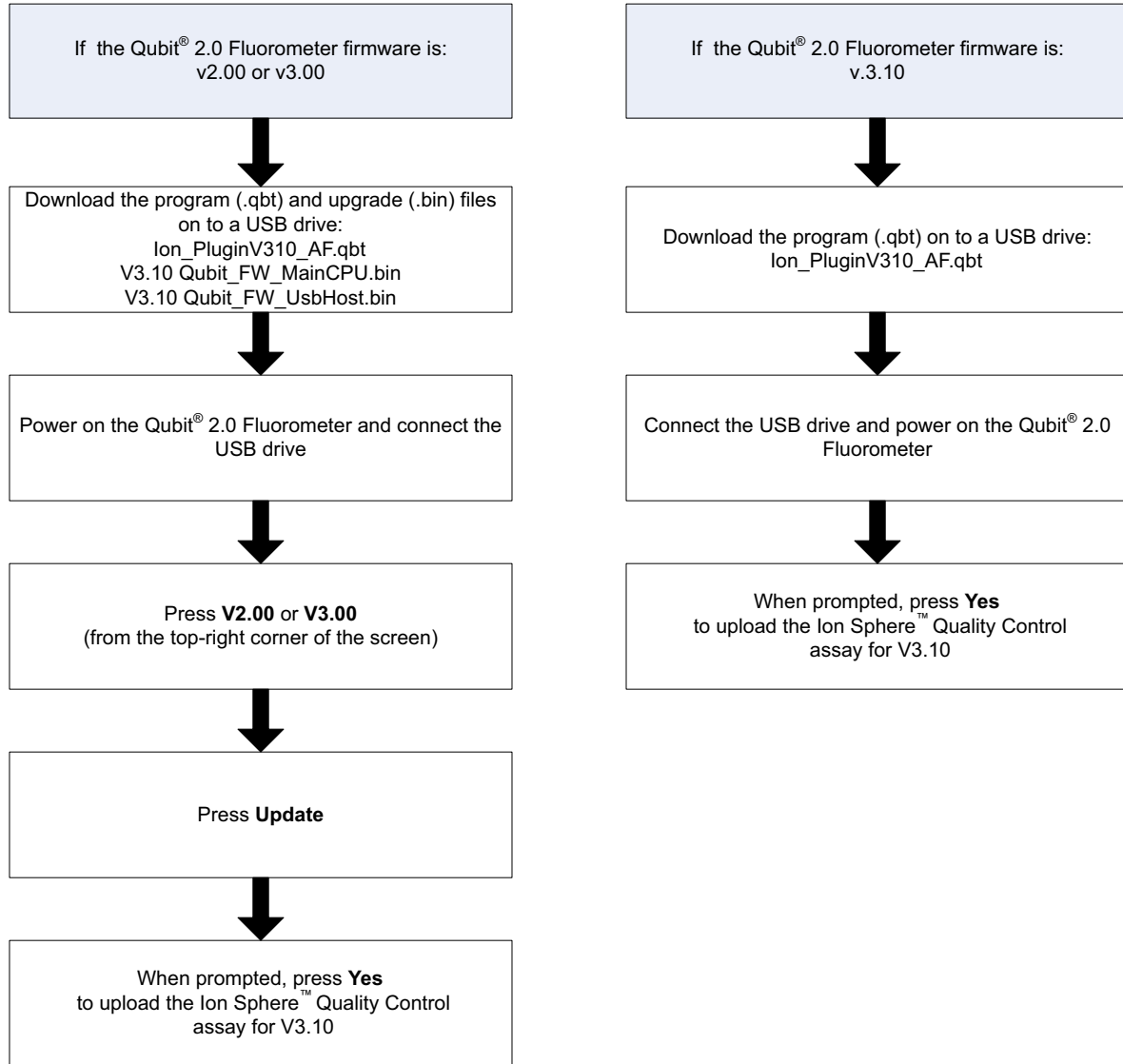
If sections 1 and 2 have been successfully completed for a particular Qubit® 2.0 Fluorometer already, then go directly to "Section 3: Measure the unenriched, templated Ion PI™ Ion Sphere™ Particles" on page 80.

**Section 1: Upgrade the Qubit® 2.0 Fluorometer firmware and software**

This section provides information about upgrading the firmware and the software of the Qubit® 2.0 Fluorometer.

The following graphic provides a summary workflow. The details are explained in the following sections:

- "Upgrade for Firmware V2.00 or V3.00" on page 72.
- "Upgrade for Firmware V3.10" on page 76.

| | |
|---|---|
| If the Qubit® 2.0 Fluorometer firmware is:<br>v2.00 or v3.00 | If the Qubit® 2.0 Fluorometer firmware is:<br>v.3.10 |
| ↓ | ↓ |
| Download the program (.qbt) and upgrade (.bin) files on to a USB drive:<br>Ion_PluginV310_AF.qbt<br>V3.10 Qubit_FW_MainCPU.bin<br>V3.10 Qubit_FW_UsbHost.bin | Download the program (.qbt) on to a USB drive:<br>Ion_PluginV310_AF.qbt |
| ↓ | ↓ |
| Power on the Qubit® 2.0 Fluorometer and connect the USB drive | Connect the USB drive and power on the Qubit® 2.0 Fluorometer |
| ↓ | ↓ |
| Press **V2.00** or **V3.00**<br>(from the top-right corner of the screen) | When prompted, press **Yes**<br>to upload the Ion Sphere™ Quality Control assay for V3.10 |
| ↓ | |
| Press **Update** | |
| ↓ | |
| When prompted, press **Yes**<br>to upload the Ion Sphere™ Quality Control assay for V3.10 | |

### Upgrade for Firmware V2.00 or V3.00

**Note:** The previous program files (.qbt) are not compatible with the new V3.10 firmware. The new program file (Ion_PluginV310_AF.qbt) is not compatible with V3.00 firmware.

**Materials required for update**:

- Qubit® 2.0 Fluorometer (Part no. Q32866)
- USB drive (included with Qubit® 2.0 Fluorometer)
- Program (.qbt) and upgrade (.bin) files:
  – Ion_PluginV310_AF.qbt
  – V3.10 Qubit_FW_MainCPU.bin
  – V3.10 Qubit_FW_UsbHost.bin

**Guidelines for downloading files**:

We recommend that you use a Windows® OS-based computer to download and transfer the Qubit® 2.0 Fluorometer files to the USB Drive.

**Note:** Do not use a Macintosh® computer to transfer the files to the USB drive if the serial number of your Qubit® 2.0 Fluorometer is lower than 1104004846.

We also recommend that you use the USB drive provided with Qubit® 2.0 Fluorometer for file transfer.

Other compatible USB drives may also be used. Visit:
**http://ioncommunity.lifetechnologies.com/community/products/pgm/user_guides_and_bulletins**
to see a list of approved USB drives. Log in is required.

1. Download the program file (.qbt) and two upgrade files (.bin) from
   **http://ioncommunity.lifetechnologies.com/community/products/pgm/user_guides_and_bulletins**
   onto a windows PC desktop. Log in is required.

2. Remove the existing program file (.qbt) and two upgrade files (.bin) from the USB drive, if any.

3. Transfer the three downloaded files to the USB drive provided with the Qubit® 2.0 Fluorometer (recommended), or another compatible USB drive.

   **Note:** Files on the USB drive must be in the root directory and cannot be in a folder.

**Upgrade the firmware from the previous versions**

1. Power on the Qubit® 2.0 Fluorometer by plugging in the unit.

2. Insert the USB drive, containing the program (.qbt) and upgrade (.bin) files, into the USB port on the instrument.

3. In the upper-right corner of the main menu, **touch V2.00, or V3.00 depending on the version**.

4. If the image of the USB drive in the update screen displays a green dot, touch Update to upgrade the instrument firmware.

   The firmware update lasts approximately 2 minutes, during which the Qubit® 2.0 Fluorometer screen flashes:

   **Note:** If the image displays a red dot, the Qubit® 2.0 Fluorometer cannot detect the USB drive. Ensure the USB drive is in place securely. If the problem persists, remove the USB drive and reinsert.
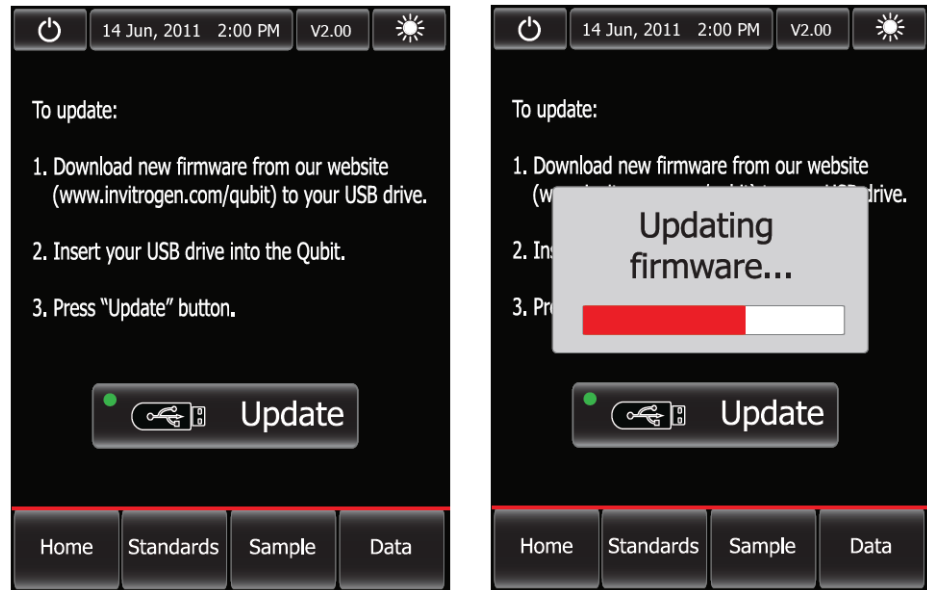
5. When the Qubit® 2.0 Fluorometer displays the main screen, confirm that V3.10 is displayed (in the upper-right corner), which confirms that the upgrade was successful.

   After the firmware has been updated, the following screen will be displayed. Confirm that V3.10 is displayed in the upper-right corner. Touch **Yes** to permanently upload the Ion Sphere™ QC program to the instrument:
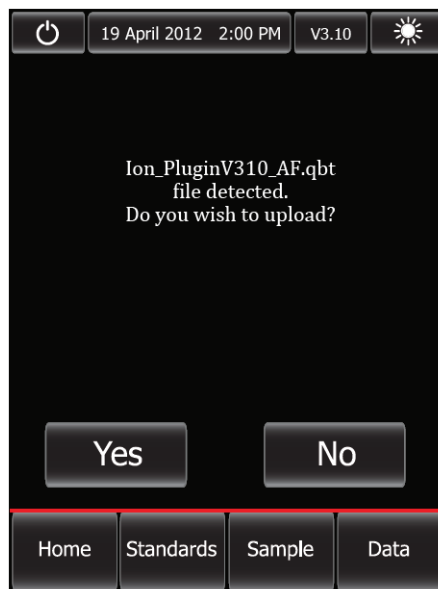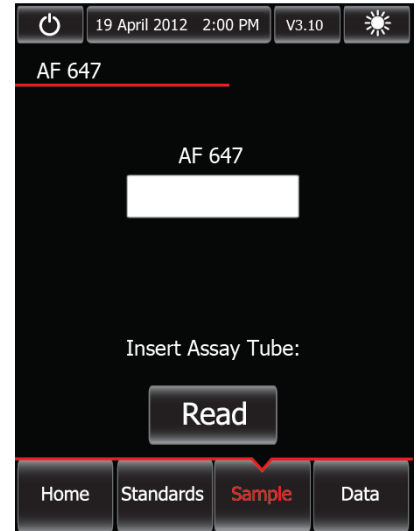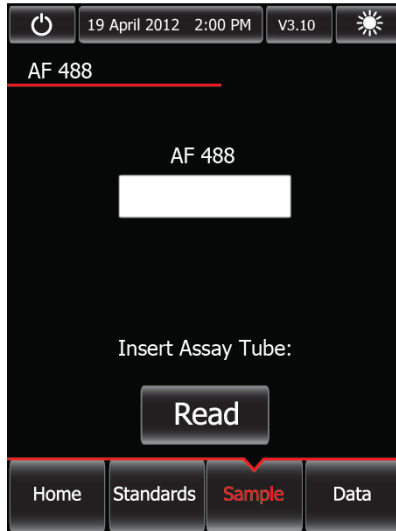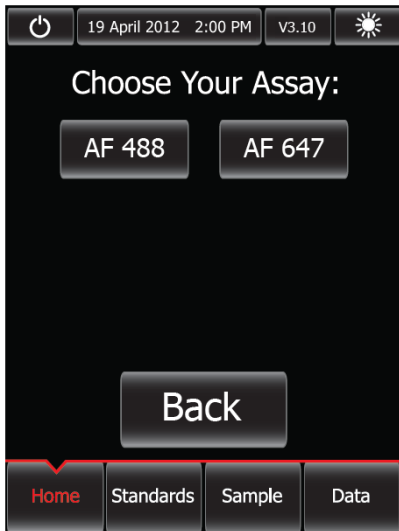
6. Confirm the Ion Sphere™ QC program (.qbt) file is functional by checking the following screens.

a. In the main menu, the Ion selection option is present:
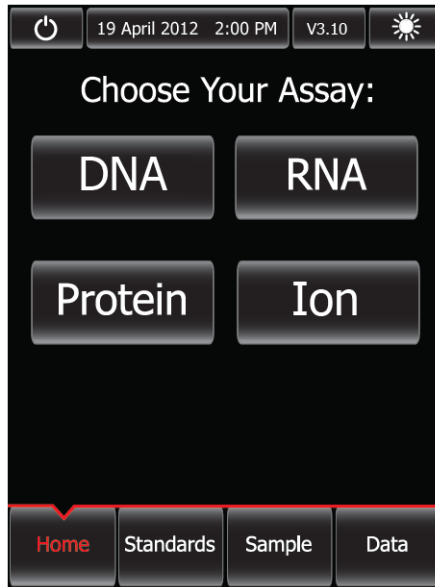


b. After touching **Ion** ( Ion ), **AF 488** ( AF 488 ) and **AF 647** ( AF 647 ) selection options appear on the screen:

**Note:** Touching AF 488 or AF 647 enters the respective measurement channels.

**7.** Proceed to complete the instruction in "Section 2: Calculate the Qubit® 2.0 Fluorometer calibration factor" on page 78.

