Ion PGM™ Template IA 500 Kit

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Ion PGM™ Template IA 500 Kit User Guide* (Pub. No. MAN0009347). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Before you begin

1. Dilute your library to 50 pM (30 × 10^6 copies/µL) in Nuclease-free Water in the clean room.
2. Preheat a heat block to 40°C in a post-PCR room. Add water to the wells to accelerate equilibration of the reaction tube.
3. Thaw the Ion PGM™ Template IA Primer Mix S or L, and keep it and the Ion PGM™ Template IA Start Solution on ice while setting up the reaction.

   Note: Use Primer Mix S if your library insert length is ≤350 bp. Use Primer Mix L if your library insert length is >350 bp.

Perform the IA reaction

1. Prepare Templating Solution in a 2-mL Eppendorf LoBind™ Tube on ice (or a cold block) using the following table. Adjust library input according to whether your library is amplified or non-amplified.

Order of addition	Component	Volume per reaction	
		Amplified library	Non-amplified library
1	Ion PGM™ Template IA ISP Dilution Buffer (yellow cap)	130 µL	128 µL
2	Ion PGM™ Template IA Primer Mix S or Primer Mix L[1]	8 µL	8 µL
3	Ion PGM™ Template IA ISPs[2] (orange cap)	21 µL	21 µL
4	Library (50 pM)	3.2 µL	4.8 µL
—	**Total**	**≈162 µL**	**≈162 µL**

[1] Use Primer Mix S if your library insert length is ≤350 bp. Use Primer Mix L if your library insert length is >350 bp.

[2] Vortex 30 seconds at maximum speed to resuspend immediately prior to addition.

2. Vortex the tube containing the Templating Solution for 2 seconds at the maximum setting to mix, pulse-spin, then return the tube to ice.
3. Invert the Ion PGM™ Template IA Rehydration Buffer (white cap) three times to mix, then add 720 µL to the tube containing the Ion PGM™ Template IA Pellet to rehydrate the pellet. Vortex for 2 seconds at maximum setting, then pulse-spin to collect the contents at the bottom of the tube. Place the rehydrated pellet on ice or in a cold block.
4. Transfer the rehydrated Ion PGM™ Template IA Pellet to the Templating Solution on ice, vortex for 2 seconds at the maximum setting, then pulse-spin.
5. Invert the Ion PGM™ Template IA Start Solution (purple cap) three times to mix, then add 300 µL to the Template/IA Solution using the reverse pipetting technique.

   Note: If you are setting up more than one IA reaction, follow steps 5 through 7 for each reaction before beginning the next reaction.
6. Vortex the tube ten times in 1 second pulses at the maximum vortexer setting. Invert the tube and repeat the ten 1 second pulses.

7. Pulse-spin the tube to collect contents, then immediately place the tube on ice.

8. Start the IA reaction by gently placing the tube in the 40°C heat block. Make sure the tube is immersed in water.

9. Incubate the IA reaction for 25 minutes at 40°C.

Recover the template-positive ISPs

1. Stop the IA reaction by removing the tube from the heat block and adding 650 uL of Ion PGM™ Template IA Stop Solution.

2. Vortex the tube well to mix contents thoroughly, then centrifuge the tube at 7,500 × *g* for 3 minutes.

3. Aspirate and discard the supernatant, being careful not to disturb the pellet. Leave ~100 µL in the tube.

4. Resuspend the pellet in 1 mL Ion PGM™ Template IA Recovery Solution.
   a. Pipette up and down to resuspend the pellet.
   b. Add an additional 700 µL Ion PGM™ Template IA Recovery Solution and vortex thoroughly.

5. Incubate for 5 minutes with vortexing 5 seconds every minute.

6. Centrifuge for 3 minutes at 12,000 × *g*.

7. Immediately remove and discard all of the supernatant without disturbing the ISP pellet. Remove any bubbles prior to removing the bulk of the liquid to avoid frothing in subsequent steps.

8. Add 100 µL of the Ion PGM™ Template IA Wash Solution to the ISP pellet.

9. Resuspend the templated ISPs completely by vortexing for 4 seconds at maximum speed, then pipet the ISP suspension up and down 4 times. Proceed to "Enrich the template-positive ISPs".

STOPPING POINT Store templated ISPs in Ion PGM™ Template IA Wash Solution at 4°C for up to one week.

Enrich the template-positive ISPs

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.

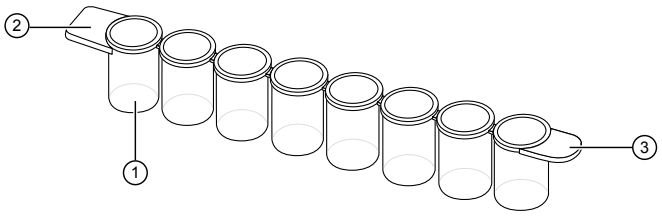
6. Remove the tube from the magnet, vortex the tube for 30 seconds, and centrifuge for 2 seconds.

Fill the 8-well strip

Note: If the template-positive ISPs were stored at 2°C to 8°C, vortex the tube to resuspend the ISPs and pulse spin to collect

contents. Pipet the solution up and down to resuspend the Ion PGM™ Template IA ISPs and transfer to Well 1 of the 8-well strip.

1. Add the entire volume (~100 µL) of template-positive ISPs from the amplification reaction into 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 Guava™ easyCyte™ 5 Flow Cytometer, or the Applied Biosystems™ Attune™ Acoustic Focusing Cytometer.

3. Fill the remaining wells in the 8-well strip as follows:

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)
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 2]
Well 3	300 µL of Ion PGM™ Template IA Wash Solution
Well 4	300 µL of Ion PGM™ Template IA Wash Solution
Well 5	300 µL of Ion PGM™ Template IA Wash Solution
Well 6	Empty
Well 7	300 µL of freshly-prepared Melt-Off Solution (prepared in "Prepare Melt-Off Solution" on page 2)
Well 8	Empty

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.

Perform the Ion OneTouch™ ES run

Confirm that a new tip and opened 0.2-mL PCR tube with the Neutralization Solution have been 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.

1. Pipet the contents of Well 2 up and down to resuspend the beads before starting the run.

2. If necessary, turn ON the Ion OneTouch™ ES and wait for the instrument to initialize. The screen displays "rdy".

3. Press **Start/Stop**.

4. At the end of the run, the instrument displays "End" and beeps every 60 seconds. Press the **Start/Stop** button to silence this alarm and reset the Ion OneTouch™ ES for the next run.

5. ***Immediately after the run***, securely close and remove the PCR tube containing the enriched ISPs.

6. Mix the contents of the PCR tube by gently inverting the tube 5 times.

7. Remove the used tip and the 8-well strip.

Sequence or store the template-positive ISPs

- Sequence using the Ion PGM™ Hi-Q™ View Sequencing Kit (Cat. No. A30044). For more information, see the *Ion PGM™ Hi-Q™ View Sequencing Kit User Guide* (Pub. No. MAN0014583).
  or
- Store the material at 2°C to 8°C for up to 3 days.

Limited product warranty

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