Ion PGM™ Template OT2 200 Kit

For use with: the Ion OneTouch™ 2 System

Catalog Number 4480974

Pub. No. MAN0007221 **Rev.** B.0

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Ion PGM™ Template OT2 200 Kit User Guide* (Pub. no. MAN0007220). 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 PGM™ Template OT2 200 Kit (Cat. no. 4480974) 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.

- Set up the Ion OneTouch™ 2 Instrument 1
- Run the Ion OneTouch™ 2 Instrument 3
- Recover the template-positive ISPs 3
- Maintain the Ion OneTouch™ 2 Instrument 4
- Enrich the template-positive ISPs with the Ion OneTouch™ ES 5

Set up the Ion OneTouch™ 2 Instrument

IMPORTANT! Ensure that the latest firmware is installed to perform the cleaning procedure with the Ion OneTouch™ 2 Cleaning Adapter (touch **Options** then touch **Info** on the instrument display). To update the firmware to the appropriate version, refer to the *Ion PGM™ Template OT2 200 Kit User Guide*.

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

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

Install the 2 Ion OneTouch™ Recovery Tubes and the Ion OneTouch™ Recovery Router, then close the centrifuge lid.

Install the Ion OneTouch™ 2 Amplification Plate

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

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

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

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

Install the disposable injector

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

You should hear a click.

Install the Reagent Tubes

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

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

Empty the waste container

Appropriately dispose of waste.

Prepare the amplification solution

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 PGM™ Template OT2 200 Reagent Mix	1. 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 during use. Store thawed reagent mix at 2ºC to 8ºC.
Ion PGM™ Template OT2 200 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 2ºC to 8ºC, heat the reagent for 1 minute in a heat block set at 75ºC. Vortex the reagent for 1 minute, then centrifuge the solution for 2 seconds. 3. Inspect the reagent. 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 PGM™ Template OT2 200 Enzyme Mix	1. Centrifuge the enzyme for 2 seconds. 2. Place on ice.
Ion PGM™ Template OT2 200 Ion Sphere™ Particles	Place the suspension at room temperature.

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

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

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

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

   b. Place the diluted library on ice.

3. In a 1.5-mL Eppendorf LoBind™ Tube at 15°C to 30°C, add the following components in the designated order (You add the ISPs in step 6 of this procedure.) Add each component, then pipet the amplification solution up and down to mix:

Order	Reagent	Cap color	Volume
1	Nuclease-free Water	—	25 µL
2	Ion PGM™ Template OT2 200 Reagent Mix	Violet	500 µL
3	Ion PGM™ Template OT2 200 PCR Reagent B	Blue	300 µL
4	Ion PGM™ Template OT2 200 Enzyme Mix	Brown	50 µL
5	Diluted library (*not* stock library)	—	25 µL
—	**Total**	—	**900 µL**

4. Vortex the solution prepared in step 3 at maximum speed for 5 seconds, then centrifuge the solution for 2 seconds.

5. Prepare the ISPs:

   a. Vortex the ISPs at maximum speed for *1 minute* to resuspend the particles.

   b. Centrifuge the ISPs for 2 seconds.

   c. Pipet the ISPs up and down to mix.

   d. *Immediately* proceed to the next step.

6. Add the ISPs to the amplification solution:

Order	Reagent	Cap Color	Volume
1	Amplification solution *without* ISPs (from step 3 of this procedure)	—	900 µL
2	Ion PGM™ Template OT2 200 Ion Sphere™ Particles	Black	100 µL
—	**Total**	—	**1000 µL**

7. Vortex the complete amplification solution prepared in step 6 at maximum speed for 5 seconds.

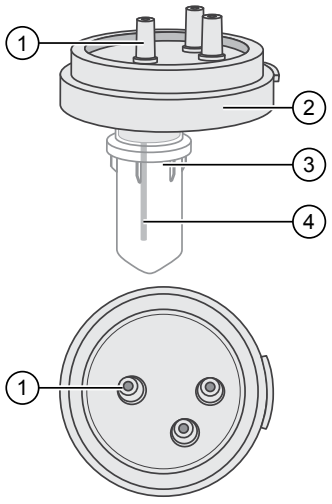
IMPORTANT! Start the run on the Ion OneTouch™ 2 Instrument ≤15 minutes after preparing the amplification solution.

