Ion PI™ Template OT2 200 Kit v3

For use with: Ion OneTouch™ 2 System

Publication Part Number MAN0009134 Rev. A.0

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Ion PI™ Template OT2 200 Kit v3 User Guide* (Pub. no. MAN0009133). For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

IMPORTANT! Use *only* the Ion PI™ Template OT2 200 Kit v3 (Cat. no. 4488318) with this user guide and with the Ion OneTouch™ 2 System. Do not use the kit with the Ion OneTouch™ System. Do ***not*** mix reactions or disposables including plates, solutions, and kit reagents from other template preparation kits. Template-positive Ion PI™ Ion Sphere™ Particles prepared with this kit should *only* be used in conjunction with the Ion PI™ Sequencing 200 Kit v3 (Cat. no. 4488315). Refer to the *Ion PI™ Sequencing 200 Kit v3 User Guide* (Pub. no. MAN0009136).

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.

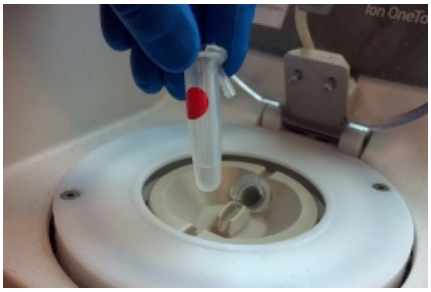
Set up the Ion OneTouch™ 2 System

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

Install the Ion PI™ Template OT2 Recovery Tubes and Ion OneTouch™ Recovery Router

Dispense 150 µL Ion PI™ OT2 Breaking Solution into each of two Recovery Tubes. Install the Ion PI™ Template OT2 Recovery Tubes and the Ion OneTouch™ Recovery Router, then close the centrifuge lid.

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



Install the Ion OneTouch™ 2 Amplification Plate

Remove the used cleaning adapter, insert the new amplification plate, then pull the handle to close the heat block. Thread the disposable tubing through the catch and pinch valve.

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

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

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

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

Install the disposable injector

Insert the disposable injector, then confirm automatic placement of the disposable injector above the router by briefly pressing then releasing the spring-loaded top of the Ion OneTouch™ DL Injector Hub. You should hear a click.

Install the reagents

1. Install the Ion Proton™ OT2 Oil on the *left* front port  . Invert the Ion Proton™ OT2 Oil bottle (450-mL size) 3 times, then fill the Reagent Tube *one-half* full with Oil. Install the Reagent Tube. Minimize bubbles.

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

2. Install the Ion PI™ OT2 Recovery Solution on the *right* front port  . Invert the bottle of Recovery Solution 3 times, then fill the Reagent Tube *one-third* full with Recovery Solution. Install the Reagent Tube. Minimize bubbles.

Empty the waste container and the oil waste tray

Appropriately dispose of waste.

Prepare the amplification solution

IMPORTANT! Use only Ion PI™ Ion Sphere™ Particles in the Ion OneTouch™ 2 kits with the Ion OneTouch™ 2 System. Do ***not*** use ISPs from other or previously used kits.

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. 2. Vortex the solution for 30 seconds, then centrifuge the solution for 2 seconds. 3. Keep the reagent mix at room temperature. After use, discard the single-use tube. **Note:** If precipitate is visible, then see the "Troubleshooting" section in the *Ion PI™ Template OT2 200 Kit v3 User Guide*.
Ion PI™ PCR Reagent B	1. Vortex the reagent for 1 minute, then centrifuge the solution for 2 seconds. 2. Inspect the reagent: • If the solution is *clear*, then prepare the amplification solution. Keep Reagent B at room temperature. • 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. 3. Inspect the reagent again. If the reagent is: • *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. • *Clear*: Equilibrate the reagent to room temperature, then prepare the amplification solution. Store Reagent B at room temperature. **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. 2. Place on ice.
Ion PI™ Ion Sphere™ Particles v3	Place the suspension at room temperature.

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.

