

Ion OneTouch™ Template Kit

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Note: For safety and biohazard guidelines, refer to the “Safety” section in the *Ion OneTouch™ Template Kit User Guide* (Part no. 4468007). For every chemical, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Set up the Ion OneTouch™ Instrument

1. Discard the OneTouch™ Reaction Tube/Reaction Filter assembly. Note that the Reaction Tube is filled with OneTouch™ Oil.
2. Install a new OneTouch™ Amplification Plate.



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

3. Install the OneTouch™ Oil on the *left* front port ():
 - If you are using a *new kit*, appropriately discard the used Reagent Tube, sipper, and Oil. Install a new sipper; do **not** let the sipper touch any surfaces. Invert the Oil bottle 10 times, then fill the Reagent Tube half-full with Oil.
 - *Between runs*, agitate the Reagent Tube of Oil for 30 seconds to generate bubbles. Next, invert the Oil bottle 10 times, then fill the Reagent Tube half-full with Oil. Install the tube.
4. Install the OneTouch™ Recovery Solution on the *right* front port ():
 - Ensure that the recovery solution is clear; if not, heat the Recovery Solution to 30°C until it is clear.
 - If you are using a *new kit*, appropriately discard the used Reagent Tube, sipper, and Recovery Solution. Install a new sipper; do **not** let the sipper touch any surfaces. Invert the Recovery Solution bottle 10 times, then fill the Reagent Tube half-full with Recovery Solution.
 - *Between runs*, invert the bottle of Recovery Solution 10 times, then add more Recovery Solution to the solution in the tube until the Reagent Tube is half-full. Install the tube.

IMPORTANT! Use only the OneTouch™ Recovery Solution in the Ion OneTouch™ Template Kit. Do not use a different recovery solution from another kit.

5. Install the 2 OneTouch™ Recovery Tubes and the OneTouch™ Recovery Router, then close the centrifuge lid.
6. Empty the Waste Container.

Prepare and install the amplification solution

IMPORTANT! Life Technologies recommends preparing the amplification solution in a room dedicated to pre-PCR activities or a controlled pre-PCR hood.

Note: If you are troubleshooting the amplification process, dilute 1 µL of the *E. coli* DH10B Control Library [Ion Control Materials Kit (Part no. 4466465)] into 260 µL of Nuclease-Free Water.

1. Equilibrate the OneTouch™ Reagent Mix to 15°C to 30°C. Place the OneTouch™ Enzyme Mix, library, and the Ion Sphere™ Particles (ISPs) on ice. Ensure that no drops are on the lids of the tubes.

Note: After first use, store the Reagent Mix at 2°C to 8°C to avoid repeated freeze/thaw cycles.

2. In a 1.5-mL LoBind Tube, add the following components at room temperature in the designated order. *You add the ISPs in step 3 below. Before* adding each component, change tips, then pipet the component up and down to mix. Add each component, then pipet the amplification solution up and down to mix:

Order	Reagent	Cap color	Volume
1	Nuclease-Free Water	—	595 µL
2	OneTouch™ Reagent Mix	Violet	200 µL
3	OneTouch™ Enzyme Mix	Brown	100 µL
4	Diluted library (<i>not</i> stock library)	—	5.0 µL ¹
	Total	—	900 µL

3. Add ISPs. Vortex the ISPs at maximum speed for **1 minute** to resuspend the particles. Pipet the ISPs up and down to mix, then add the ISPs:

Order	Reagent	Cap color	Volume
1	Amplification solution <i>without</i> ISPs (from step 2 above)	—	900 µL
2	Ion Sphere™ Particles	Black	100 µL
	Total	—	1000 µL

IMPORTANT! Use only ISPs in the Ion OneTouch™ Template Kit with the Ion OneTouch™ System. Do *not* use ISPs from other or previously used kits.

- Pipet the complete amplification solution up and down to mix.
- Ensure that the amplification solution is at 15°C to 30°C.
- 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.
- Place the OneTouch™ Reaction Tube in a secure holder, then pipet the entire volume of vortexed amplification solution into the Reaction Tube.
- Using a new tip each time to prevent contamination of the Oil, gently layer and fill the tube with OneTouch™ Reaction Oil (from the small bottle) over the amplification solution. Do *not* invert the OneTouch™ Reaction Oil (small bottle). Do *not* mix the Oil and amplification solution. Minimize bubbles:



¹ The 5 µL aliquot is from a diluted library. The library was diluted according to the calculated template dilution factor to target 10–30% of positive ISPs.

- Secure a OneTouch™ Reaction filter to the Reaction Tube. Ensure that the Reaction Filter is firmly and evenly seated against the reaction tube:



- Immediately before installation on the Ion OneTouch™ Instrument, *slowly* invert the Reaction Filter and Reaction Tube assembly to this position. Do **not** mix the Oil and amplification solution.
- Firmly insert the 3 prongs of the inverted Reaction Filter into the three ports on the top stage of the Ion OneTouch™ Instrument.
- Push down on the Reaction Tube to confirm that it is firmly seated on the Reaction Filter.

