

Ion PGM™ Checklist —

Ion One Touch™ ES Enrichment

Pub. no. MAN0009379

Rev. 1.0

Use this checklist if you are an experienced user of the Ion PGM™ Template OT2 200 Kit (Catalog no. 4480974).

For complete kit and protocol information, including precautions and safety information, refer to the *Ion PGM™ Template OT2 200 Kit User Guide* (Pub no. MAN0007220), available on the [Ion Community website](#).

Enrich template-positive OT2 200 Ion Sphere™ Particles (ISPs) with the Ion OneTouch™ ES

Perform the residual volume test on the Ion OneTouch™ ES

Perform the residual volume test on the Ion OneTouch™ ES if necessary (refer to the *Ion PGM™ Template OT2 200 Kit User Guide*).

Prepare reagents

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

Order	Component	Volume
<input type="checkbox"/> 1	Tween® Solution	280 µL
<input type="checkbox"/> 2	1 M NaOH	40 µL
—	Total	320 µL

Wash and resuspend Dynabeads® MyOne™ Streptavidin C1 Beads

1. Vortex the tube containing Dynabeads® MyOne™ Streptavidin C1 Beads for 30 seconds, then centrifuge the tube 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.
3. Transfer 13 µL of the beads to a new 1.5-mL Eppendorf LoBind® Tube.
4. Place the tube on a magnet for 2 minutes, then carefully remove and discard the supernatant without disturbing the pellet.
5. Add 130 µL of MyOne™ Beads Wash Solution to the beads.
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. Obtain an 8-well strip from the Ion OneTouch™ ES Supplies Kit. Ensure that the square-shaped tab of an 8-well strip is on the *left*.
2. Obtain suspensions of template-positive Ion Sphere Particles (ISPs), prepared as described in the *Ion PGM™ Checklist — Ion OneTouch™ 2 Template Preparation* (MAN0009128).
3. If ISPs were stored at 2–8°C, centrifuge (15,500 × *g* for 2.5 minutes), remove all but 100 µL, and resuspend (pipet up and down).
4. Transfer the ISP suspensions from both tubes (1 tube if stored) into Well 1 of the 8-well strip (total of 100 µL in the well).
5. Fill the remaining wells as follows, then immediately proceed to step 6:

Well number	Reagent to dispense in well
<input type="checkbox"/> Well 1 (next to square tab)	Entire template-positive ISP sample (100 µL)
<input type="checkbox"/> Well 2	130 µL of Dynabeads® MyOne™ Streptavidin C1 Beads resuspended in MyOne™ Beads Wash Solution
<input type="checkbox"/> Well 3	300 µL of Ion OneTouch™ Wash Solution (W)
<input type="checkbox"/> Well 4	
<input type="checkbox"/> Well 5	
<input type="checkbox"/> Well 6	Empty
<input type="checkbox"/> Well 7	300 µL of freshly-prepared Melt-Off solution
<input type="checkbox"/> Well 8	Empty

6. Confirm that the square-shaped tab is on the left, then insert the filled 8-well strip pushed to the far right end of the tray slot.

Prepare the Ion OneTouch™ ES

1. Place a new tip in the Tip Loader, and press the Tip Arm firmly down onto the tip to load the tip.
2. Return the arm with the installed tip to its cradle.
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 run

1. Confirm that the new tip, opened 0.2-mL PCR tube with Neutralization Solution, and 8-well strip have been correctly loaded.
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. Press **Start/Stop** to begin the run.
4. *Immediately after the run*, close and remove the PCR tube containing the enriched ISPs. Press **Start/Stop** to reset the instrument.
5. Ensure the 0.2-mL PCR tube contains >200 µL and mix the contents of the tube by gently inverting the tube 5 times.
6. Remove and discard the used tip by twisting the tip counterclockwise and pulling it downward.
7. Remove and discard the used 8-well strip.

Sequence or store the enriched ISPs

- Proceed to sequencing using the Ion PGM™ Sequencing 200 Kit v2 (Cat. no. 4482006) or store the material at 2°C to 8°C for up to 3 days.

Perform ISP quality control

- Optional:* Determine the enrichment efficiency. Refer to the **Ion Community** website.

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

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

© 2013 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation and/or its affiliate(s) or their respective owners.

Tween is a registered trademark of Uniqema Americas, LLC.

Eppendorf LoBind is a registered trademark of Eppendorf AG.

Guava is a registered trademark and easyCyte is a trademark of Merck KGaA.

DISCLAIMER

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit iontorrent.com/support or email ionsupport@lifetech.com

lifetechnologies.com

4 September 2013