	Ion AmpliSeq™ DNA Library†	Ion AmpliSeq™ RNA Library	gDNA Fragment Library	Ion TargetSeq™ Exome-Enriched Library	Ion Total RNA-Seq Library
Final library concentration	100 pM	100 pM	100 pM	100 pM	100 pM
Volume of library	8–10 µL	8–10 µL	8–10 µL	8–10 µL	8–10 µL
Volume of Nuclease-Free Water	90–92 µL	90–92 µL	90–92 µL	90–92 µL	90–92 µL
Total volume of diluted library to add to the amplification solution	**100 µL**	**100 µL**	**100 µL**	**100 µL**	**100 µL**

† Includes Ion AmpliSeq™ Exome libraries.

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

   b. Place the diluted library on ice.

3. Vortex the Ion PI™ Ion Sphere™ Particles (ISPs) at maximum speed for ***1 minute***, centrifuge the ISPs for 2 seconds, pipet the ISPs up and down to mix; then *immediately* proceed to the next step.

4. In a 2.5-mL Reaction 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	Nuclease-Free Water	—	160 µL
2	Ion PI™ Reagent Mix TL	Violet	1200 µL
3	Ion PI™ PCR Reagent B	Blue	720 µL
4	Ion PI™ Enzyme Mix TL	Brown	120 µL
5	Ion PI™ Ion Sphere™ Particles v3	Black	100 µL
6	Diluted library (*not* stock library)	—	100 µL
—	**Total**	—	**2400 µL**

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 and install the Ion PI™ Plus Reaction Filter Assembly on the Ion OneTouch™ 2 Instrument".

Fill and install the Ion PI™ Plus Reaction Filter Assembly on the Ion OneTouch™ 2 Instrument

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

1. 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.

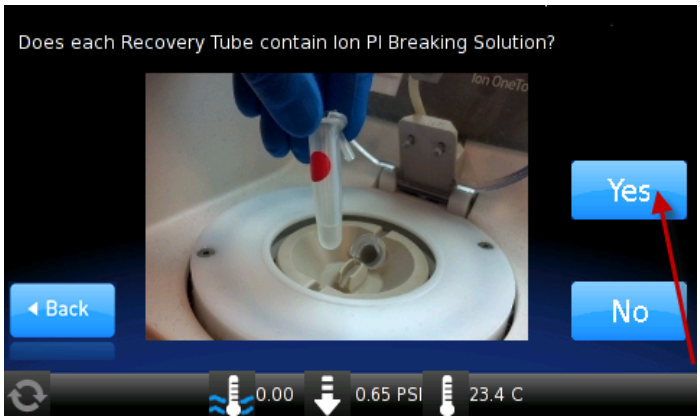
2. Pipet the amplification solution up and down to mix, then pipet 800 µL of the amplification solution through the sample port.

3. Repeat step 2 **two more** times (for a total of 3 times) to load the entire amplification solution volume into the Ion PI™ Plus Reaction Filter Assembly.
4. Invert then install the filled Ion PI™ Plus Reaction Filter Assembly into the three holes on the top stage of the Ion OneTouch™ 2 Instrument.

Run the Ion OneTouch™ 2 Instrument

1. Close the centrifuge lid, touch **Run**, then select **Proton: Ion PI™ Template OT2 200 Kit v3**, touch **Next**, touch **Assisted** or **Expert**. To cancel a run, touch **Abort**, then touch **Yes**.

   Note: Remember to add 150 µL of Ion PI™ OT2 Breaking Solution to each Recovery Tube before the starting the run. On the reminder screen, confirm and touch **Yes**:



2. Remove the samples ≤16 hours after starting the run.

Recover the template-positive ISPs

1. At the end of the run, follow the screen prompts to centrifuge the sample. If you removed the Reaction Tubes at the end of the run before the Ion OneTouch™ 2 Instrument had spun the sample or have not processed the sample after 15 minutes, centrifuge the sample on the instrument: On the home screen, touch **Options**, then touch **Final Spin**, then follow any screen prompts to centrifuge the sample.