### Upgrade for Firmware V3.10

If your Qubit® 2.0 Fluorometer is equipped with V3.10 firmware, you *only* need to upload the Ion Sphere™ Quality Control assay into your fluorometer:



Firmware version

---
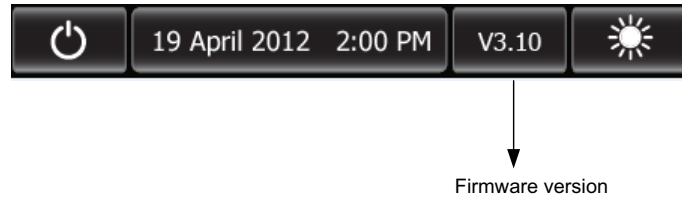
**IMPORTANT!**  Do not re-upgrade the firmware if the instrument is shipped with V3.10 version.

---

To upload the Ion Sphere™ Quality Control assay into your fluorometer, use the following steps:

**1.** Download the new Ion_PluginV310_AF.qbt file (from:
**http://ioncommunity.lifetechnologies.com/community/products/pgm/user_guides_and_bulletins**)
to your USB drive to activate the application to accept the new names. Log in is required.

**Note:**  We recommend that you use the USB drive provided with Qubit® 2.0 Fluorometer for file transfer; however, other compatible USB drives may also be used. Visit:
**http://ioncommunity.lifetechnologies.com/community/products/pgm/user_guides_and_bulletins**
to see a list of approved USB drives. Log in is required.

2. With the USB drive (containing the Ion_PluginV310_AF.qbt file) inserted into the Qubit® 2.0 Fluorometer, power-cycle the Qubit® 2.0 Fluorometer by unplugging and plugging it back in. Touch **Yes** to permanently upload the program to the instrument:
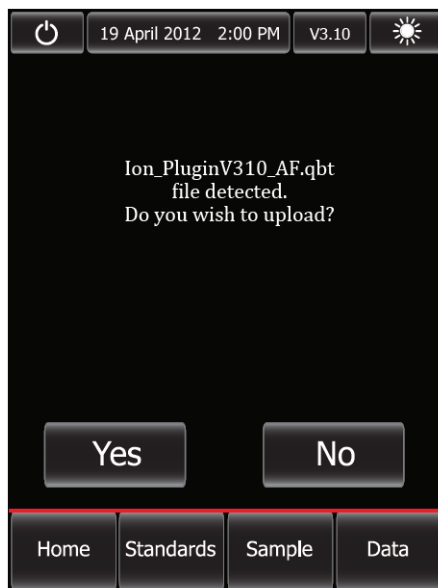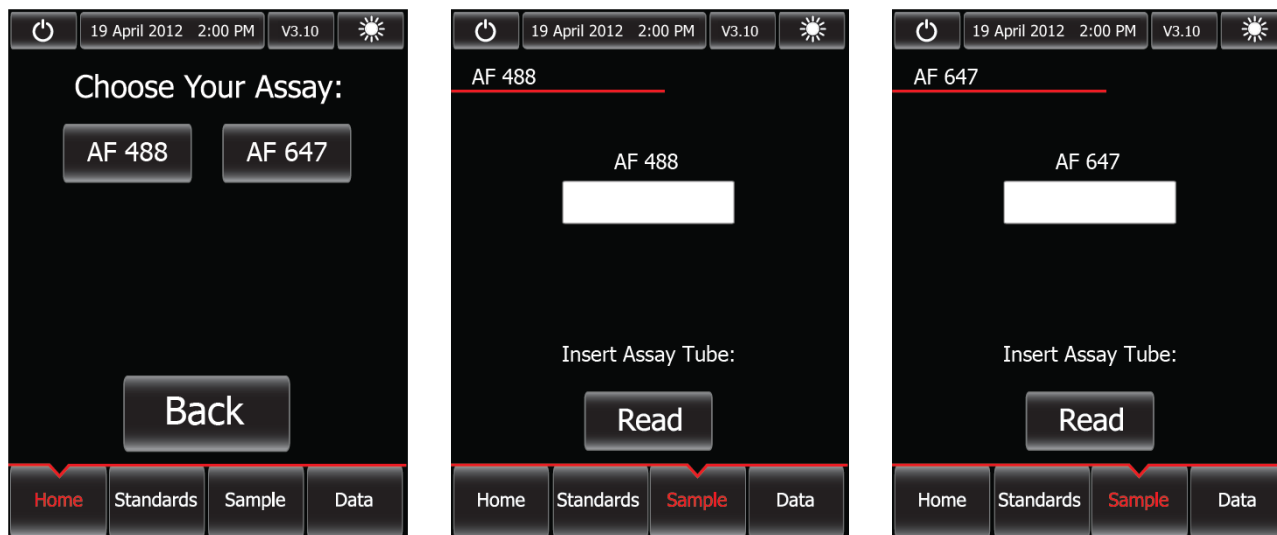


3. Confirm the Ion Sphere™ QC program (.qbt) file is functional by checking the following screens:

   a. In the main menu, the Ion selection option is present.
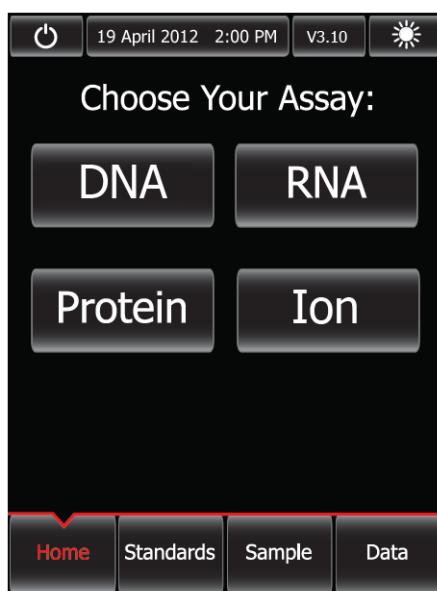
b. After touching **Ion** ( Ion ), **AF 488** ( AF 488 ) and **AF 647** ( AF 647 ) selection options appear on the screen:

**Note:** Touching AF 488 or AF 647 enters the respective measurement channels.



4. Proceed to complete the instruction in "Section 2: Calculate the Qubit® 2.0 Fluorometer calibration factor".

## Section 2: Calculate the Qubit® 2.0 Fluorometer calibration factor

Section 2 describes the procedure to determine the Qubit® 2.0 Fluorometer instrument-specific calibration factor.

---

**IMPORTANT!** You must upgrade the Qubit® 2.0 Fluorometer firmware and software prior to performing the following procedure. See "Section 1: Upgrade the Qubit® 2.0 Fluorometer firmware and software" on page 71 for more information.

---

Each Qubit® 2.0 Fluorometer has a unique Calibration Factor that must be calculated and applied to all Percent Templated ISPs calculations.

**Note:** It is only necessary to calculate the calibration factor once for a particular instrument, unless a problem is suspected.

### Materials required

- Qubit® 2.0 Easy Calculator Microsoft® Excel® Spreadsheet (Log in is required.)
- Qubit® 2.0 Fluorometer (Part no. Q32866) with V3.10 firmware
- USB drive containing the ".qbt" file
- Qubit® Assay Tubes (Part no. Q32856)
- Ion Sphere Quality Control Kit (Part no. 4468656)

### Download Qubit® 2.0 Easy Calculator

Download the Qubit® 2.0 Easy Calculator Microsoft® Excel® Spreadsheet file from: **http://ioncommunity.lifetechnologies.com/community/products/pgm/ user_guides_and_bulletins**
(Log in is required), and save the file to the computer used for Qubit® 2.0 data analysis.

### Calibration standard preparation

1. From the Ion Sphere™ Quality Control Kit, thaw the Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standard reagents.

   **Note:** Both the Alexa Fluor® 488 and Alexa Fluor® 647 molecules are photosensitive, so avoid exposure to light for long periods of time and direct sunlight.

2. Vortex well to mix and pulse-spin the tube to remove any liquid trapped in the cap.

3. Transfer 200 μL of each standard into two separate Qubit® assay tubes. Pulse-spin to bring all the liquid to the bottom of the tube.

### Calibration standard measurement

---

**IMPORTANT!** Prior to using the Qubit® 2.0 Fluorometer, ensure that the instrument is running Firmware V3.10, and the Ion_PluginV310_AF.qbt file has been permanently uploaded to the instrument. See section "Section 1: Upgrade the Qubit® 2.0 Fluorometer firmware and software" on page 71 for more information about managing Qubit® 2.0 Fluorometer firmware versions.

---

1. Touch **Ion** to access Alexa Fluor® 488 and Alexa Fluor® 647 measurement options.

2. Touch **AF 488** and insert the Alexa Fluor® 488 Calibration Standard reagent into the Qubit® 2.0 Fluorometer, close the lid, and touch **Read**.

   **Note:** The lettering on the Read ( Read ) selection option changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

3. Record the RFU value and remove sample from Qubit® 2.0 Fluorometer.

4. Touch **Home**, touch **Ion**, and then touch **AF 647**. Insert the Alexa Fluor® 647 Calibration Standard into the Qubit® 2.0 Fluorometer, close the lid, and touch **Read**.

   **Note:** The lettering on the Read ( Read ) selection option changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

5. Record the RFU value and remove sample from Qubit® 2.0 Fluorometer.

### Calibration Factor calculation

1. In the Qubit® 2.0 Easy Calculator, enter each recorded RFU value in the appropriately labeled green cell to display the Calibration Factor specific for the Qubit® 2.0 Fluorometer.

2. Save a copy of the Qubit® 2.0 Easy Calculator containing the Calibration Factor for use as a template for future Percent Templated ISPs calculations:

   **Note:** Affix a sticker with the instrument-specific Calibration Factor to the Qubit® 2.0 Fluorometer.

**Qubit Calibration Factor Calculation**

| Calibration Standard | RFU | Calibration Factor |
|---|---|---|
| Alexa Fluor® 488 Calibration Standard | | #DIV/0! |
| Alexa Fluor® 647 Calibration Standard | | |

**Templated Signal Ratio**

| Calibration Standard | Raw RFU Value | | Background RFU (Negative Control Tube) | | | |
|---|---|---|---|---|---|---|
| Sample ID | Alexa Fluor® 488 | Alexa Fluor® 647 | Alexa Fluor® 488 | Alexa Fluor® 647 | Conversion Factor* | Percent Templated ISPs |
| | | | | | | #DIV/0! |
| | | | | | | #DIV/0! |
| | | | | | | #DIV/0! |
| | | | | | | #DIV/0! |

\* Conversion factor can be found on the Ion Community website (www.iontorrent.com/qubit_files/) and is Templated Kit LOT specific.

**Legend**

| | |
|---|---|
| | Green cells = Raw RFU values of Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standards supplied in the Ion Sphere Quality Control Kit. |
| | Red cells = Raw RFU values measured in Section 3: Measure the Ion Sphere™ Particles (ISPs) 200 sample. |
| | Purple cells = Raw RFU values measured for negative control in Section 3: Measure the Ion Sphere™ Particles (ISPs) 200 sample. |
| | Blue cells = Template kit LOT specific conversion factor. |

**IMPORTANT!** For each Qubit® 2.0 Fluorometer used, save a separate Qubit® 2.0 Easy Calculator Microsoft® Excel® Spreadsheet file containing the Calibration Factor specifically calculated for that particular instrument.

## Section 3: Measure the unenriched, templated Ion PI™ Ion Sphere™ Particles

Section 3 describes the procedure for determining the Percent Templated ISPs for unenriched Ion PI™ Ion Sphere™ Particles.

### Materials required

- Qubit® 2.0 Fluorometer (Part no. Q32866) with the V3.10 firmware and the Ion Sphere™ Quality Control assay
- Qubit® Assay Tubes (Part no. Q32856)
- PCR tubes, 0.2 mL (Axygen Part no. PCR-02-L-C or BioExpress Part no.T-3035-1)
- Qubit® 2.0 Easy Calculator Microsoft® Excel® Spreadsheet file containing the instrument specific Calibration Factor. See "Section 2: Calculate the Qubit® 2.0 Fluorometer Calibration Factor" for details.
- Ion Sphere™ Quality Control Kit (Part no. 4468656)
- Unenriched Ion PI™ Ion Sphere™ Particles
- Ion PI™ ISP Resuspension Solution from the Ion PI™ Template OT2 200 Kit v2 (Cat. no. 4485146)

### Sample preparation

1. From the Ion Sphere™ Quality Control Kit, thaw the Ion Probes tube, Annealing Buffer, and Quality Control Wash Buffer.

2. For unenriched Ion PI™ Ion Sphere™ Particles, if not 100 µL, adjust sample volume to 100 µL in Ion PI™ ISP Resuspension Solution from the Ion PI™ Template OT2 200 Kit v2 (Cat. no. 4485146) and transfer 2 µL to a 0.2-mL PCR tube.

3. Add Ion Probes to sample(s).

   a. If processing one sample, add 19 µL Annealing Buffer and 1 µL Ion Probes directly to the 0.2-mL PCR tube containing the Ion PI™ Ion Sphere™ Particles and mix well by pipetting up and down.

   b. If processing more than one sample, generate an Ion Probe Master Mix:

      • (21 µL Annealing Buffer * # samples) + (1 µL Ion Probes * # samples) = total volume required

      • Add 20 µL of Ion Probe Master Mix to the 0.2-mL PCR tubes containing the Ion PI™ Ion Sphere™ Particles, then mix well by pipetting up and down.

4. Load the tube(s) into a thermal cycler, then perform the following protocol to anneal the Ion Probes:

   | Temperature | Time |
   | --- | --- |
   | 95°C | 2 min |
   | 37°C | 2 min |

5. Remove unbound probes by washing the sample(s) three times with 200 µL Quality Control Wash Buffer.

   a. Add 200 µL Quality Control Wash Buffer to the 0.2-mL tube(s).

   b. Vortex properly to mix and centrifuge down at 15,500 × g for 3 minutes.

   c. Being careful not to disturb the pelleted Ion PI™ Ion Sphere™ Particles, remove the supernatant and leave behind 10 µL.

   **Note:** Compare to a 10 µL standard for reference.

   d. Repeat substeps a through c of step 5 two times for a total of three Quality Control Wash Buffer washes.

6. After the final wash, add 190 µL of Quality Control Wash Buffer for a total volume of 200 µL, mix by pipetting up and down five times and transfer the entire sample to a Qubit® assay tube.

   **IMPORTANT!** Ensure that you measure the volumes accurately.

7. To generate a negative control, add 200 µL of Quality Control Wash Buffer to a fresh Qubit® assay tube.

8. Read the sample(s) using the Qubit® 2.0 Fluorometer as described below.

### Sample measurement

---

**IMPORTANT!**  Prior to using the Qubit® 2.0 Fluorometer, ensure that the instrument is running Firmware V3.10, and the Ion_PluginV310_AF.qbt file has been permanently uploaded to the instrument. See section "Section 1: Upgrade the Qubit® 2.0 Fluorometer firmware and software" on page 71 for more information about managing Qubit® 2.0 Fluorometer firmware versions.