Run the Ion OneTouch™ Instrument

Press the drop-down menu, then select **Assisted Run** or **Expert Run**. Press **Start Run**. Complete the listed tasks, then click **Next**. The run begins (run time is 4 hours). To cancel a run, press **Cancel**, then press **Yes**.

Note: Remove the samples ≤ 16 hours after starting the run.

Recover the Ion Sphere™ Particles

- After the run is complete and the centrifuge has stopped, press **Centrifuge Sample**.



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

IMPORTANT! If you accidentally press **Next** before pressing **Centrifuge Sample**, follow the steps below:

- Remove the 2 Recovery Tubes.
- Pipet the solution up and down in one Recovery Tube to resuspend the solution.
- Transfer the solution from *one* Recovery Tube to a new 1.5-mL LoBind Tube.
- Spin the LoBind Tube at $15,500 \times g$ for 3 minutes.
- Remove all but 50 μL of solution from the LoBind Tube.
- Pipet the solution up and down in the other Recovery Tube to resuspend the solution.
- Transfer the solution from the other Recovery Tube to the 1.5-mL LoBind Tube.
- Spin the LoBind Tube at $15,500 \times g$ for 3 minutes.
- Remove all but 100 μL of solution from the LoBind Tube.
- Clean the centrifuge lid of the Ion OneTouch™ Instrument.
- Remove and discard the Recovery Router.
- Carefully clean the injector tubing. Do **not** deflect it.
- Skip to [“Enrich the Ion Sphere™ Particles with the Ion OneTouch™ ES”](#) on page 4)

- Immediately* after the centrifuge has stopped, review the listed, displayed tasks, then remove and discard the Recovery Router.
- Carefully* remove the Recovery Tubes.
- Use the same pipette tip to remove all but 50 μL of OneTouch™ Recovery Solution from each Recovery Tube. Remove any white flocculent material. Do **not** disturb the pellet of ISPs.
- Use the same tip to pipet each pellet up and down until the pellet disperses in the solution. Retain the samples to assess quality and to enrich the ISPs (see [“Enrich the Ion Sphere™ Particles with the Ion OneTouch™ ES”](#) on page 4).

STOPPING POINT To store the recovered ISP, transfer the suspension from both tubes into a new 1.5-mL LoBind Tube. Add 1 mL of OneTouch™ Wash Solution to the ISPs, then store the ISPs at 2°C to 8°C for up to 3 days. After storage, proceed to [“Maintain the Ion OneTouch™ Instrument”](#) below. After storage, spin the templated ISPs at $15,500 \times g$ for 3 minutes. Remove all but 100 μL of solution from the tube. Proceed to [“Enrich the Ion Sphere™ Particles with the Ion OneTouch™ ES”](#) on page 4.

IMPORTANT! Do **not** store the recovered ISPs at -30°C to -10°C and do **not** store the ISPs in OneTouch™ Recovery Solution.

Maintain the Ion OneTouch™ Instrument

1. On the OneTouch™ Instrument display, press **Clean Instrument** on the drop-down menu, then press **Start Run**. To complete each of the displayed tasks, follow the steps below.
2. Remove and dispose of the OneTouch™ Amplification Plate.



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

Note: You may keep the OneTouch™ Reaction Tube-Reaction Filter assembly in the instrument until the next run.

3. Ensure that there is ≥ 3 mL of OneTouch™ Oil in the 50-mL conical tube, and ensure that there is ≥ 3 mL of OneTouch™ Recovery Solution in the Reagent Tube.
4. Lift the centrifuge lid, then *carefully* install an empty cap **threaded-side up** under the sample injector. Slowly lower the lid.

IMPORTANT! Do *not* let the sample injector touch any surfaces.

5. Load 3 mL of the Oil into the OneTouch™ Cleaning Apparatus syringe.
6. Insert the elbow of the Cleaning Apparatus into the *right* hole in the plate chamber, then close the clamp.
7. Inject the 3 mL of Oil in 6 seconds (0.5 mL/second) into the instrument.
8. Ensure that the task, "Finish cleaning (syringe may be removed)" displays. Click **Next**. Recovery solution flows through the instrument tubing for 2 minutes.
9. During the flow of Recovery Solution, open the clamp, then remove the Cleaning Apparatus from the instrument.
10. Open the centrifuge lid, then *carefully* remove the cap and appropriately dispose of the waste in the cap.
11. Wipe the residue from the centrifuge lid and sample injector. Inspect the sample injector, then close the lid.