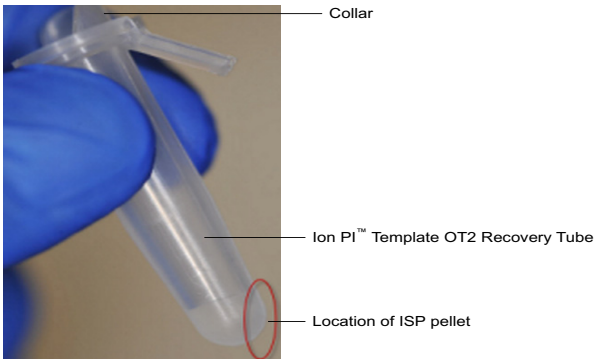
CAUTION! **ROTATION HAZARD.** Wait until rotation stops before opening. Rotating parts can cause injury.

2. Discard the Recovery Router, then carefully remove the Ion PI™ Template OT2 Recovery Tubes and put them in a tube rack.

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 ISPs". There is a stopping point in the next section.

Wash the template-positive ISPs

1. Label a new 1.5-mL Eppendorf LoBind® Tube for the template-positive ISPs.
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 ISPs:



3. Resuspend the template-positive ISPs in the remaining Recovery Solution in each tube by pipetting up and down.
4. Transfer the suspension from each Recovery Tube to a 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.

STOPPING POINT Bring the volume of the suspension to 1 mL with Nuclease-Free Water. Store the ISPs 2°C to 8°C for up to 3 days. If the template-positive ISPs were stored at 2°C to 8°C, proceed to step 8.

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

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 ISPs, then centrifuge the tube for 2 seconds.
9. (*Optional*) Assess the quality of the unenriched, template-positive Ion PI™ Ion Sphere™ Particles using the Guava® easyCyte™ Flow Cytometer. Transfer a 2.0-µL aliquot of the diluted, unenriched Ion PI™ Ion Sphere™ Particles (from step 8) 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).
10. Centrifuge the template-positive ISP suspension for 8 minutes at 15,500 × *g*.
11. Remove all but 20 µL of supernatant.
12. Bring the combined washed suspensions in the new labeled tube to 100 µL in Ion PI™ ISP Resuspension Solution.
13. Vortex the pellet for 30 seconds to completely resuspend the template-positive ISPs, then centrifuge the tube for 2 seconds.
14. If you have not used the Guava® easyCyte™ 5 flow Cytometer to perform quality control on the ISPs, you can retain a sample to assess quality of the resuspended, unenriched template-positive ISPs (from step 13) by the Qubit® 2.0 Fluorometer. Transfer a 2.0-µL aliquot of the resuspended, template-positive ISPs to a 0.2-mL PCR Tube, then refer to the *Ion PI™ Template OT2 200 Kit v3 User Guide*.
15. Enrich the template-positive ISPs (see "Enrich the template-positive ISPs with the Ion OneTouch™ ES" on page 7).

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.

Maintain the Ion OneTouch™ 2 Instrument

IMPORTANT! 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 v3 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 v3.

2. Ensure that there is ≥20 mL of Ion Proton™ OT2 Oil (*left* Reagent Tube). If not, pour oil into the Oil 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*.
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 9).
6. Firmly insert the 3 ports of the Ion OneTouch™ 2 Cleaning Adapter into the three holes on the top stage of the Ion OneTouch™ 2 Instrument. The tab protruding from the outer edge of the Ion OneTouch™ 2 Cleaning Adapter fits into the front notch of the stage.
7. Remove the disposable injector from the Ion OneTouch™ DL Injector Hub: Place one hand on the centrifuge lid. With the other hand, firmly grip the rigid plastic connector at the top of the disposable injector, then 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.