---

1. Power on the Qubit® 2.0 Fluorometer.

2. Touch **Ion** to access Alexa Fluor® 488 and Alexa Fluor® 647 measurement options.

3. Touch **AF 488** and insert the sample into the Qubit® 2.0 Fluorometer, close the lid, and touch **Read**.

   **Note:**  The lettering on the Read ( Read ) selection option changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

   **Note:**  If more than one sample is being processed, all samples can be read with the AF 488 setting before moving on to the AF 647 setting.

4. Record the value.

   **Note:**  The data retained on the Qubit® 2.0 Fluorometer can be transferred to a USB drive. See the "(Optional) Data Transfer to USB Drive" for details. If more than one sample is being processed, all samples can be read with the AF 488 setting before moving on to the AF 647 setting.

5. Touch **Home**, touch **Ion**, and then touch **AF 647**. Insert the sample into the Qubit® 2.0 Fluorometer, close the lid, and touch **Read**.

   **Note:**  The lettering on the Read ( Read ) selection option changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

6. Record the value.

---

**IMPORTANT!**  Ensure that you read the negative control (Quality Control Wash Buffer only) in both the Alexa Fluor® 488 and Alexa Fluor® 647 settings and record the RFU values.

---

### (*Optional*) Data Transfer to USB Drive

1. Ensure that the USB drive is inserted in the instrument.

2. In the main menu, touch Data (at the bottom-right corner of the screen).

3. In the data screen, touch **USB drive**, then wait for the instrument to download the data to the USB drive:

   **Note:**  The download creates a ".csv" file that can be opened on your computer using any spreadsheet software, such as Microsoft® Excel® software.
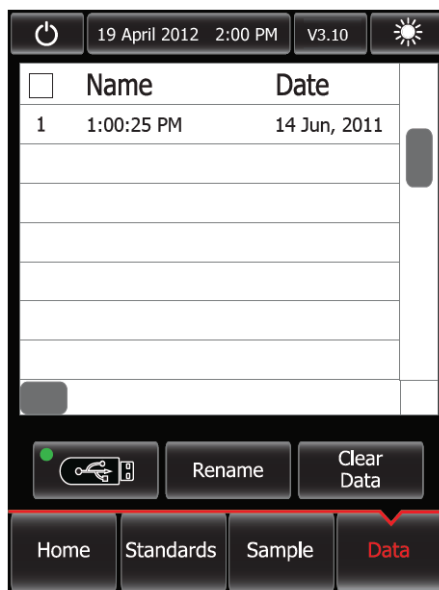
## Templated Ion PI™ Ion Sphere™ Particle evaluation

1. Open the saved Qubit® 2.0 Easy Calculator Microsoft® Excel® Spreadsheet file containing the Calibration Factor specifically calculated for the Qubit® 2.0 Fluorometer used.

2. Enter the raw RFU values from Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standards measurements in the appropriate fields for both the ISPs containing samples (red cells) and negative control sample (purple cells).

   **IMPORTANT!** The Alexa Fluor® 488 value must be >100 counts to produce a valid % Templated ISPs value. If the Alexa Fluor® 488 value is <100 counts, see the "Ion Sphere™ Quality Control assay troubleshooting table" on page 85.

| Fluorophore | Acceptable RFU Range |
|---|---|
| Alexa Fluor® 488 | > 100 counts; no upper limit |
| | Samples with <100 counts usually correlate with no or very few ISPs in the assay. |
| Alexa Fluor® 647 | Any value, with the condition that the Alexa Fluor® 488 RFU value is >100 counts. |

3. In the appropriate field (blue cells), enter the lot-specific conversion factor for *unenriched* Ion PI™ Ion Sphere™ Particles, available at:

   **http://ioncommunity.lifetechnologies.com/docs/DOC-1420**

   (Log in is required).

4. The Percent Templated ISPs calculates automatically and is displayed for each sample:



### Acceptance criteria for *unenriched* Ion PI™ Ion Sphere™ Particle samples

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.

| 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). |

# Ion Sphere™ Quality Control assay troubleshooting table

The following table contains a troubleshooting guide for the Ion Sphere™ Quality Control assay on the Qubit® 2.0 Fluorometer.

| Sample | Qubit® 2.0 Fluorometer Phenotype | Ion Proton™ Sequencer Possible Observation | Possible Root Cause | Recommended Action |
|---|---|---|---|---|
| Unenriched | <10% Templated ISPs | • Lower loading<br>• Lower % enriched<br>• Lower key signal<br>• Lower throughput | Too little library input into template preparation | • Increase library input to target 20–25% templated ISPs.<br>*or*<br>• 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.<br>*or*<br>• Continue with sequencing; expect lower throughput. |
| | >70% Templated ISPs | • Low loading<br>• Low % enriched<br>• Lower throughput<br>• High % filtered reads | Ion OneTouch™ 2 Instrument underperformance | Troubleshoot with Life Technologies Technical Support or a Field Application Scientist. |
| | | • Increased % primer dimer filtered reads<br>• Lower throughput | Adapter dimer contaminating library, more likely in short amplicon, Ion AmpliSeq™ or miRNA libraries | • Check Bioanalyzer® traces for adapter dimer peak.<br>• Re-purify library using Agencourt® AMPure® XP Kit clean-up steps as outlined in the appropriate user guides. |

# 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 from up to 200 base-pair average insert libraries on the Ion Proton™ System. For details, refer to the *Ion PI™ Ion Sphere™ Particles Quality Assessment Using the Guava® easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. no. MAN0007496), available on the Ion Community website:

**ioncommunity.lifetechnologies.com**

# B Set up, calibrate, and maintain the Ion OneTouch™ ES

> **IMPORTANT!** Ensure that the fuse module for the Ion OneTouch™ ES is properly installed, according to 110–120 V or 240 V line voltages. Refer to the *Ion Proton™ System Site Preparation Guide* (Pub. no. 4478733).

## Set up the Ion OneTouch™ ES

Before the first ISP enrichment run, set up the instrument.

**Materials required for this procedure**

**Provided in the Ion PI™ Template OT2 Solutions 200 Kit v2 (Part no. 4485143):**

Nuclease-Free Water

**Provided in the Ion PI™ Template OT2 Supplies 200 Kit v2 (Part no. 4485144):**

• Stripwell™ Plate, No Lid (8-well flat-bottom strips; Corning Cat. no. 2593)
• Eppendorf® LoRetention Dualfilter Tips (P300)

**Provided in the OneTouch™ 2 System (Part no. 4474779):**

Corning Brand 96-Well Strip Ejector (Fisher Cat. no. 07-200-22), unless 8-well strips are supplied loose and out of the frame

**Other Materials and Equipment:**

• Ion OneTouch™ ES
• Elbow fitting for Ion OneTouch™ ES
• 0.2-mL PCR tube
• Pipettes
• Vortexer
• (*Optional*) Felt-tipped pen

**Set up the instrument**

1. Locate the Tray, Tip Arm, and Tip Loader of the Ion OneTouch™ ES.

2. Install the tray:

   a. Wipe the instrument and the bottom of the Tray with a damp lab wipe to remove any packaging debris.

   b. Place the Tray on the Ion OneTouch™ ES with the calibration shelf on the left as shown in the following photograph.

   c. Push the Tray down firmly so that the Tray fits snugly in the clamps, then confirm that the Tray is level:

   ---
   **IMPORTANT!**  For proper operation of the Ion OneTouch™ ES, the tray must be firmly and uniformly seated in the cutout for the tray.

   ---
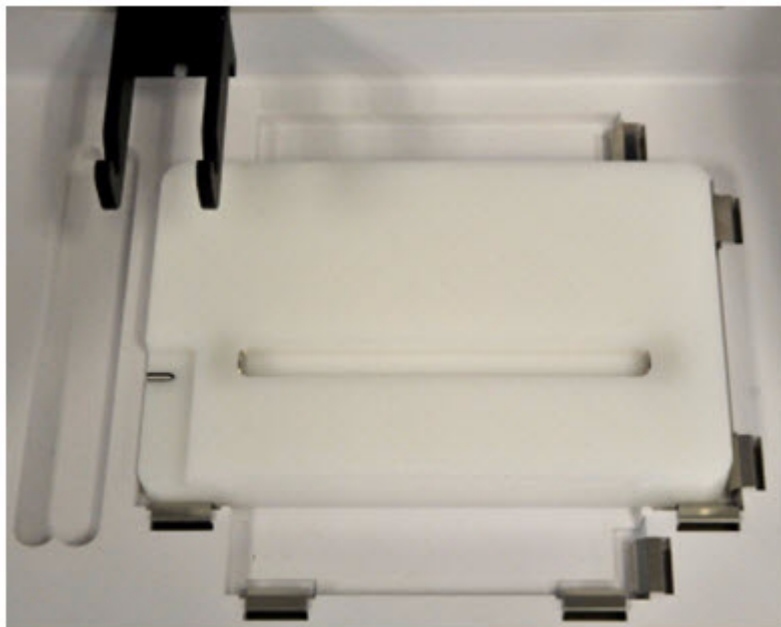
3.  Install the supplied elbow fitting and place the Tip Arm in the cradle.

    a.  Insert the male end of the elbow fitting into the female connector on the Tip Arm (see Figure a1), then finger tighten the lock ring on the elbow fitting (see Figure a2):
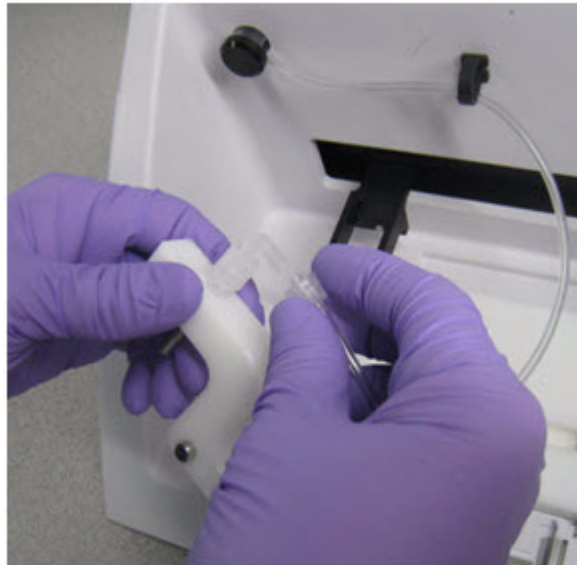
**a1**                                                            **a2**



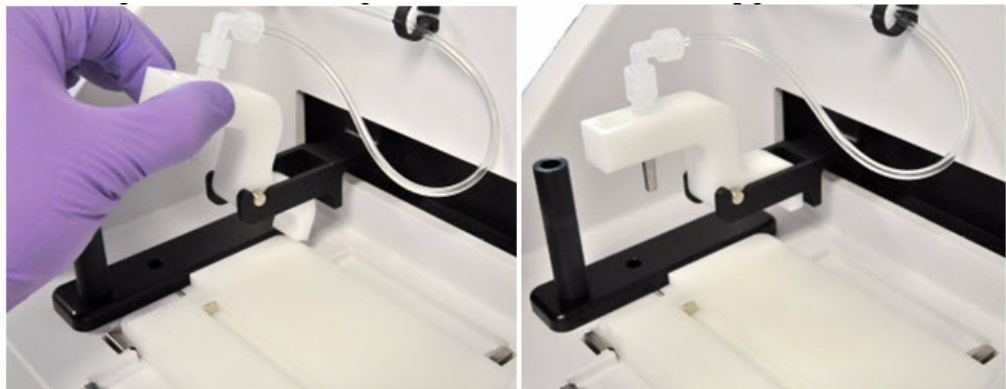    b.  Connect the tubing to the elbow fitting on the top of the Tip Arm:

c. Finger-tighten the connectors *at both ends* of the elbow. Do not twist or coil the tubing:



d. Tilt the Tip Arm back. Align the pins with the round notches in the cradle (see Figure d1), then lower the Tip Arm into position. Move the Tip Arm forward into the working position (see Figure d2):
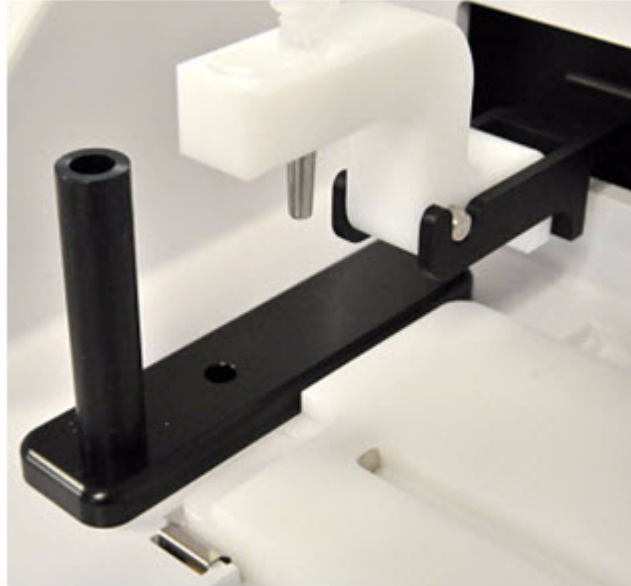
**d1**                                                    **d2**



**Note:** 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. Do *not* adjust the angle of the arm unless you need to calibrate the instrument.

4. Place the Tip Loader in the slot on the left side of the instrument deck:



5. Ensure that the AC line voltage fuse module is installed. Refer to the *Unpack and Install the Ion OneTouch™ 2 System Product Information Sheet* (Pub. no. 4481875).

6. Ensuring that the power switch is turned OFF, plug the female end (no pins) of the power cord into the instrument.

7. Plug the multi-pin end of the instrument power cord into the surge protector or line conditioner.

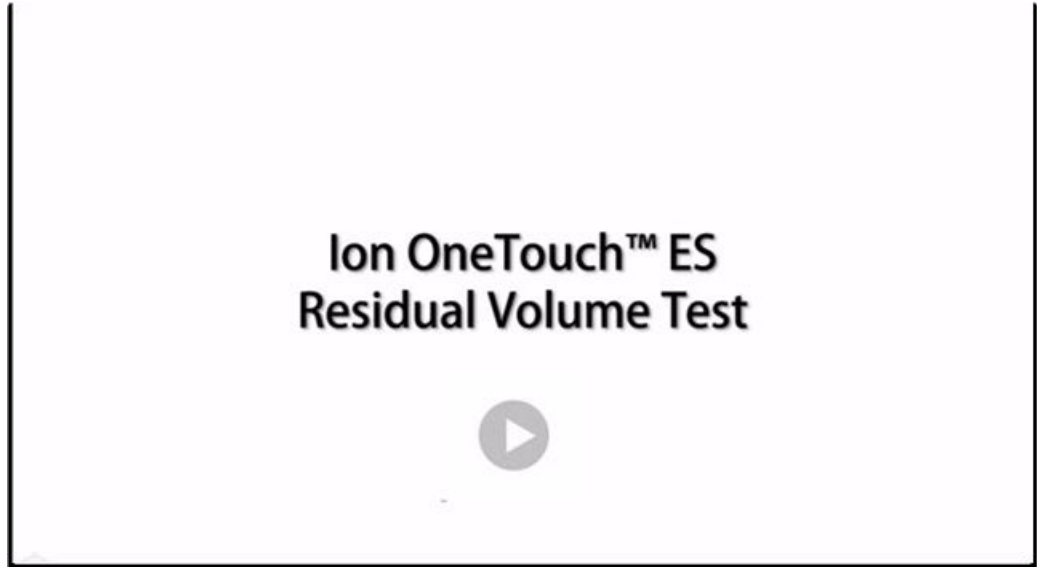8. Plug the surge protector or line conditioner into an electrical outlet.

