

Ion 540™ Kit – OT2

Catalog Number A27753

Pub. No. MAN0010852 Rev. D.0

Note: For safety and biohazard guidelines, see the “Safety” appendix in the *Ion 540™ Kit – OT2 User Guide* (Pub. No. MAN0010850). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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Create a Planned Run

1. Open Torrent Browser on a computer connected to your Ion S5™ or Ion S5™ XL Sequencer.
2. Select the **Plan** tab, select **Templates**, find the application you want to run (for example, **AmpliSeq DNA**), then select:
 - **Upload Plans** to upload a .csv format file containing your run information.
 - **Plan New Run** to plan a new run using the generic template for the selected application.
 - **Add New Template** to create a new Planned Run template that you can reuse for the selected application.
 - Other options that may be available depending on your selected application, such as downloading designs from AmpliSeq.com.
3. In the wizard, review each screen and edit as needed.
4. After you complete your selections, click **Plan Run** to save your selections. The run is listed on the Planned Runs page under the name you specified.

Run the Ion OneTouch™ 2 Instrument

IMPORTANT! Ensure that the latest firmware is installed. To update the firmware to the current version, see the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388).

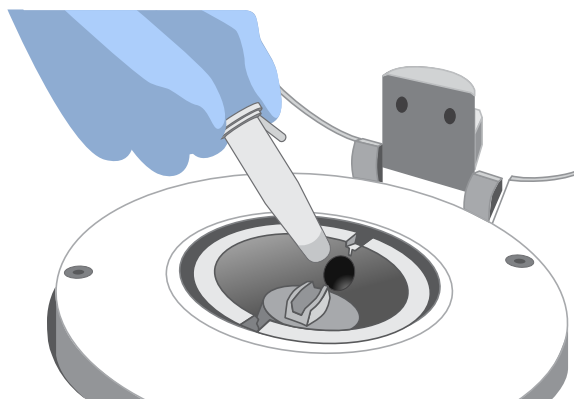
1. Close the centrifuge lid, then touch **Run** on the home screen.
2. Touch the dropdown menu, then select **Proton: Ion 540™ Kit – OT2**, then touch **Next**.
3. Touch **Assisted**, then complete the setup tasks listed onscreen, or touch **Expert**, then set up the instrument according to the following instructions.

Set up the Ion OneTouch™ 2 Instrument

- If this is the first use of the instrument, perform the one-time initialization procedure (refer to the *Ion 540™ Kit – OT2 User Guide*, Pub. No. MAN0010850) at any time before the first run.
- If this is the first run after initialization, proceed to "Prepare and install the amplification solution". The instrument consumables are already installed and ready for the run.

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


1. Dispense 150 µL Ion OneTouch™ Breaking Solution into each of two Ion OneTouch™ Recovery Tubes.
2. Insert the Recovery Tubes containing Ion OneTouch™ Breaking Solution into the two centrifuge positions.





3. Install the Ion OneTouch™ Recovery Router into the center slot of the centrifuge.
4. Close the centrifuge lid.

Install the Ion OneTouch™ 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 OneTouch™ 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

1. Pull the external tubing from the port of the Waste Container.
2. Empty the Waste Container into the appropriate receptacle.
3. Reinstall the empty Waste Container.

Prepare the amplification solution

IMPORTANT! Use *only* the Ion 540™ Kit – OT2 with this user guide and with the Ion OneTouch™ 2 Instrument. Do not use the kits with the Ion OneTouch™ System.

1. Prepare the reagents as follows:

Reagents	Preparation
Ion S5™ Reagent Mix	<ol style="list-style-type: none"> 1. Allow the tube of Ion S5™ Reagent Mix (2 mL) to come to room temperature before use. 2. Vortex the solution for 30 seconds, then centrifuge the solution for 2 seconds. 3. Keep the tube of Ion S5™ Reagent Mix at room temperature during use. Store thawed Ion S5™ Reagent Mix at 2°C to 8°C.
Ion S5™ Enzyme Mix	<ol style="list-style-type: none"> 1. Flick the tube with your finger 4 times to mix. 2. Centrifuge the enzyme for 2 seconds. 3. Place on ice.
Ion Sphere™ Particles	Place the suspension at room temperature.

2. Dilute 6-8 µL of your 100 pM library with Nuclease-free Water in a 1.5-mL Eppendorf LoBind™ Tube to give a total volume of 100 µL. Vortex the diluted library for 5 seconds, centrifuge for 2 seconds, then place the diluted library on ice.
3. Vortex the ISPs at maximum speed for 1 minute, centrifuge for 2 seconds, pipet the ISPs up and down to mix; then *immediately* proceed to the next step.
4. To the tube containing 2 mL of Ion S5™ Reagent Mix 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	—	80 µL
2	Ion S5™ Enzyme Mix	Brown	120 µL
3	Ion Sphere™ Particles	Black	100 µL
4	Diluted library (<i>not</i> stock library)	—	100 µL
—	Total volume (including Master Mix)	—	2400 µL

5. Proceed *immediately* to the next section.

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

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

IMPORTANT! We recommend filling the Ion OneTouch™ Reaction Filter in a room dedicated to pre-PCR activities or in a controlled pre-PCR hood. Do *not* use a reaction filter assembly from any other template preparation 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 OneTouch™ Reaction Filter.
4. Pipet 200 µL of Ion OneTouch™ Reaction Oil through the sample port.
5. Invert then install the filled Ion OneTouch™ Reaction Filter into the three holes on the top stage of the Ion OneTouch™ 2 Instrument.