8. Gently pull the disposable tubing downwards on the both sides of the pinch valve until the disposable tubing is out of the valve.
9. Place the used, disposable injector into an empty 50-mL conical tube in a tube rack by the instrument. The conical tube will be used for waste:

10. On the Ion OneTouch™ 2 Instrument home screen, touch **Clean**. Complete each task, then touch **Next**. After you touch **Next** on the last task, you see a progress bar, and the cleaning begins.
11. 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.
12. Appropriately dispose of the waste in the 50-mL conical tube.
13. 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.

14. Wipe the residue from the centrifuge lid with dry Kimwipes® disposable wipes, then close the centrifuge lid.
15. Touch **Next** to return to the home screen on the instrument.

Enrich the template-positive ISPs with the Ion OneTouch™ ES

Perform the residual volume test on the Ion OneTouch™ ES

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 the Ion OneTouch™ ES" on page 11).
Routine use and residual volume in Well 1 and Well 8 is > 5.0 µL	
Routine use and residual volume in Well 1 and Well 8 is ≤ 5.0 µL	Operate the instrument without performing the residual volume test. Proceed to "Prepare reagents then fill the 8-well strip" on page 7.

Prepare reagents then fill the 8-well strip

Prepare Melt-Off Solution

Prepare fresh Melt-Off Solution by combining in this order:

Order	Component	Volume
1	Tween® Solution	280 µL
2	1 M NaOH	40 µL
	Total	**320 µL**

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

Wash and resuspend the Dynabeads® MyOne™ Streptavidin C1 Beads

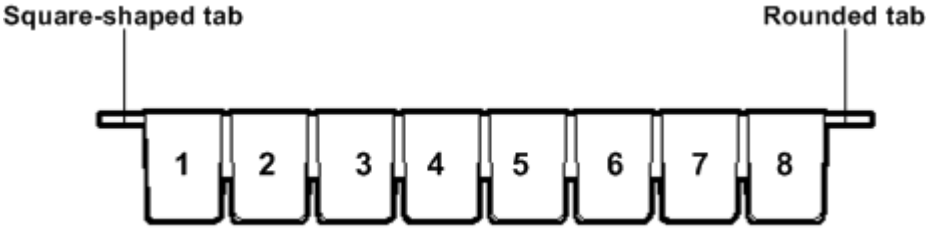
1. Vortex the bottle containing the Dynabeads® MyOne™ Streptavidin C1 Beads for 30 seconds to thoroughly resuspend the beads, then *immediately* proceed to the next step.
2. Transfer 100 µL of Dynabeads® MyOne™ Streptavidin C1 Beads to a new 1.5-mL Eppendorf LoBind® Tube.
3. Place the tube on a magnet 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 ISPs from the Ion OneTouch™ 2 Instrument are in 100 µL of Ion PI™ ISP Resuspension Solution. If the template-positive ISPs were stored at 2°C to 8°C, follow the appropriate washing procedure for 100 µL of washed ISPs (see "Wash the template-positive ISPs" on page 5).
2. Obtain an 8-well strip from the Ion OneTouch™ kit. Ensure that the square-shaped tab of the 8-well strip is on the *left*:



3. Pipet the ISPs up and down 10 times to mix, then transfer the entire volume (100 µL) of resuspended, template-positive ISPs in on PI™ ISP Resuspension Solution (step 1 of this procedure) into Well 1 of the 8-well strip.
4. If you have not already, assess the quality of the unenriched, template-positive Ion PI™ Ion Sphere™ Particles using the Qubit® 2.0 Fluorometer or the (*optional*) Guava® easyCyte™ Flow Cytometer.
5. Fill the remaining wells as follows, then *immediately* proceed to step 6 (see the figure following step 6):

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 MyOne™ Beads Wash Solution (prepared in "Wash and resuspend the Dynabeads® MyOne™ Streptavidin C1 Beads" on page 7] (B)
Well 3	300 µL of Ion PI™ ES Wash Solution (W)
Well 4	300 µL of Ion PI™ ES Wash Solution (W)
Well 5	300 µL of Ion PI™ ES Wash Solution (W)
Well 6	Empty
Well 7	300 µL of freshly-prepared Melt-Off solution (prepared in "Prepare Melt-Off Solution" on page 7) (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. Align the pins with the round notches in the cradle, then lower the Tip Arm into position. Rock the Tip Arm forward into the working position.
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.
3. ***Insert a new 0.2-mL opened PCR tube into the hole in the base of the Tip Loader.***