   IMPORTANT! The voltage of the electrical outlet must match the voltage of the surge protector and the selected voltage of the installed AC line voltage fuse module in the Ion OneTouch™ ES.
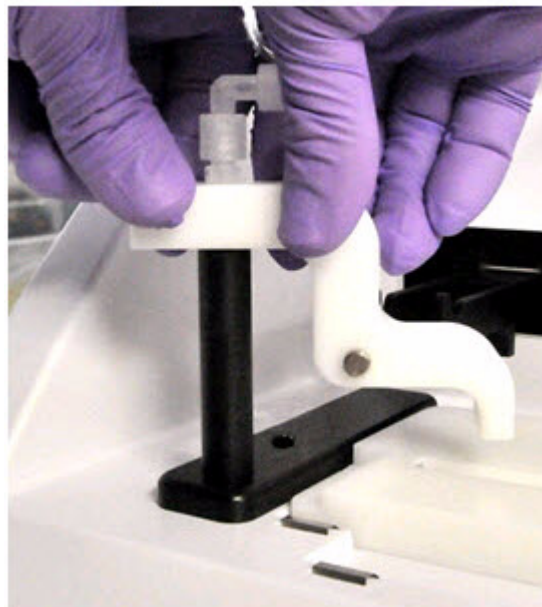
9. Turn the power switch ON. (*Optional*) Leave the instrument ON indefinitely.

# Perform a residual volume test

**Note:** To see a video about performing the residual volume test, click the following photograph. If a pop-up window appears after you click the photograph, click **Allow** to access the video:



1. Set up the Ion OneTouch™ ES (see ).

2. Install a new tip in the Tip Arm:

   a. Place a new tip in the Tip Loader. Remove the Tip Arm from the cradle and align the metal fitting of the Tip Arm with the tip. 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. Lift the Tip Arm *straight* up to pull the installed tip from the Tip Loader tube:

b. Return the Tip Arm to the cradle: Tilt the Tip Arm back (see Figure b1). Align the pins with the round notches in the cradle (see Figure b2), then lower the Tip Arm into position (see Figure b3). Move the Tip Arm forward into the working position:

**b1**                    **b2**                    **b3**



**Note:** 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. The following photograph shows the correct position of the Tip Arm:



**Note:** For the residual volume test, you do ***not*** need to put a 0.2-mL PCR into the hole of the Tip Loader.

3. Load an 8-well strip on the Ion OneTouch™ ES:

   a. Load 80 μL water or Ion OneTouch™ Wash solution into the second well (Well 2) from the square-tabbed end of the 8-well strip:
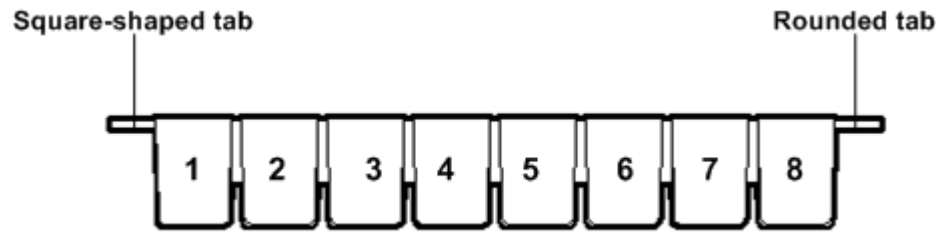


   b. Load the 8-well strip into the slot of the Tray so that the square-tabbed end is to the *left* and the 8-well strip is pushed all the way to the right until it touches the end of the slot.

   ---

   **IMPORTANT!** Before running the residual volume test, carefully read and familiarize yourself with step 4 of this procedure.

   ---

4. Run the residual volume test. During the test, confirm that the tip is centered between the sides of the wells when moving in or out of a well.

   a. Turn the instrument ON.

   b. Wait for the instrument to initialize: The screen displays "rdy". The Tip Arm performs a series of movements and returns to the home position (~5 seconds).

   c. Press **Start/Stop**.

   d. Wait for the instrument to aspirate the solution from Well 2 and completely remove the tip from Well 2, then *manually* push the 8-well strip to the left so that Well 4 is positioned directly under the Tip Arm.

   e. Wait for the instrument to dispense the tip contents into Well 4.

   f. Press **Start/Stop** to stop the test run, then press **Start/Stop** again to return the Tip Arm to the home position.

   g. Place a P10 pipette at the front bottom of Well 2, aspirate the entire residual water or Ion OneTouch™ Wash Solution from the well, then estimate the residual volume.

5. Remove the used tip: With the Tip Arm in its cradle and while standing above the Tip Arm, twist the tip *counterclockwise* and pull it downward to remove and discard the tip:
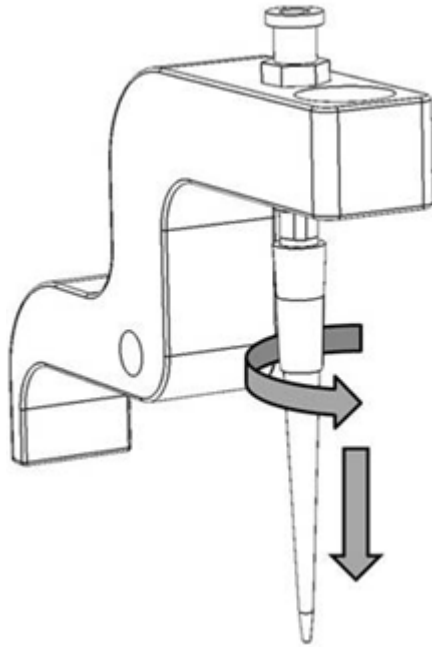


**IMPORTANT!** Improper removal of tips can loosen the metal tip adapter fitting on the Tip Arm and affect instrument operation.

6. Remove and discard the used 8-well strip.

7. After performing the residual volume test, take one or more of the following actions:

| Observation | Possible cause | Recommended actions |
|---|---|---|
| Residual volume in Well 2 is ≤ 5 µL | — | Proceed to, "Prepare reagents then fill the 8-well strip" on page 50. |
| Residual volume in Well 2 is >5 µL<br><br>**IMPORTANT!** The volume is measured from the *bottom* of the well, not from the sides. | The tip height is too high during aspiration. | Restore defaults, then calibrate the instrument (see "Calibrate the Ion OneTouch™ ES"). |
| Aspiration is irregular | This instrument out of calibration. | Restore defaults, then calibrate the instrument (see "Calibrate the Ion OneTouch™ ES"). |
| The 8-well strip lifts as the tip rises to the top of the well | The tip is angled too far forward or the tip height is set too low. | Verify that the tip is vertical and positioned directly over the notch in the calibration shelf. If the tip is positioned correctly. Restore defaults, then calibrate the instrument (see "Calibrate the Ion OneTouch™ ES"). |

# Calibrate the Ion OneTouch™ ES

Perform horizontal and vertical calibrations so that during operation the tip is optimally positioned in the well of the 8-well strip:
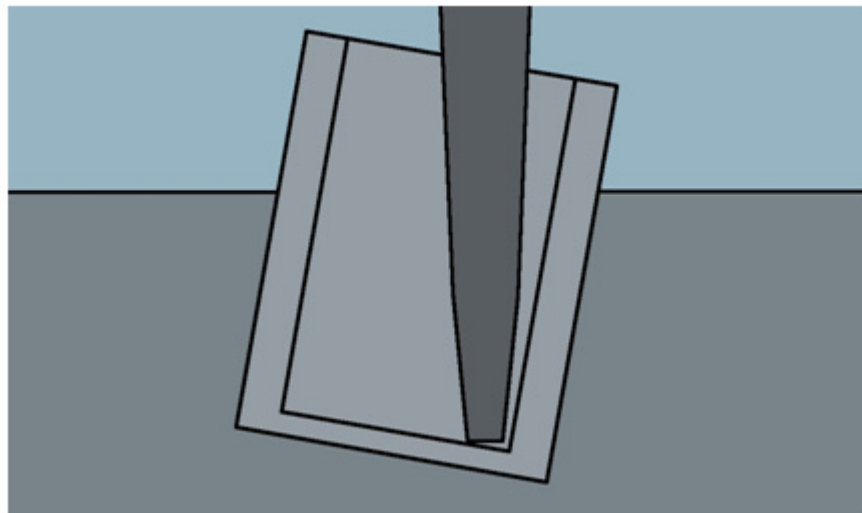
Note that the 8-well strip is always tilted at a fixed 10-degree angle in the slot. The pipette tip is vertical. When the tip is aligned properly during calibration so that it is in line with the notch in the calibration shelf, the tip touches the bottom of the well during the run, close to the front bottom edge of the well.

---

**IMPORTANT!** If you use more than one Ion OneTouch™ ES, do not switch Trays or Tip Arms between instruments. Each Tray and Tip Arm is calibrated with a particular instrument. To track the Tray and Tip Arm, each component has a printed label with the matching serial number of the instrument.

---

**Perform vertical calibration**

1. Set up the Ion OneTouch™ ES (see page 87).

2. Install a new tip (see page 92).

3. Restore the factory default settings:

   a. Power OFF the instrument. and wait 3 seconds.

   b. While holding down **Vert. Adjust**, power ON the instrument.

4. Put the instrument into calibration mode:

   a. Power the instrument OFF.

   b. While holding down **Select/Calibrate**, power the instrument ON. Keep holding down **Select/Calibrate** until "P1" is displayed.

   c. Press **Select/Calibrate** for ~3 seconds until the instrument beeps 2 times and "CAL" is displayed.

      **Note:** The instrument will cycle through several values before "CAL" is displayed.

5. Press **Vert. Adjust**. The instrument displays "ASP" (Aspirate or z-bottom position).

6. Press **Start/Stop**. The Tip Arm lowers to bring the tip near the notch in the calibration shelf on the left side of the Tray.

7.  Adjust if needed the position of the bottom of the pipette tip:

    a.  If the pipette tip is not positioned *in line with* the slot in the calibration shelf, adjust the thumb screw at the back of the Tip Arm (turn counterclockwise to move the tip forward or clockwise to move the tip back; see the right following photograph), until the tip is *in line with* the notch in the calibration shelf (black outline and encircled in red in the left following photograph):



Tip in line with notch                    Fingers on thumbscrew
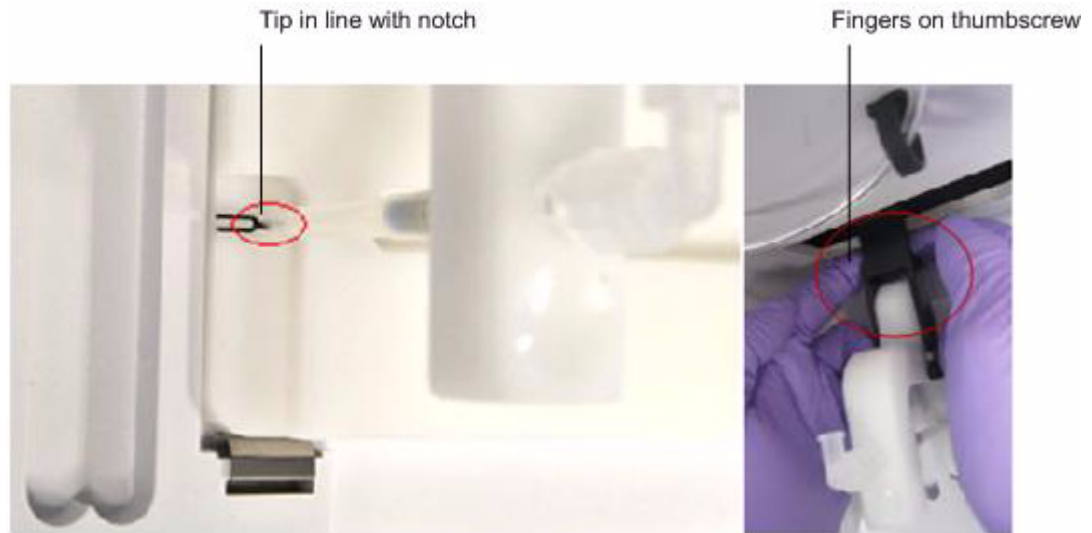
    b.  Adjust the height of the pipette tip in relation to the calibration shelf:

    1. Press the ▼ (minus) button repeatedly until the tip touches the shelf.

    2. Press the ▼ (minus) button *8* more times to lower the tip further. Pressing the ▼ (minus) additional times button accounts for different tip lengths and variations during tip installation:

    **Note:** It is better to have the ASP (aspiration) height be too low than too high.

8. Press **Start/Stop**, then wait for the Tip Arm to stop moving and for "P1" to display.

**Perform horizontal axis calibration**

1. Press **Select/Calibrate** for ~3 seconds until the instrument beeps 2 times and "CAL" is displayed.

   **Note:** The instrument will cycle through several values before "CAL" is displayed.

2. Press **Horiz. Adjust**. Instrument displays "FLA", then press **Start/Stop**.

3. Place an empty 8-well strip in the slot in the Tray, with the square tab on the left.

4. Push the 8-well strip to the far left in the slot as possible.

5. Observe the position of the 8-well strip relative to the position of the tip. When properly calibrated, the 8-well strip is ≤1 mm of touching but not pushing on the tip. To clearly see the relationship between the pipette tip, calibration shelf, and notch during calibration, mark each of them with a felt-tip pen:



6. Adjust the horizontal position of the Tip Arm so that the tip just touches the left tab of the 8-well strip when the 8-well strip is pushed to the far left of the slot in the Tray:

   a. Apply slight pressure to keep the 8-well strip to the far left.

   b. Press the ⛰ (plus) button repeatedly until the tip touches the 8-well strip. Each press of the ⛰ (plus) key moves the Tip Arm to the *right* by ~0.002 inches (~50 µm), which may be difficult to detect.