Enrich the Ion Sphere™ Particles 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 page 9).
Routine use and residual volume in Well 1 and Well 8 is >5 μ L	
Routine use and residual volume in Well 1 and Well 8 is ≤ 5 μ L	Operate the instrument without performing the residual volume test. Proceed to " Prepare the reagents " below.

Prepare the reagents

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

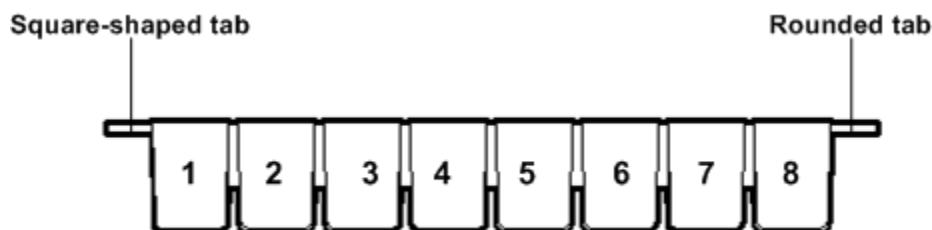
Order	Component	Volume
1	Nuclease-Free Water	865 μ L
2	1 M NaOH	125 μ L
3	10% Tween® 20 in Nuclease-Free Water	10.0 μ L
	Total	1.0 mL

IMPORTANT! Prepare Melt-Off Solution daily.

2. Wash and resuspend the Dynabeads® MyOne™ Streptavidin C1 magnetic beads (to fill Well 2, described below):
 - a. Add 130 µL of MyOne™ Beads Wash Solution to a new 1.5-mL LoBind Tube.
 - b. Vortex the bottle containing the MyOne™ beads.
 - c. Transfer 13 µL of MyOne™ beads to the tube containing the MyOne™ Beads Wash Solution, then pipet the solution up and down 10 times to mix.
 - d. Place the tube on a magnet such as a DynaMag™-2 magnet for 2 minutes, then remove and discard the supernatant.
 - e. Add 130 µL of MyOne™ Beads Wash Solution to the MyOne™ beads, then pipet up and down to resuspend the beads.

Note: You add the resuspended MyOne™ beads in the 130 µL MyOne™ Beads Wash Solution to Well 2 of the 8-well strip in step 7 below.
3. Ensure that the templated ISPs from the Ion OneTouch™ Instrument are in 50 µL of OneTouch™ Recovery Solution in each tube (see step 4 on page 3). If the volume of templated ISPs is <50 µL, add sufficient OneTouch™ Wash Solution to bring the volume to in each tube to 50 µL. If the templated ISPs were stored at 2°C to 8°C, centrifuge the ISPs at 15,500 × g for 3 minutes, carefully remove the supernatant, then add sufficient OneTouch™ Wash Solution to bring the volume to 100 µL.

Note: If the templated ISPs were transferred from the Recovery Tubes and pooled in a 1.5-mL LoBind Tube before spinning, ensure that the volume of templated ISPs in the 1.5-mL LoBind Tube is 100 µL. Pipet the ISPs up and down to resuspend in the 100 µL volume of Recovery Solution.
4. Obtain an 8-well strip from the Ion OneTouch™ Template Kit. Ensure that the square-shaped tab of the strip is on the left:



5. With a new tip and using the same tip for both tubes, transfer the entire volume (50 µL) of resuspended ISPs from each Recovery Tube directly into Well 1 of an 8-well strip well for a total volume of 100 µL. If the templated ISPs are in a single tube, transfer the entire volume (100 µL) of resuspended ISPs to Well 1.
6. (Optional) From Well 1, take a 2.0 µL aliquot to assess the quality of the ISPs by the Qubit® 2.0 Fluorometer or take a 10.0 µL aliquot to assess quality by sequencing (refer to the *Ion OneTouch™ Template Kit User Guide*).
7. Fill the remaining wells as follows, then immediately proceed to the next step:

Well number	Reagent to dispense in well
Well 1 (well closest to the square-shaped tab)	Entire ISP sample (100 µL; see step 3 above)
Well 2	130 µL of MyOne™ Beads resuspended in MyOne™ Beads Wash Solution (prepared in step 2 above)
Well 3	300 µL of Ion OneTouch™ Wash Solution
Well 4	300 µL of Ion OneTouch™ Wash Solution
Well 5	300 µL of Ion OneTouch™ Wash Solution
Well 6	Empty
Well 7	300 µL of freshly-prepared Melt-Off solution
Well 8	Empty

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

Install 0.2-mL PCR tube in hole of tip holder

Install 8-well strip with square-shaped tab on the left and strip pushed to the right end of the slot



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.

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.
2. *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 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 the Ion OneTouch™ ES ON 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.