8. Proceed *immediately* to "Fill and install the Ion PGM™ OneTouch Plus Reaction Filter Assembly".

Fill and install the Ion PGM™ OneTouch Plus Reaction Filter Assembly

IMPORTANT! We recommend filling the Ion PGM™ OneTouch 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 any other template preparation kit.

1. Pipet 1000 µL of the amplification solution, prepared in the previous procedure, through the sample port.



① Sample port
② Ion PGM™ OneTouch Plus Reaction Filter Assembly
③ Ion OneTouch™ Reaction Tube
④ Short tubing from sample port to Ion OneTouch™ Reaction Tube

2. Pipet 1000 µL of Ion OneTouch™ Reaction Oil (27-mL size) through the sample port.
3. Pipet an additional 500 µL of Ion OneTouch™ Reaction Oil through the sample port.
4. Invert then install the filled Ion PGM™ OneTouch 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. Ensure that the centrifuge lid of the Ion OneTouch™ 2 Instrument is closed.
2. On the home screen, touch **Run**.
3. Touch the drop-down menu, then select **PGM: Ion PGM™ Template OT2 200 Kit**.

   Note: The **PGM: Ion PGM™ Template OT2 200 Kit for Hi-Q** script is discontinued in Torrent Suite™ Software (TSS) v5.0. If you need to continue using the Ion PGM™ Hi-Q™ Sequencing Kit with the Ion PGM™ Template OT2 200 Kit, upgrade to TSS v.5.0 only after you no longer require the mixed workflow.

4. Touch **Next**.
5. Touch **Assisted** or **Expert**. Complete the listed tasks, if listed, then touch **Next**. The run begins. To cancel a run, touch **Abort**, then touch **Yes**.
6. Remove the samples within 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.

   Note: 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 within 15 minutes, centrifuge the sample on the instrument. On the home screen, touch **Options**, then touch **Final Spin**, then follow the screen prompts to centrifuge the sample.

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

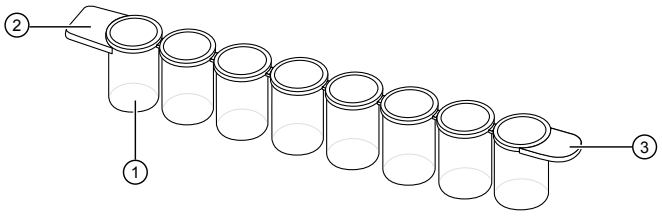
2. Immediately after the centrifuge stops, touch **Open Lid**, wait until the lid opens, then remove and discard the Recovery Router.
3. Remove both Recovery Tubes, and put the tubes in a tube rack.
4. Remove all but 50 µL of OT2 Recovery Solution from each Recovery Tube.

5. With a new tip and using the same tip for both tubes, resuspend the ISPs in the remaining Ion PGM™ OT2 Recovery Solution. Pipet the pellet up and down until each pellet disperses in the solution.

STOPPING POINT Transfer the suspensions from both tubes into a new 1.5-mL Eppendorf LoBind™ Tube for a total of 100 µL. Add 1 mL of Ion OneTouch™ Wash Solution to the 100-µL ISP suspension. Store the ISPs 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 2.5 minutes, then carefully remove all but 100 µL of supernatant. With a new tip, pipet up and down to resuspend the ISPs. Proceed to step 6.

IMPORTANT! Do not store the recovered ISPs in Ion PGM™ OT2 Recovery Solution and do not store the recovered ISPs at –30°C to –10°C.

6. Pipet the ISPs ups and down 10 times to mix, then transfer the suspensions from both tubes (1 tube if stored) into Well 1 of an 8-well strip for a total of 100 µL of suspension of ISPs in the well:



① Well 1
② Square-shaped tab
③ Rounded tab

7. Assess the quality of the unenriched, template-positive ISPs using the Qubit™ 2.0 or Qubit™ 3.0 Fluorometer, (optional) Guava™ easyCyte™ 5 Flow Cytometer, or the (demonstrated protocol) Applied Biosystems™ Attune™ Acoustic Focusing Cytometer.