7. Press **Start/Stop** to save the setting and for "P1" to display.

8. Power the instrument OFF, wait >3 seconds, then power the instrument ON to return to normal operating mode.

9. Perform a residual volume test (see page 92).

# Maintain the Ion OneTouch™ ES

**Materials and equipment required**

Xiameter® PMX-200 Silicone Fluid

**Ion OneTouch™ ES back panel layout**



FUSE MODULE

SYRINGE PUMP

SYRINGE ASSY

SYRINGE VALVE & VALVE COVER

TUBING CONNECTION
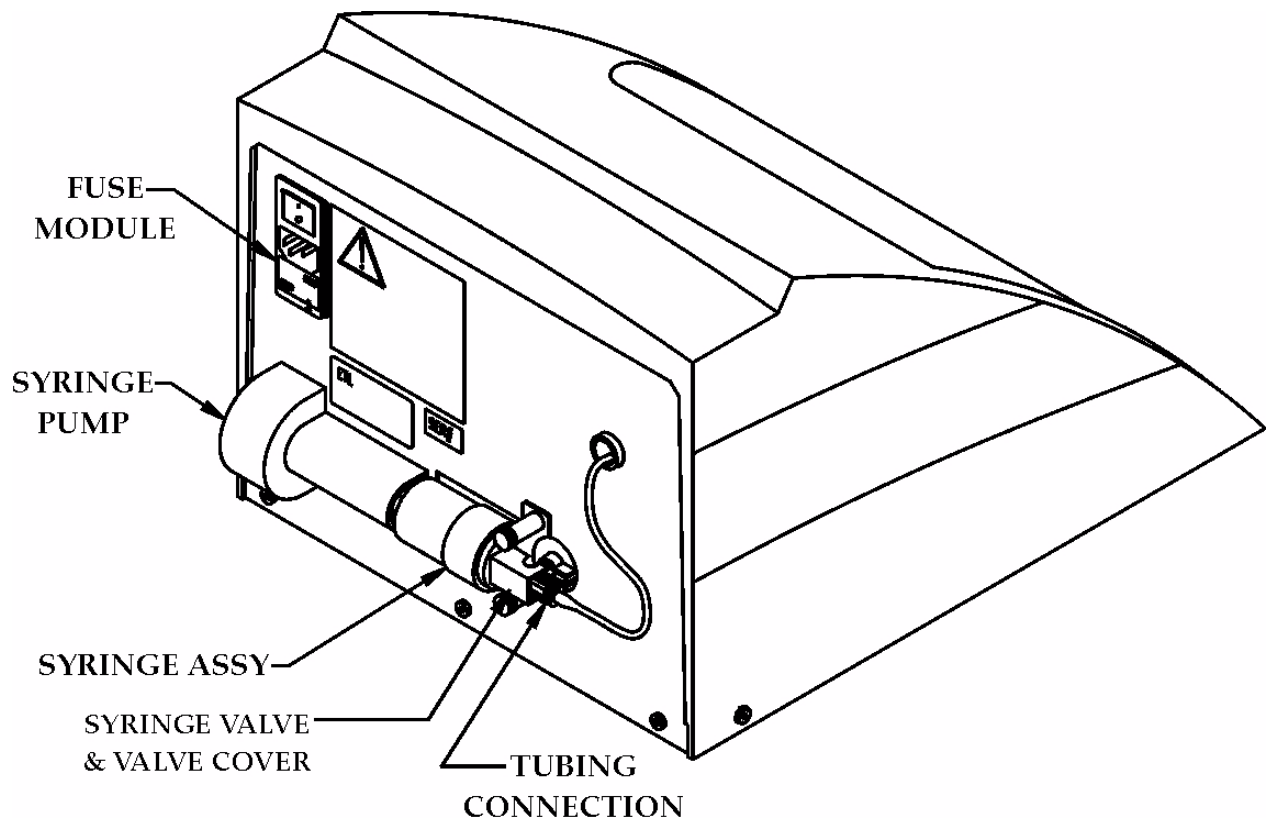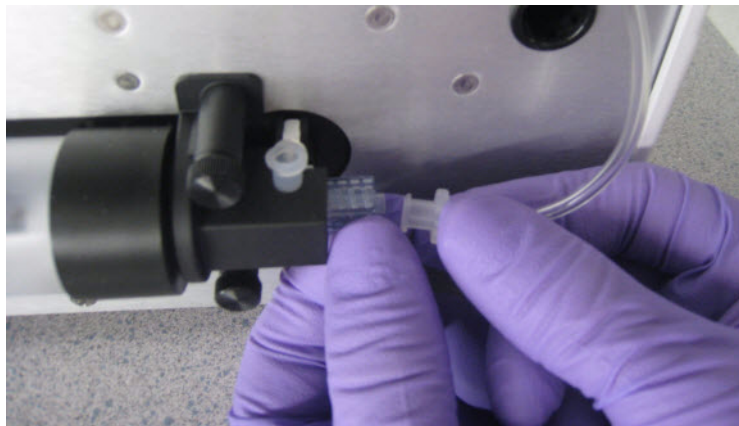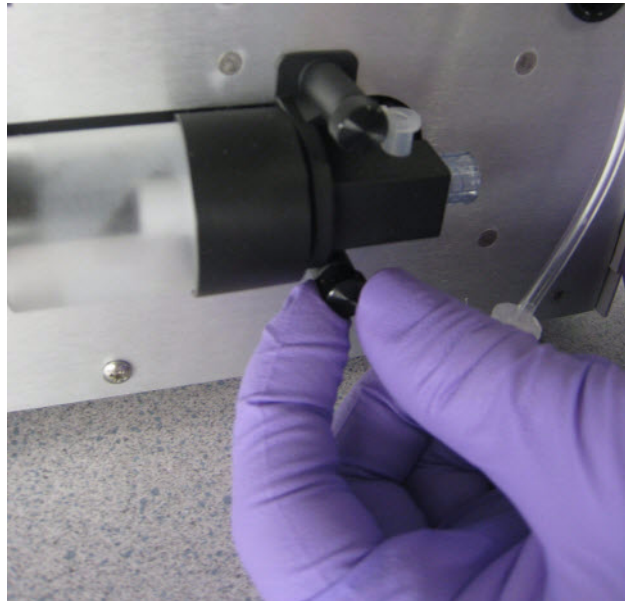
**Lubricate the syringe annually**

1. Disassemble the syringe (located on the back of the instrument):
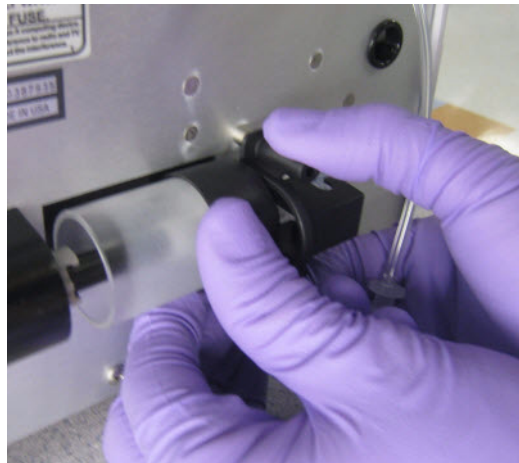


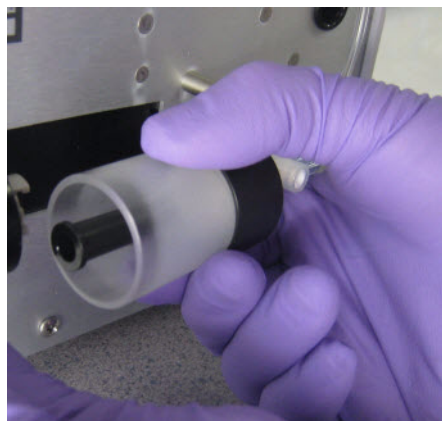   a. Disconnect the tubing:

**b.** Remove the 2 screws:



**c.** Remove the retainer:



**d.** Pull the syringe body toward you to remove from the instrument:

e. Remove the plunger from the syringe body:



f. Apply a thin layer of Xiameter® PMX-200 Silicone Fluid to the inside of the syringe body:
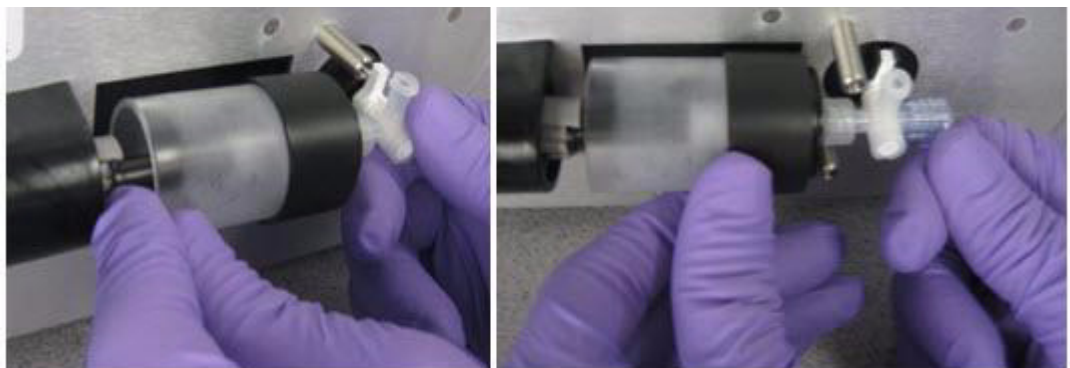


2. Reassemble the syringe:

a. Push the plunger all the way into the syringe body, then pull back out approximately ¼ inch.

b. Engage the plunger with its mate end (see Figure b1), then insert the valve into its docking position (see Figure b2):

**b1**                                                    **b2**



c. Replace the retainer.

d. Replace the 2 screws (finger-tighten).

e. Reconnect the tubing.

# Decontaminate the Ion OneTouch™ ES

Before returning the instrument to Life Technologies, decontaminate it according to the procedure below.

**Materials and equipment required**

- Disposable rubber gloves
- Safety glasses
- Lab coat
- Bleach
- Water
- Paper towels

---

**IMPORTANT!** This procedure does not guarantee total decontamination of the Ion OneTouch™ ES.

---

**Decontaminate the instrument**

1. Wear disposable rubber gloves, safety glasses, and a lab coat.

2. Use a cleaning pad wetted with a solution of 1 part chlorine bleach in 9 parts water (10% bleach solution) to clean all outside surfaces of the Ion OneTouch™ ES. Avoid getting bleach solution inside the chassis.

3. Dry the surfaces of the Ion OneTouch™ ES with paper towels or other disposable wipes.

4. Use cotton swabs to clean and dry areas that are difficult to reach.

5. Properly dispose of used cleaning materials to ensure that no one becomes exposed to contaminants.

# C

# Supplemental procedures

## Unpack and install the Ion OneTouch™ 2 System

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

- Site preparation and installation requirements: Refer to the *Ion Proton™ System Site Preparation Guide* (Pub. no. 4478733).

- Unpacking and installation instructions: See "Unpack and install the Ion OneTouch™ 2 System".

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

**Unpack an Ion OneTouch™ 2 Instrument**

1. Unpack and install the OneTouch™ 2 Instrument in a location different from the location used to prepare the amplification solution. Refer to the *Unpack and Install the Ion OneTouch™ 2 System Product Information Sheet* (Pub. no. 4481875) and the *Ion Proton™ System Site Preparation Guide* (Pub. no. 4478733). Remove the instrument from the carton by pulling the cardboard lifting handles.

   **IMPORTANT!** Do not lift the instrument by the metal handle used to access the Ion OneTouch™ 2 Amplification Plate.

2. Ensure that the power switch is turned OFF, then plug the 3-prong adapter of the power cord into the instrument.

3. Plug the other end of the power cord into an electrical outlet of the appropriate voltage.

   **IMPORTANT!** The Ion OneTouch™ 2 Instrument draws 6 amps of current. Do not exceed the circuit breaker limit for current. If necessary, plug multiple instruments into different circuits.

4. Turn the power switch ON. Initial start-up takes ~3 minutes. You may hear sounds from the instrument. This is normal.

   **IMPORTANT!** Leave ON the Ion OneTouch™ 2 Instrument. If the instrument is turned off, critical log files are lost. If you need to turn OFF the instrument or if a run fails, download the log files (see "Download the log files from the Ion OneTouch™ 2 Instrument" on page 121).

### Unpack and install the Ion OneTouch™ ES

1. Unpack and install the OneTouch™ ES in a location different from the location used to prepare the amplification solution. Refer to the *Unpack and Install the Ion OneTouch™ 2 System Product Information Sheet* (Pub. no. 4481875) and the *Ion Proton™ System Site Preparation Guide* (Pub. no. 4478733).

   **IMPORTANT!** Do *not* lift the instrument by holding the syringe located at the back of the instrument.

2. Install the AC line voltage fuse module (refer to the *Unpack and Install the Ion OneTouch™ 2 System Product Information Sheet).*

3. Ensure that the power switch is OFF, then plug the female end (no pins) of the power cord into the instrument.

4. Plug the multi-pin end of the instrument power cord into the surge protector or line conditioner.

5. Plug the surge protector or line conditioner into an electrical outlet.

   **IMPORTANT!** The voltage of the electrical outlet must match the voltage of the surge protector and the selected voltage of the installed AC line voltage fuse module in the Ion OneTouch™ ES.

6. Ensure that the Ion OneTouch™ ES is in a room at an operating temperature of 15°C to 25°C (60°F to 77°F).

7. Turn the power switch ON.

8. Set up the Ion OneTouch™ ES (see "Set up the Ion OneTouch™ ES" on page 87), then perform the residual volume test (see "Perform a residual volume test" on page 92).

   **Note:** Leave ON the Ion OneTouch™ ES.

9. Set up and run the Ion OneTouch™ 2 Instrument (see "Getting started" on page 15).

# Install a firmware update

**Install a firmware update with a USB flash drive**

IMPORTANT! Reformatting the USB flash drive erases data on the drive.

1. Ensure that the USB flash drive is FAT32-formatted, according to the operating system where the USB flash drive is installed:

   • *Macintosh® operating system*:

   a. Insert a USB flash drive into a USB port.

   b. Double-click the **Macintosh HDD** icon.

   c. Navigate to the **Applications** folder, then click: **Utilities** > **Disk Utility**.

   d. Double-click the **Disk Utility** application.

   e. Highlight the hard drive (the *USB flash drive*) to format and click the **Erase** button.

   f. From the volume format, select **MS-DOS (FAT)**, then enter a name for the USB flash drive.

   g. Click **Erase**.

   h. Confirm the FAT32 formatting.