Note: At the end of the run, Well 1 contains only residual MyOne™ beads ($\leq 2 \mu\text{L}$). Well 2 also contains a visible amount of MyOne™ beads. Wells 2-5 and 7 contain waste or residual reagents. Excess reagents are used to avoid aspirating air during the run.
6. *Immediately after the run*, close and remove the PCR tube containing the enriched ISPs. Close and remove the tube.

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.

7. Proceed immediately to “**Remove and wash the enriched ISPs**” below.

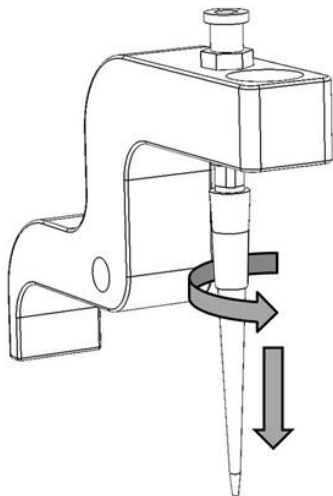
Note: Ensure that the 0.2-mL PCR tube has $\geq 200 \mu\text{L}$ of solution containing the enriched ISPs. If the tube has $\ll 200 \mu\text{L}$ of solution, check Well 8 for ISPs. If there are ISPs in Well 8, transfer the ISPs from Well 8 to the PCR tube, then re-calibrate the Ion OneTouch™ ES and perform a residual volume test (refer to “Perform a residual volume test” in the *Ion OneTouch™ Template Kit User Guide*).

Remove and wash the enriched ISPs

1. Spin the enriched ISPs at 15,500 x g for 1.5 minutes.
2. Ensure that there are no MyOne™ Beads with the ISPs (a brown-tinted pellet). If there are MyOne™ Beads with the ISPs, place the 0.2-mL PCR tube containing the MyOne™ Beads and ISPs against a magnet such as a DynaMag™-2 magnet for 4 minutes, then transfer the ISPs to a new tube without disturbing the pellet of MyOne™ Beads.
3. Wash the enriched ISPs in OneTouch™ Wash Solution once:
 - a. Remove all but $\sim 10 \mu\text{L}$ of supernatant without disturbing the pellet, then add 200 μL of OneTouch™ Wash Solution. Pipet up and down 10 times to resuspend the pellet (pellet may be difficult to see).
 - b. Spin the enriched ISP tube at 15,500 x g for 1.5 minutes.
 - c. Remove all but $\sim 10 \mu\text{L}$ of supernatant without disturbing the pellet.
 - d. Add sufficient OneTouch™ Wash Solution for a final volume of 100 μL . Pipet up and down 10 times to resuspend the pellet.
4. Sequence the library or store the washed ISPs:

Next action	Then...
Sequencing	Proceed immediately to the instructions listed in the <i>Ion Sequencing Kit User Guide v2.0</i> (Part no. 4469714).
Storage	STOPPING POINT Store the material at 2°C to 8°C for up to 3 days.

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.

Perform Ion Sphere™ Particles quality control

You can determine the appropriate library dilution and/or the enrichment efficiency using *one* of the methods below. See the following sections for details:

- “Quality control of Ion Sphere™ Particles using a Qubit® 2.0 Fluorometer” (refer to the *Ion OneTouch™ Template Kit User Guide*).
- “Determine recovery and percentage unenriched template-positive Ion Sphere™ Particles (ISPs) by sequencing” (refer to the *Ion OneTouch™ Template Kit User Guide*).
- *Ion Sphere™ Particles Quality Assessment Using the Guava® EasyCyte™ 5 Flow Cytometer User Bulletin* (Part no. 4470082). To access this protocol, visit the Ion Community website (ioncommunity.iontorrent.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 OneTouch™ Template Kit User Guide*).
2. Install a tip on the Tip Arm.
3. Remove an 8-well strip from the frame.
4. 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 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 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 below.

5. 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 the instrument ON, 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 OneTouch™ Wash Solution from the well, then estimate the residual volume.
6. 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 7).

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

7. Remove and discard the used 8-well strip.
8. 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	—	Operate the instrument. Proceed to " Prepare the reagents " on page 4.
Residual volume in Well 2 is > 5 µL IMPORTANT! The volume is measured from the <i>bottom</i> of the well, not from the sides.	The tip height is too high during aspiration.	Decrease the ASP (aspiration) height ~5 steps to lower the tip (refer to "Perform vertical calibration" in the <i>Ion OneTouch™ Template Kit User Guide</i>). Re-run the residual volume test.
Aspiration is irregular	Instrument out of calibration	Perform vertical calibration (refer to "Perform vertical calibration" in the <i>Ion OneTouch™ Template Kit User Guide</i>).
The 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, then try increasing the ASP (aspiration) height by ~5 steps to raise the tip (refer to "Perform vertical calibration" in the <i>Ion OneTouch™ Template Kit User Guide</i>).

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