8. Enrich the template-positive ISPs (see "Enrich the template-positive ISPs with the Ion OneTouch™ ES" on page 5).

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. Follow the cleaning procedure in this section to clean the Ion OneTouch™ 2 Instrument with the Ion OneTouch™ 2 Cleaning Adapter. *Perform the cleaning procedure after every run.*

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

If you are...	Then...
Switching to the Ion PGM™ Template OT2 200 Kit from another kit	Refer to Chapter 5 of the *Ion OneTouch™ 2 System User Guide* (Pub. no. MAN0014388). Use the reagents from the appropriate kit to maintain the Ion OneTouch™ 2 Instrument.
Already using the Ion PGM™ Template OT2 200 Kit	Proceed to step 2. Continue to use the reagents provided in the Ion PGM™ Template OT2 200 Kit.

2. Ensure that there is ≥20 mL of Ion OneTouch™ 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 PGM™ OneTouch Plus Reaction Filter Assembly. Remove the assembly from the instrument by grasping the *filter*.

4. Place a 50-mL conical tube in a tube rack, then place the tube rack with the tube adjacent to the instrument.

5. Firmly insert the 3 ports of the single-use Ion OneTouch™ 2 Cleaning Adapter into the three holes on the top stage of the Ion OneTouch™ 2 Instrument.

6. Remove the tubing from the pinch valve, then remove the disposable injector from the Ion OneTouch™ DL Injector Hub and place the injector into the empty 50-mL conical tube adjacent to the instrument. *Do not* remove the amplification plate from the heat block.

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.

7. Touch **Clean**. Complete each task when prompted, then touch **Next**. After you touch **Next** on the last task, a progress bar appears, and the cleaning begins.

8. At the end of the cleaning run, the screen displays "**Time Remaining 00:00:00, Cleaning Run Complete**". Press **Next**, then ensure that the task in bold displays: **"Remove plate, injector, conical tube, and waste"**.

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

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

10. Remove and appropriately dispose of the used Ion OneTouch™ 2 Amplification Plate, disposable injector, and tubing from the instrument.

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

11. Wipe the residue from the centrifuge lid with a Kimwipes™ disposable wipe, then close the centrifuge lid.

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

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

Perform a 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. Refer to Appendix C of the *Ion PGM™ Template OT2 200 Kit User Guide* (Pub. no. MAN0007220) for the procedure.
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	Proceed to "Prepare reagents then fill the 8-well strip".

Prepare reagents then fill the 8-well strip

Prepare Melt-Off Solution

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

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

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

Wash and resuspend the Dynabeads™ MyOne™ Streptavidin C1 Beads

1. Vortex the tube for 30 seconds to thoroughly resuspend the beads, then centrifuge the tube of Dynabeads™ MyOne™ Streptavidin C1 Beads for 2 seconds.

2. Open the tube, then use a new tip to pipet up and down the dark pellet of beads until the pellet disperses. *Immediately* proceed to the next step.

3. Transfer 13 µL of Dynabeads™ MyOne™ Streptavidin C1 Beads to a new 1.5-mL Eppendorf LoBind™ Tube.

4. Place the tube on a magnet such as a DynaMag™-2 magnet for 2 minutes, then *carefully* remove and discard the supernatant without disturbing the pellet of Dynabeads™ MyOne™ Streptavidin C1 Beads.

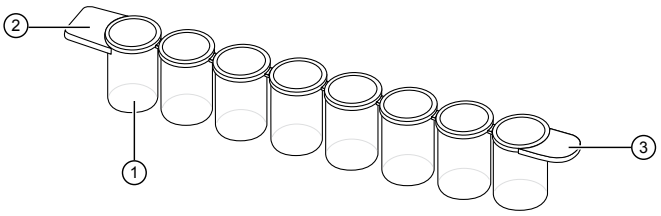
5. Add 130 µL of MyOne™ Beads Wash Solution to the Dynabeads™ MyOne™ Streptavidin C1 Beads.