   • *Microsoft® Windows® operating system*:

   a. Insert the USB flash drive into a USB port.

   b. Double-click **My Computer**.

   c. Right-click the USB flash drive icon, then select **Format**.

   d. Click the drop-down menu and select **FAT32**, then click **Start**.

   e. Confirm the FAT32 formatting.

2. Copy the file containing the firmware update (.bz2 file) to the top-level directory of the USB flash drive.

3. Turn the power switch ON on the Ion OneTouch™ 2 Instrument.

4. Ensure that the instrument is not performing a run and that the home screen displays:
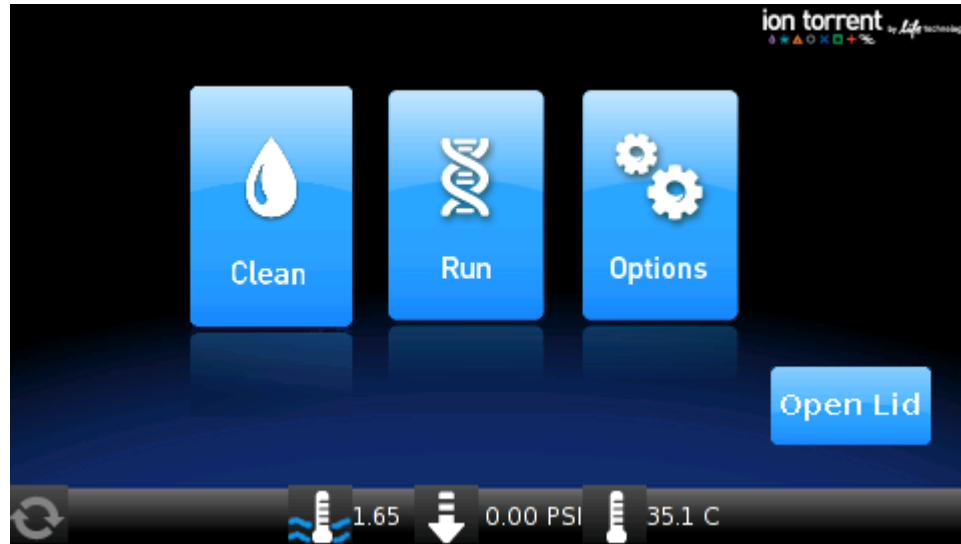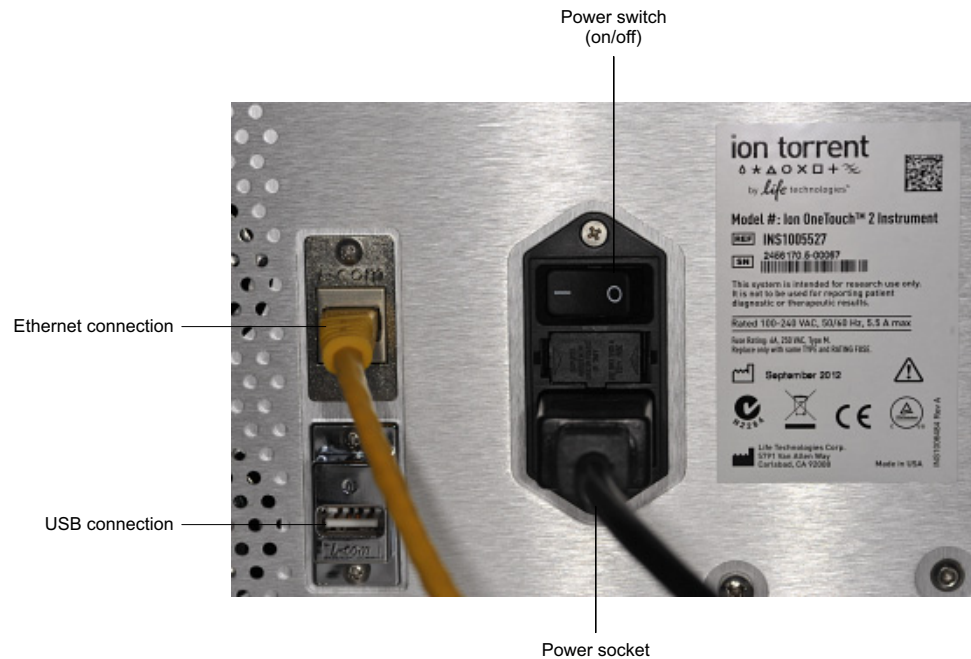


5. On the home screen, touch **Options**, then touch **Upgrade**:
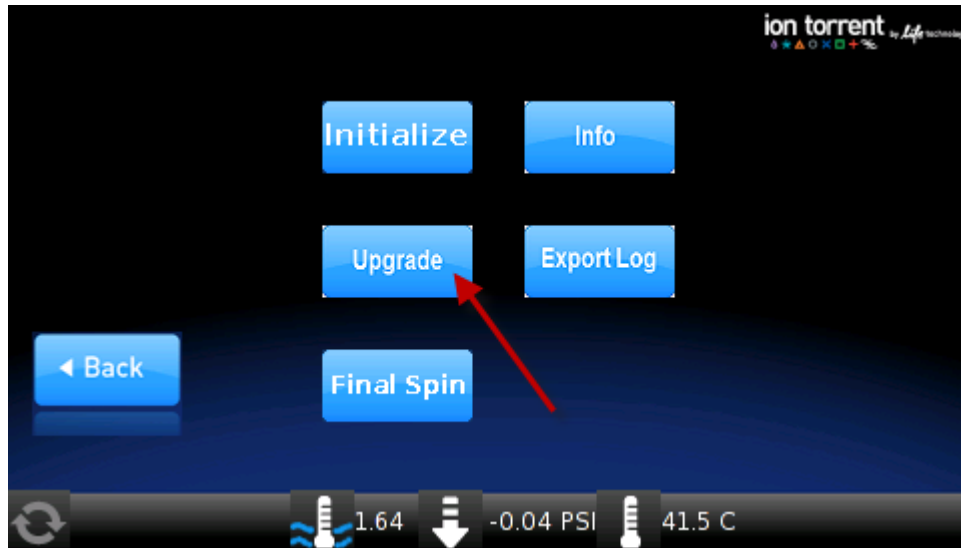


**Note:** The Upgrade screen asks if you would like to upgrade the firmware, but *immediately* proceed to the next step.

6. Insert the USB flash drive into the USB connection, located on the back of the Ion OneTouch™ 2 Instrument:



Power switch
(on/off)

Ethernet connection

USB connection

Power socket

7. On the Upgrade screen, touch **Yes**. If you touch **No**, the Options screen displays.

8. Wait 10 seconds for the screen to display the software status update:
   - If the status update displays, proceed to step 9.
   - If the Options screen displays, then there are no firmware updates.

9. Touch **Options** then touch **Info** to ensure that the new version of the firmware update is installed.

10. Turn the power switch OFF, wait several seconds, then remove the USB flash drive.

11. Turn the power switch ON.

12. Prepare for the next run (see "Set up the Ion OneTouch™ 2 Instrument" on page 20).

**Alternative method: Install a firmware update using an Ethernet connection**

**Note:** Use a shielded Ethernet cable to connect to the Ion OneTouch™ 2 Instrument.

1. Turn the power switch OFF.

2. Plug an Ethernet cable from the Ion OneTouch™ 2 Instrument into one of the Proton™ ports on the Torrent Server.

3. Turn the power switch ON. Initial start-up takes ~3 minutes.

   **Note:** You may hear sounds from the instrument. This is normal.

4. Log in to the Torrent Browser with your ionadmin account username and password.

5. Click the gear icon at top-right of the screen, then select **Configure** from the drop-down menu.

6. Click **Admin Interface** at the bottom of the page.

7. Click **Update OneTouch Device**.

8. Click **Update**. You see the progress bar during the update displayed on the Ion OneTouch™ 2 Instrument. Wait for the update to complete and the instrument to reset.

9. Touch **Options** on the Ion OneTouch™ 2 Instrument home page to ensure that the new version of the firmware update is installed.
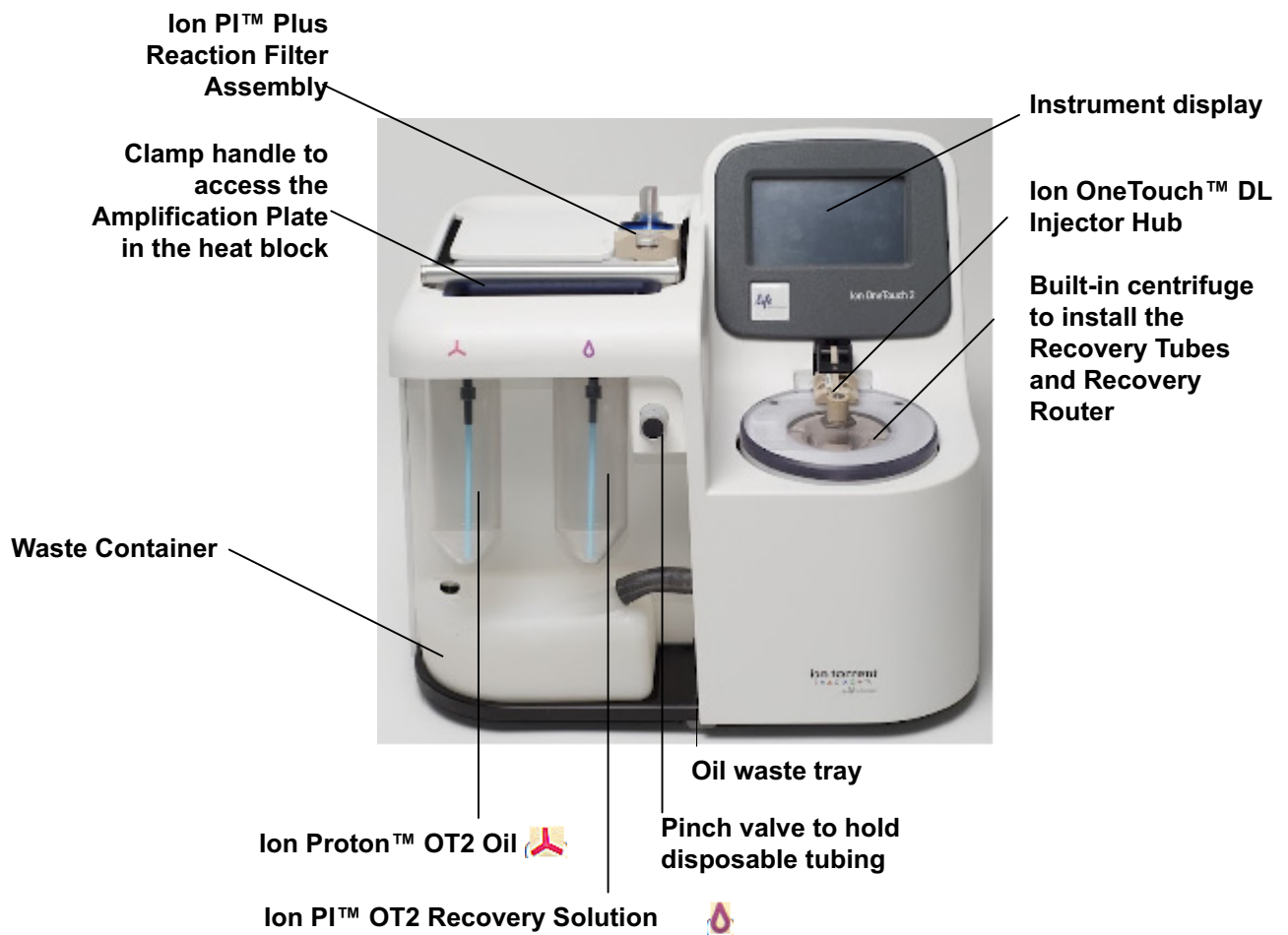
# Initialize the Ion OneTouch™ 2 Instrument

IMPORTANT! Before operating the Ion OneTouch™ 2 Instrument for the first time, you must perform the one-time initialization on the instrument. Initialization primes the pumps and tubing lines for reliable operation.

**Set up the Ion OneTouch™ 2 Instrument**

Ion OneTouch™ 2 Instrument layout



**Ion PI™ Plus Reaction Filter Assembly**

**Clamp handle to access the Amplification Plate in the heat block**

**Instrument display**

**Ion OneTouch™ DL Injector Hub**

**Built-in centrifuge to install the Recovery Tubes and Recovery Router**

**Waste Container**

**Oil waste tray**

**Ion Proton™ OT2 Oil**

**Pinch valve to hold disposable tubing**

**Ion PI™ OT2 Recovery Solution**

Note: The photograph does not show the disposable tubing.

## Materials required for this procedure

**Provided in the Ion PI™ Template OT2 Supplies
200 Kit v2 (Part no. 4485144):**

- 2 Ion OneTouch™ Reagent Tubes
- Ion OneTouch™ Recovery Router
- 2 Ion PI™Template OT2 Recovery Tubes
- Ion OneTouch™ 2 Amplification Plate
- 2 Ion OneTouch™ Sipper Tubes
- Ion OneTouch™ 2 Cleaning Adapter

**Provided in the Ion PI™ Template OT2 Solutions
200 Kit v2 (Part no. 4485143):**

- Ion Proton™ OT2 Oil (450-mL size)
- Ion PI™ OT2 Recovery Solution

**Other Materials:**

18 MΩ water

**Note:** Life Technologies has validated this protocol using only the material specified. Substitution may adversely affect performance and safety.

---

**IMPORTANT!** Use *only* the Ion PI™ Template OT2 200 Kit v2 (Cat. no. 4485146) 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.
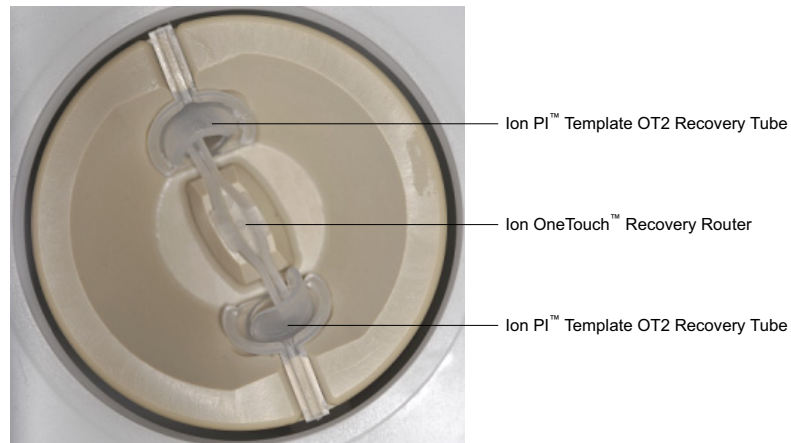
---

## Install the Ion PI™ Template OT2 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. Do *not* use the sample injector tubing to lift the lid.