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

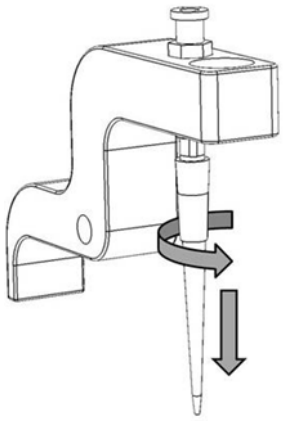
Note: If necessary to stop a run, press **Start/Stop**. The instrument completes the current step, then stops the run and displays "End". Press **Start/Stop** again to return the Tip Arm to the home position. It is not possible to restart (where you left off) after stopping a run.

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 ISPs.

   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:



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 ISPs".

Remove and wash the enriched ISPs

1. Centrifuge the enriched ISPs in the 0.2-mL PCR tube at 15,500 x *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 enriched ISPs in 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:

Dynabeads® MyOne™ Streptavidin C1 Beads (brown pellet) in tube?	Then...
No	1. Remove all but ~10 µL of supernatant without disturbing the pellet. 2. Add sufficient Nuclease-Free Water for a final volume of 100 µL. 3. Pipet up and down 10 times to resuspend the pellet. 4. Sequence or store the template-positive ISPs: • For the Ion PI™ Template OT2 200 Kit v3, use the Ion PI™ Sequencing 200 Kit v3 (Cat. no. 4488315). Refer to the *Ion PI™ Sequencing 200 Kit v3 User Guide* (Pub. no. MAN0009136). *or* • Store the enriched ISPs at 2°C to 8°C for up to 3 days.
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 ISPs: • For the Ion PI™ Template OT2 200 Kit v3, use the Ion PI™ Sequencing 200 Kit v3 (Cat. no. 4488315). Refer to the *Ion PI™ Sequencing 200 Kit v3 User Guide* (Pub. no. MAN0009136). *or* • Store the enriched ISPs at 2°C to 8°C for up to 3 days.

(*Optional*) Perform ISP quality control

You can determine the appropriate library dilution and/or the enrichment efficiency by using the Guava® easyCyte™ 5 Flow Cytometer. Transfer a 2.0-µL aliquot of the enriched ISPs to a 1.5-mL Eppendorf LoBind® Tube. 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

Supplemental procedure

Perform a residual volume test on the Ion OneTouch™ ES

1. Set up the Ion OneTouch™ ES. Refer to "Set up the Ion OneTouch™ ES" in the *Ion PI™ Template OT2 200 Kit v3 User Guide*.
2. Install a tip on the Tip Arm.
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 5 of this procedure.

4. Run the residual volume test. During the test, confirm that the tip is centered with respect to the sides of the wells when moving in or out of a well.
   a. Turn ON the instrument, and 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).
   b. Press **Start/Stop**.
   c. 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.
   d. Wait for the instrument to dispense the tip contents into Well 4, press **Start/Stop** to stop the test run, then press **Start/Stop** again to return the Tip Arm to the home position.
   e. 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 (see diagram in "Remove and wash the enriched ISPs" on page 10).

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 7.
Residual volume in Well 2 is >5 µL **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. Refer to "Calibrate the Ion OneTouch™ ES" in the *Ion PI™ Template OT2 200 Kit v3 User Guide*.
Aspiration is irregular	This instrument out of calibration.	Restore defaults, then calibrate the instrument Refer to "Calibrate the Ion OneTouch™ ES" in the *Ion PI™ Template OT2 200 Kit v3 User Guide*.
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. Refer to "Calibrate the Ion OneTouch™ ES" in the *Ion PI™ Template OT2 200 Kit v3 User Guide*.

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

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