   Note: You add the resuspended Dynabeads™ MyOne™ Streptavidin C1 Beads in the 130 µL MyOne™ Beads Wash Solution to Well 2 of the 8-well strip.

6. 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 PGM™ OT2 Recovery Solution and are in Well 1 of the 8-well strip. Well 1 with the ISPs is on the left:



① Well 1
② Square-shaped tab
③ Rounded tab

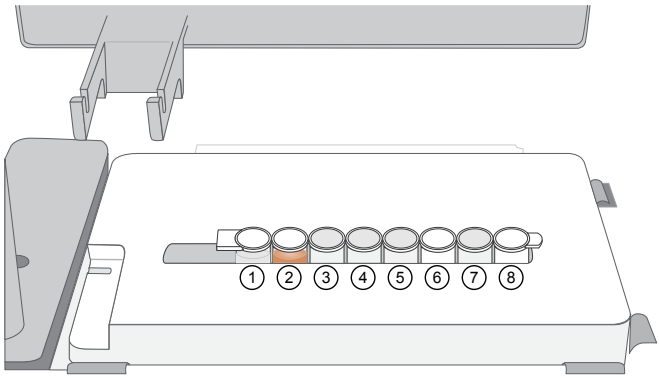
2. If you have not done so already, assess the quality of the unenriched, template-positive ISPs using the Qubit™ 2.0 or Qubit™ 3.0 Fluorometer, (optional) Guava™ easyCyte™ 5 Flow Cytometer, or the (demonstrated protocol) Applied Biosystems™ Attune™ Acoustic Focusing Cytometer.

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

Well number	Reagent to dispense in well
Well 1[1]	*Entire* template-positive ISP sample [100 µL; prepared in step 1 of this procedure (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 5 (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 5 (M)]
Well 8	Empty

[1] Well closest to the square-shaped tab

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



Prepare the Ion OneTouch™ ES

1. Load a new tip in the Tip Arm.
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. Add 10 µL of Neutralization Solution to a new 0.2-mL PCR tube.
4. *Insert the opened 0.2-mL PCR tube with the Neutralization Solution 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 with the Neutralization Solution 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.

   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 and remove the PCR tube containing the enriched ISPs.
7. Mix the contents of the PCR tube by gently inverting the tube 5 times.

   Note: Ensure that the 0.2-mL PCR tube has >200 µL of solution containing the enriched ISPs. After a successful run on the instrument, the sample is in ~230 µL of Melt-Off Solution, Ion OneTouch™ Wash Solution, and Neutralization Solution. If the tube has <<200 µL of solution containing the enriched ISPs, contact Technical Support.
8. Remove the used tip and the 8-well strip.

Sequence or store the template-positive ISPs

- Proceed to sequencing using the Ion PGM™ Sequencing 200 Kit v2 (Cat. no. 4482006). Refer to the *Ion PGM™ Sequencing 200 Kit v2 User Guide* (Pub. no. MAN0007273).

  or

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

Perform Ion Sphere™ Particles quality control

Determine the enrichment efficiency using one of the following methods:

Quality assessment by...	Then...
Guava™ easyCyte™ 5 Flow Cytometer	Transfer a 1.0-µL aliquot of the enriched ISPs to a 1.5-mL Eppendorf LoBind™ Tube. Refer to the *Ion Sphere™ Particles (ISPs) Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. no. 4470082), available on the Ion Community website: **ioncommunity.thermofisher.com**
Demonstrated protocol: Quality assessment by the Applied Biosystems™ Attune™ Acoustic Focusing Cytometer	Transfer a 1.0-µL aliquot of the enriched ISPs to a 1.5-mL Eppendorf LoBind™ Tube. Put the sample on ice, then refer to *Demonstrated Protocol: Ion Sphere™ Particles (ISPs) Quality Assessment using the Applied Biosystems™ Attune™ Acoustic Focusing Cytometer User Bulletin* (Pub. no. 4477181), available on the Ion Community website: **ioncommunity.thermofisher.com**

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

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288

For support visit **thermofisher.com/support** or email **techsupport@lifetech.com**

thermofisher.com

19 November 2015