2. Insert a Recovery Tube into each of the slots in the centrifuge.

3. 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 is seated flat and secure in the center of the rotor:



Ion PI™ Template OT2 Recovery Tube

Ion OneTouch™ Recovery Router

Ion PI™ Template OT2 Recovery Tube

**Note:** The Recovery Router is intentionally offset from the Recovery Tubes.

4. Close the lid of the centrifuge.

## Install the Ion OneTouch™ 2 Amplification Plate

1. If there is a used Ion OneTouch™ 2 Cleaning Adapter on the instrument, remove and appropriately discard it.

   **Note:** The Cleaning Adapter may be filled with Ion Proton™ OT2 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. Obtain an Ion OneTouch™ 2 Amplification Plate. The disposable tubing and disposable injector are attached to the Amplification Plate. An Amplification Plate supplied in the Ion PI™ Template OT2 200 Kit v2 has "ion" on the top tab:



   **Note:** Before use, inspect the plate port of the Amplification Plate to ensure that it is straight and perpendicular to the plate. If you have questions about the plate, contact Life Technologies Technical Support.

4. Insert the Amplification Plate:

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

   b. 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:
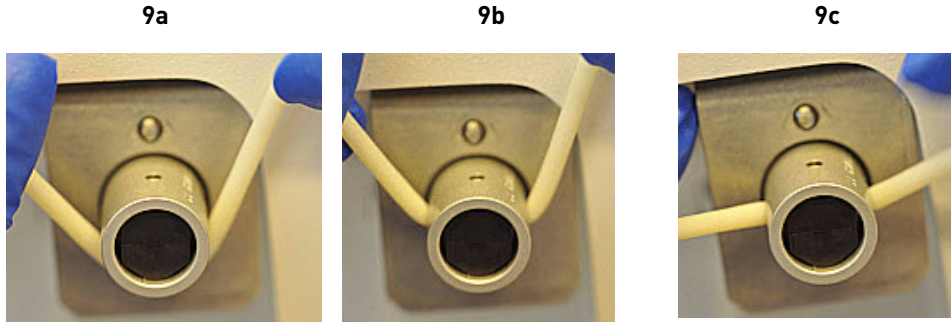
   

5. Pull the handle of the heat block to close the block. The disposable tubing is under the handle:

   

6. Thread the disposable tubing through the Ion OneTouch™ DL Tubing Catch:

**7.** 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 (see figure 9a):

   **b.** Gently pull the disposable tubing upwards on the both sides of the pinch valve (see figure 9b) until the disposable tubing is in the slot and secured in the round notch on each side of the pinch valve (see figure 9c):

            **9a**                         **9b**                         **9c**



   **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" on page 116):

### Install the disposable injector

**Note:**  Before use, inspect the long metal shaft of the disposable injector. Some disposable injectors may be slightly bent, which is normal. If you have questions about the disposable injector, contact Life Technologies Technical Support.

1.  Place one hand on the centrifuge lid. Place the other hand at the top of the disposable injector, and insert the disposable injector straight into the port of the Ion OneTouch™ DL Injector Hub:
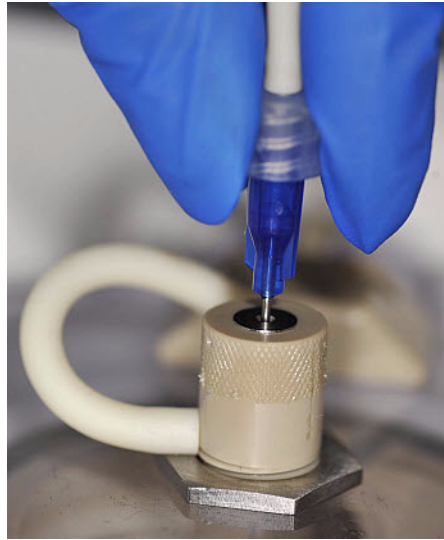
> ⚠ **CAUTION!  PHYSICAL INJURY HAZARD**. The pointed end of the disposable injector can puncture your skin. Keep your hand away from the point of the disposable injector.



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

2. Keep your hand on the centrifuge lid, then push the disposable injector through the port until the disposable injector just stops at the base of the router:
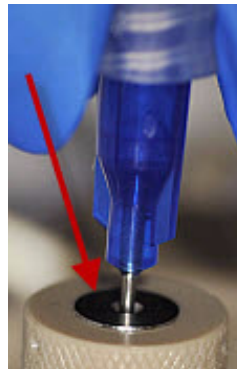


3. Release the disposable injector.

4. Confirm automatic placement of the disposable injector above the router. Briefly press then release the spring-loaded top of the Injector Hub at the point indicated by the arrows in figures 4a–4c. You should hear a click:
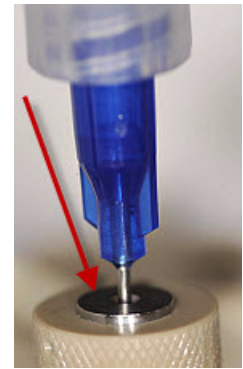
| 4a (up position) | 4b (down position) | 4c (up position) |
|---|---|---|



IMPORTANT! If the Injector Hub remains in the down position, see "Troubleshooting" on page 61.

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, amplification plate, and tubing. Use a new disposable injector and Ion OneTouch™ 2 Amplification Plate.

### Install the Ion Proton™ OT2 Oil

**Note:** The Ion Proton™ OT2 Oil is yellow. This is normal.

1. Fill the appropriate Ion OneTouch™ Reagent Tube with Ion Proton™ OT2 Oil on the *left*

   front port ▲:

   a. Use a new Reagent Tube from the kit.

   b. 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.

   c. Invert the Ion Proton™ OT2 Oil bottle (450-mL size) 3 times to mix, then fill the Reagent Tube *half*-full with Oil. Minimize bubbles.

   d. Insert the filled Reagent Tube into the *left* front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument.

2. Insert the filled Reagent Tube into the *left* front port, and screw the Reagent Tube firmly into place, quarter-turn on the instrument.

### Install the Ion PI™ OT2 Recovery Solution

**IMPORTANT!** Use only the Ion PI™ OT2 Recovery Solution in the Ion PI™ Template OT2 200 Kit v2 that you are currently using. Do *not* use a different recovery solution from another kit.

1. Ensure that the Ion PI™ OT2 Recovery Solution is clear. If it is clear, proceed to the next step. If the Recovery Solution is not clear, heat 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 PI™ OT2 Recovery

   Solution on the *right* front port ⬤:

   a. Use a new Reagent Tube from the kit.

   b. 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. Minimize bubbles.

   c. Invert the container of Recovery Solution bottle 3 times, then fill the Reagent Tube a *quarter*-full with Recovery Solution. Minimize bubbles.

   d. Insert the filled Reagent Tube into the *right* front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument.

### Install the Ion OneTouch™ 2 Cleaning Adapter

1. Obtain a new Ion OneTouch™ 2 Cleaning Adapter:



2. Firmly insert the 3 ports of the Cleaning Adapter into the three holes (see Figure 2a) on the top stage of the Ion OneTouch™ 2 Instrument (see Figure 2b). The tab protruding from the outer edge of the Cleaning Adapter fits into the front notch of the stage:
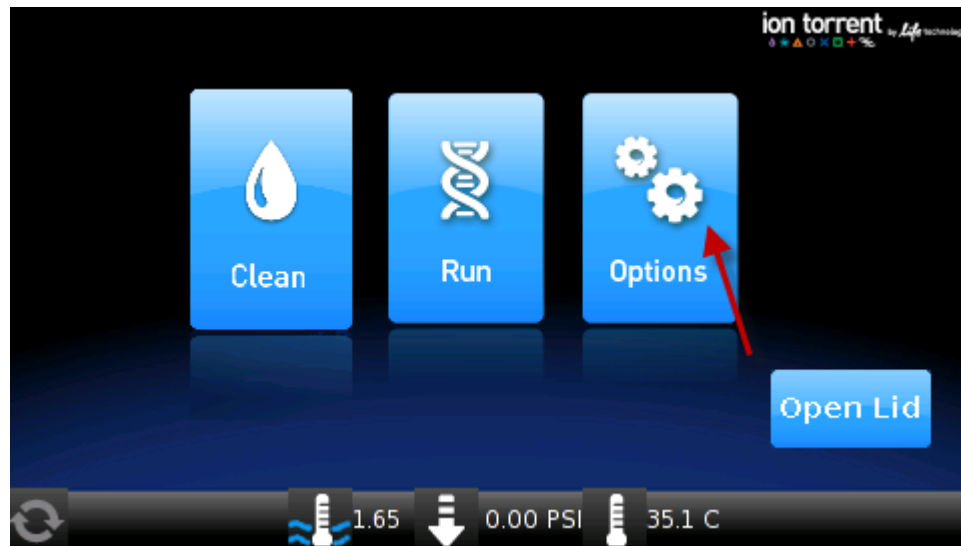
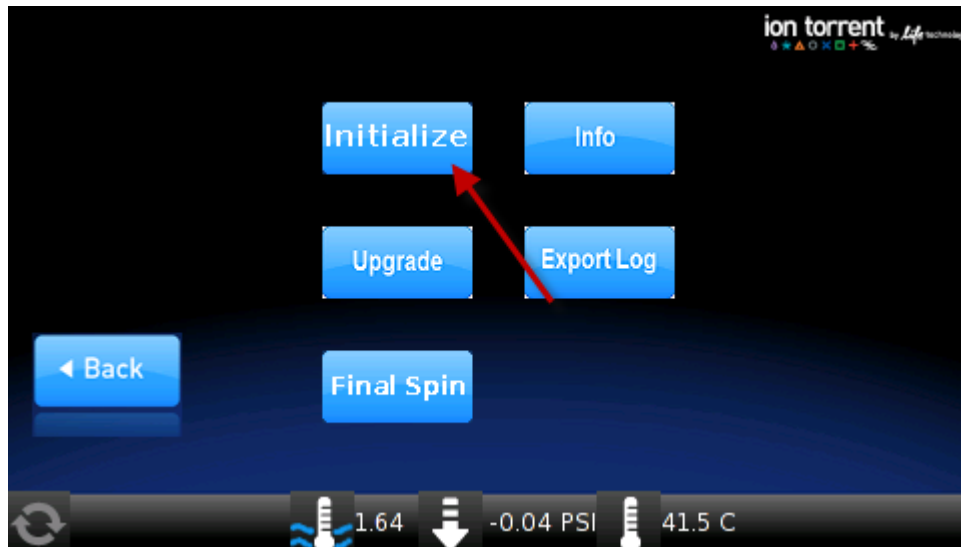**2a**                                            **2b**



Tab

Notch

### Initialize the instrument

1. On the home screen, touch **Options**:



2. On the Options screen, touch **Initialize**, then follow the screen prompts to initialize the instrument:



Initialization takes 10 minutes.

### Continue to set up the Ion OneTouch™ 2 Instrument

1. Remove and retain the Ion OneTouch™ 2 Cleaning Adapter. You can reuse the Cleaning Adapter one time for the instrument maintenance protocol after the first run, but you must appropriately dispose of the used Cleaning Adapter after running the maintenance protocol (see "Maintain the Ion OneTouch™ 2 Instrument" on page 43).

2. Keep all of the disposable components that were used for initialization in place, including the Ion OneTouch™ 2 Amplification Plate, disposable line, disposable injector, Ion OneTouch™ Router, and Ion PI™ Template OT2 Recovery Tubes.

> IMPORTANT!  After the first run on the Ion OneTouch™ 2 Instrument, appropriately dispose of all components as directed.

3. Complete the remaining tasks to set up for a sample run (see "Perform a verification run" on page 16).

# Download the log files from the Ion OneTouch™ 2 Instrument

Log files capture important information regarding instrument operation. Should the Ion OneTouch™ 2 Instrument fail, downloaded log files are used to troubleshoot the instrument.

> IMPORTANT!  Until the log files are downloaded, leave ON the Ion OneTouch™ 2 Instrument. If the instrument is turned off, critical log files are lost. If you need to turn OFF the instrument or if a run fails, download the log files.

1. Ensure that the Ion OneTouch™ 2 Instrument is not performing a run.

2. Format a USB flash drive, if necessary (see "Install a firmware update" on page 107). Confirm that a formatted USB flash drive has no data files on it.

3. Insert the USB flash drive into the USB connection, located on the back of the Ion OneTouch™ 2 Instrument:
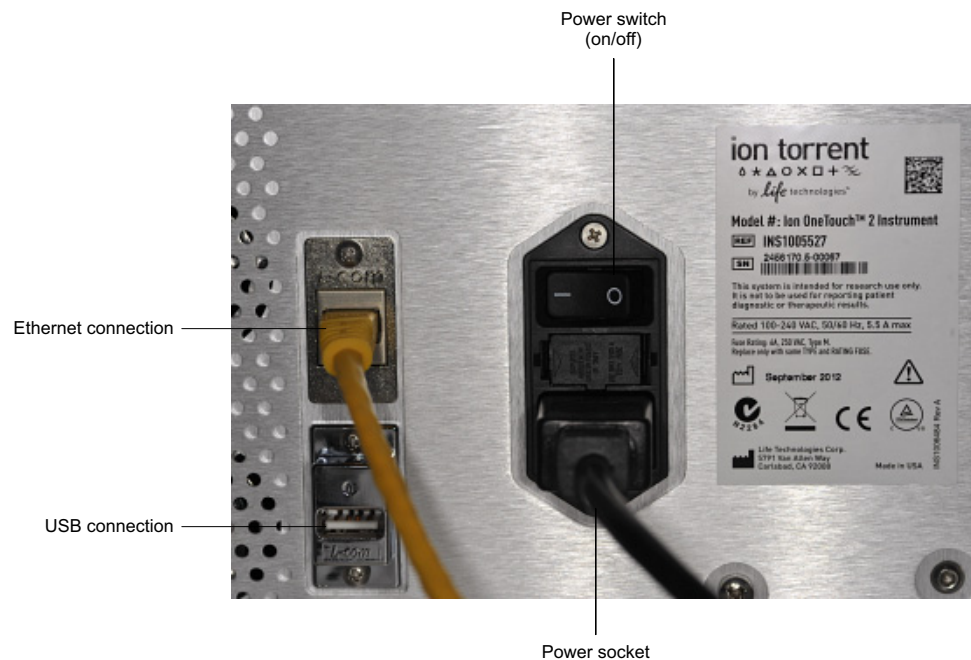
4. On the home screen, touch **Options**, then touch **Export Log**.
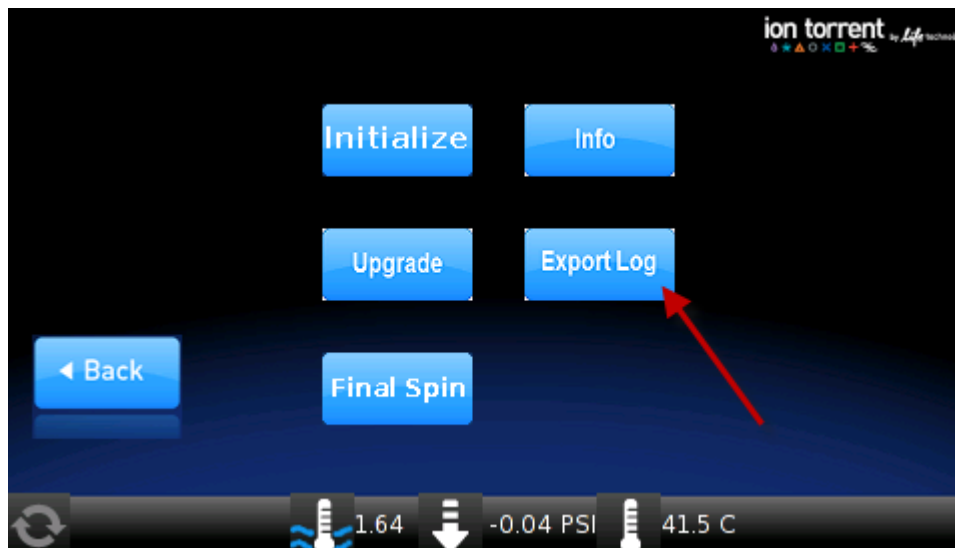


The Export Log screen asks if you would like to export the log files.

5. On the Export log screen, touch **Yes**. The log files export to the USB flash drive and the status update displays. After export, the Options screen displays.

6. Remove the USB flash drive from the instrument, then verify on a computer that the instrument downloaded the log files to the flash drive. If the files are:

   • Downloaded: Proceed to the next step.

   • Not downloaded: Repeat steps 3–6 until the log files are downloaded.

7. Save the log files to a computer.

8. Turn OFF the instrument. The instrument deletes the log files. If necessary, contact Life Technologies Technical Support.

# Ion OneTouch™ 2 Instrument operations

| If the on-screen comment is... | Then the instrument is... |
|---|---|
| Priming and Filling Recovery Solution | Priming the filter and filling the collection tubes with Ion PI™ OT2 Recovery Solution. |
| Sample Injection | Injecting sample and starting emulsification. |
| Amplification | Amplifying the sample by PCR. |
| Filling Recovery Solution | Completely filling the Ion PI™ Template OT2 Recovery Tubes with Ion PI™ OT2 Recovery Solution. |
| ISP Collection | Breaking the emulsion and collecting the Ion PI™ Ion Sphere™ Particles. |
| Wash Cycles | Washing the instrument and draining the washes. |
| Final Spin | Performing final centrifugation to pellet the Ion PI™ Ion Sphere™ Particles in the Ion PI™ Template OT2 Recovery Tubes. |

# Decontaminate the Ion OneTouch™ 2 Instrument

Before returning the instrument to Life Technologies, decontaminate it according to the procedure below.

**Materials and equipment required**

- Disposable rubber gloves
- Safety glasses
- Lab coat
- Bleach
- Water
- Paper towels

**IMPORTANT!** This procedure does not guarantee total decontamination of the Ion OneTouch™ 2 Instrument.

**Decontaminate the instrument**

1. Wear disposable rubber gloves, safety glasses, and a lab coat.

2. Use a cleaning pad wetted with a solution of 1 part chlorine bleach in 9 parts water (10% bleach solution) to clean all outside surfaces of the Ion OneTouch™ 2 Instrument. Use care to avoid getting bleach solution inside the chassis.

3. Dry the surfaces of the Ion OneTouch™ 2 Instrument with paper towels or other disposable wipes.

4. Use cotton swabs to clean and dry areas that are difficult to reach.

5. Properly dispose of used cleaning materials to ensure that no one becomes exposed to contaminants.

# Instrument warranty

For new Ion Torrent™ instruments, Life Technologies warrants to and only to buyer for twelve (12) months from the date of shipping, that the Ion Torrent™ software and Ion Torrent™ instruments are free from defects in material and workmanship and conform to Life Technologies' published specifications in all material respects. Where a valid and timely claim in respect of breach of Ion Torrent™ warranty is submitted to Life Technologies, Life Technologies may, at its discretion, replace, repair or modify the Ion Torrent™ instrument. Any agreed replacement shall be at 1:1, like-kind basis, at no cost to the buyer. For Ion Torrent™ chips or reagents reasonably determined by Life Technologies to be defective, independent of user error, shall be replaced by Life Technologies on a 1:1, like-kind basis at no cost to buyer, provided that such defective Ion Torrent™ chips or reagents were used by buyer prior to their expiration date, or if there is no expiration date, the Ion Torrent™ chips or reagents were used within six (6) months of receipt, and the defect was promptly reported with appropriate detail to Life Technologies' technical support.

NO OTHER WARRANTIES SHALL BE APPLICABLE TO ION TORRENT PRODUCTS (WHETHER OR NOT ANY FURTHER WARRANTY DOCUMENTATION MAY BE INCLUDED IN THE SHIPMENT), WITH THE EXCEPTION OF THIRD PARTY WARRANTIES WITH RESPECT TO THIRD PARTY PRODUCT. ANY THIRD PARTY PRODUCTS ARE NOT COVERED BY THIS SECTION AND ANY WARRANTIES FOR THIRD PARTY PRODUCTS ARE PROVIDED BY THE ORIGINAL MANUFACTURER OF THE THIRD PARTY PRODUCT. Warranties are made only to buyer purchasing the Ion Torrent™ Product directly from Life Technologies, are not transferable and do not extend to the benefit of any other person or entity, unless otherwise expressly stated in writing by Life Technologies. ANY PRODUCT NOT COVERED BY AN EXPRESS WRITTEN WARRANTY IS SOLD AND PROVIDED "AS IS," WITHOUT WARRANTY OF ANY KIND, STATUTORY, EXPRESS OR IMPLIED. Any description of Ion Torrent™ Product recited in Life Technologies' quotation is for the sole purpose of identifying Ion Torrent™ Product, and any such description is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product shall conform to that description. Any sample or model used in connection with Life Technologies' quotation is for illustrative purposes only, and is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product will conform to the sample or model. No affirmation of fact or promise made by Life Technologies, whether or not in Life Technologies' quotation, shall constitute a warranty that Ion Torrent™ Product will conform to the affirmation or promise. Unless otherwise specified in writing in documentation shipped with Ion Torrent™ Product or otherwise agreed by Life Technologies in writing, Life Technologies does not provide service or support for custom products or other products made to buyer's specifications. THE WARRANTIES IDENTIFIED IN THIS CLAUSE ARE LIFE TECHNOLOGIES' SOLE AND EXCLUSIVE WARRANTIES WITH RESPECT TO ION TORRENT™ PRODUCT AND ARE IN LIEU OF ALL OTHER WARRANTIES, STATUTORY, EXPRESS OR IMPLIED, ALL OF WHICH OTHER WARRANTIES ARE EXPRESSLY DISCLAIMED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTY OF MERCHANTABILITY, FITNESS FOR

A PARTICULAR PURPOSE, NON-INFRINGEMENT, OR REGARDING RESULTS OBTAINED THROUGH THE USE OF ANY PRODUCT (INCLUDING, WITHOUT LIMITATION, ANY CLAIM OF INACCURATE, INVALID OR INCOMPLETE RESULTS), WHETHER ARISING FROM A STATUTE OR OTHERWISE IN LAW OR FROM A COURSE OF PERFORMANCE, DEALING OR USAGE OF TRADE.

# E  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.

## Symbols on this instrument: Ion OneTouch™ 2 Instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

• **CAUTION**! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

• **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

• **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

⚠

The following table gives the meaning of the hazard symbols.

| Symbol | English | Français |
|---|---|---|
| ⚠ | Caution, risk of danger<br><br>Consult the manual for further safety information. | Attention, risque de danger<br><br>Consulter le manuel pour d'autres renseignements de sécurité. |
| | Caution, hot surface | Attention, surface chaude |
| | Rotation hazard | Danger rotation potentiel |
| ⏻ | On/Off | On/Off (marche/arrêt) |
| ⏚ | Protective conductor terminal (main ground) | Borne de conducteur de protection (mise à la terre principale) |
| | Do not dispose of this product in unsorted municipal waste<br><br>**CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options. | Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.<br><br>**CAUTION!** Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable. |

| Conformity mark | Description |
|---|---|
| | Indicates conformity with safety requirements for Canada and U.S.A. |
| CE | Indicates conformity with European Union requirements for safety and electromagnetic compatibility. |
| | Indicates conformity with Australian standards for electromagnetic compatibility. |

# Symbols on this instrument: Ion OneTouch™ ES

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the symbol is used along with user attention words described in the "About this guide" section to highlight important safety information. The following table gives the meaning of these symbols.

| Symbol | English | Français |
|--------|---------|----------|
| ⚠ | Caution, risk of danger<br><br>Consult the manual for further safety information. | Attention, risque de danger<br><br>Consulter le manuel pour d'autres renseignements de sécurité. |
| ⏻ | On/Off | On/Off (marche/arrêt) |
| ⏚ | Protective conductor terminal (main ground) | Borne de conducteur de protection (mise à la terre principale) |

| Conformity mark | Description |
|-----------------|-------------|
| ETL LISTED c/us | Indicates conformity with safety requirements for Canada and U.S.A. |
| CE | Indicates conformity with European Union requirements for safety and electromagnetic compatibility. |

# Safety information for instruments not manufactured by Life Technologies

Some of the accessories provided as part of the instrument system are not designed or built by Life Technologies. Consult the manufacturer's documentation for the information needed for the safe use of these products.

# Instrument safety

**General**

⚠ **CAUTION! Do not remove instrument protective covers.** If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

⚠ **CAUTION!**  **Solvents and Pressurized fluids.** Wear eye protection when working with any pressurized fluids.  Use caution when working with any polymeric tubing that is under pressure:

· Extinguish any nearby flames if you use flammable solvents.

· Do not use polymeric tubing that has been severely stressed or kinked.

· Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.

· Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.

· Be aware that high solvent flow rates (~40 mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.

## Physical injury

⚠ **CAUTION!**  **Moving and Lifting Injury.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

· Depending on the weight, moving or lifting may require two or more persons.

· If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.

· Ensure you have a secure, comfortable grip on the instrument or accessory.

· Make sure that the path from where the object is to where it is being moved is clear of obstructions.

· Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.

· Participants should coordinate lift and move intentions with each other before lifting and carrying.

· For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

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

## Electrical

⚠ **WARNING!**  **Voltage AC line voltage fuse module.** Before installing the instrument, verify that the AC line voltage fuse module is installed in the correct orientation for the supplied voltage. This will prevent damage to the instrument, reduce risk of fire, and enable proper operation.

⚠ **WARNING!**  **Fuse Module.** Before installing the instrument, verify that the Fuse Module is set for the supply voltage. This will prevent damage to the instrument, reduce risk of fire, and enable proper operation.

⚠ **WARNING!  FIRE HAZARD**. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

⚠ **WARNING!  Ensure appropriate electrical supply.** For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

⚠ **WARNING!  Power Supply Line Cords.** Use properly configured and approved line cords for the power supply in your facility.

⚠ **WARNING!  Disconnecting Power.** To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

⚠ **CAUTION!** Cleaning and Decontamination. Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

# Safety and electromagnetic compatibility (EMC) standards: Ion OneTouch™ 2 Instrument

The instrument design and manufacture complies with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

**Safety**

| Reference | Description |
|---|---|
| Directive 2006/95/EC | *European Union "Low Voltage Directive"* |
| IEC 61010-1<br>EN 61010-1<br>CSA C22.2 61010-1 | *Safety requirements for electrical equipment for measurement, control, and laboratory use* |
| IEC 61010-2-010<br>EN 61010-2-010<br>CSA C22.2 61010-2-010 | *Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials* |
| IEC 61010-2-020<br>EN 61010-2-020<br>CSA C22.2 61010-2-020 | *Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-020: Particular requirements for laboratory centrifuges* |
| IEC 61010-2-081<br>EN 61010-2-081<br>CSA C22.2 61010-2-081 | *Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes* |

**EMC**

| Reference | Description |
|---|---|
| Directive 2004/108/EC | *European Union "EMC Directive"* |
| EN 61326-1 | *Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements* |
| FCC Part 18 (47 CFR) | *U.S. Standard "Industrial, Scientific, and Medical Equipment"* |
| AS/NZS 2064 | *Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment* |
| ICES-001, Issue 3 | *Industrial, Scientific and Medical (ISM) Radio Frequency Generators* |

**Environmental design**

| Reference | Description |
|---|---|
| Directive 2006/95/EC | *European Union "WEEE Directive" – Waste electrical and electronic equipment* |

# Safety and electromagnetic compatibility (EMC) standards: Ion OneTouch™ ES

The instrument design and manufacture complies with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

### Safety

| Reference | Description |
|---|---|
| EU Directive 2006/95/EC | European Union "Low Voltage Directive" |
| IEC 61010-1<br>EN 61010-1<br>UL 61010-1<br>CSA C22.2 No. 61010-1 | *Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements* |
| IEC 61010-2-081<br>EN 61010-2-081 | *Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes* |

### EMC

| Reference | Description |
|---|---|
| Directive 2004/108/EC | European Union "EMC Directive" |
| EN 61326-1 | *Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements* |
| FCC Part 18 (47 CFR) | U.S. Standard "Industrial, Scientific, and Medical Equipment" |
| AS/NZS 2064 | *Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment* |
| ICES-001, Issue 3 | *Industrial, Scientific and Medical (ISM) Radio Frequency Generators* |

### Environmental design

| Reference | Description |
|---|---|
| Directive 2002/95/EC | *European Union "WEEE Directive" – Waste electrical and electronic equipment* |

# 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, and 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.

⚠️ **WARNING!  HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

# Specific chemical handling

| CAS | Chemical | Phrase |
|---|---|---|
| 26628-22-8 | Sodium Azide | Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. |
| 8042-47-5 | Mineral Oil | Aspiration of mineral oil dust may cause lipoid pneumonia. To the best of our knowledge, the chemical, physical, biological, and toxicological properties of this product have not been thoroughly investigated. |

# Documentation and support

## Obtaining SDSs

Safety Data Sheets (SDSs) are available from **lifetechnologies.com/iontorrent**.

For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

## Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

## Obtaining support

For the latest services and support information for all locations, go to:

**www.lifetechnologies.com/support**

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

# Ion contact information

**Web site:** lifetechnologies.com/iontorrent

**Ion community:** ioncommunity.lifetechnologies.com

**Support email:** ionsupport@lifetech.com

**Phone numbers**
In North America: 1-87-SEQUENCE (1-877-378-3623)
Outside of North America: +1-203-458-8552