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**, touch **Final Spin**, then follow any screen prompts to centrifuge the sample.



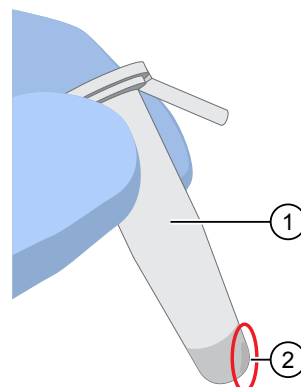
CAUTION! ROTATION HAZARD. Wait until rotation stops before opening. Rotating parts can cause injury.

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

IMPORTANT! Do not store the recovered, template-positive 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. Use a pipette to remove all but ~100 µL of Ion OneTouch™ Recovery Solution from each Ion OneTouch™ 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:



- ① Ion OneTouch™ Recovery Tube
② Location of ISP pellet

2. Resuspend the template-positive ISPs in the remaining Recovery Solution in each tube by pipetting the suspension up and down.
3. Combine the suspension from each Recovery Tube into a new labeled 1.5-mL Eppendorf LoBind™ Tube.
4. Add 100 µL of Nuclease-free Water to each Recovery Tube, pipet up and down to mix, then transfer the residual beads into the labeled 1.5-mL tube.
5. Bring the combined suspensions in the labeled 1.5-mL tube to 1 mL with Nuclease-free Water.

STOPPING POINT The ISPs can be stored at 2°C to 8°C for up to 3 days. If you stored the template-positive ISPs at 2°C to 8°C, proceed to step 8. Do not store the recovered ISPs in Ion OneTouch™ Recovery Solution.

6. Vortex for 30 seconds to completely resuspend the template-positive ISPs, then centrifuge the tube for 2 seconds.
7. (Optional) Assess the quality of the unenriched, template-positive ISPs using the Guava™ easyCyte™ 5 Flow Cytometer. Transfer a 2.0-µL aliquot of the diluted, unenriched ISPs (from step 6) to a 1.5-mL Eppendorf LoBind™ Tube. See the *Ion PI™ Ion Sphere™ Particles (ISPs) Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. No. MAN0007496).
8. Centrifuge the template-positive ISP suspension for 8 minutes at 15,500 × g.
9. Remove all but 20 µL of supernatant.
10. Bring the combined washed suspensions in the new labeled tube to 100 µL in ISP Resuspension Solution.
11. Vortex the pellet for 30 seconds to completely resuspend the template-positive ISPs, then centrifuge the tube for 2 seconds.

12. (Optional) If you have not used the Guava™ easyCyte™ 5 Flow Cytometer to perform quality control on the ISPs, you can retain a sample at this point to evaluate quality of the resuspended, unenriched template-positive ISPs (step 11) using the Qubit™ 2.0 Fluorometer or Qubit™ 3.0 Fluorometer. Transfer a 2.0-µL aliquot of the resuspended, unenriched ISPs to a 0.2-mL PCR tube, then see the

- *Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide* (Pub. No. MAN0016387), or
- *Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide* (Pub. No. MAN0016388),

available at thermofisher.com/order/catalog/product/4468656, for further details.

Enrich the template-positive ISPs with the Ion OneTouch™ ES". You may start the enrichment procedure while the Ion OneTouch™ 2 Instrument cleaning is in progress.

IMPORTANT! Do not store the recovered, template-positive ISPs at -30°C to -10°C. Do not store the recovered ISPs in Ion OneTouch™ Recovery Solution.

Maintain the Ion OneTouch™ 2 Instrument

IMPORTANT! To ensure continued safe operation, visually inspect the rotor assembly and casing periodically to ensure that 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™ 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	Action
Switching to the Ion 540™ Kit – OT2 from another kit	See Chapter 5 of the <i>Ion OneTouch™ 2 System User Guide</i> (Pub. No. MAN0014388). Use the reagents from the appropriate kit to maintain the Ion OneTouch™ 2 Instrument.
Already using the Ion 540™ Kit – OT2	Proceed to step 2. Continue to use the reagents provided in the Ion 540™ Kit – OT2.

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 OneTouch™ Reaction Filter. 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 next to the instrument.
5. Firmly insert the 3 ports of the single-use Ion OneTouch™ 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, then place the injector into the empty 50-mL conical tube next 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 starts.
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 discard the waste in the 50-mL conical tube.
10. Remove and appropriately discard the used Ion OneTouch™ Amplification Plate, disposable injector, and tubing from the instrument.



CAUTION! Hot Surface. Use care when working near this area to avoid injury from contact with 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 Instrument

Determine if a residual volume test is necessary

If the condition is	Then
First use of the instrument and during monthly maintenance	Perform a residual volume test. Refer to "Ion OneTouch™ ES Instrument installation, setup, and maintenance" in the <i>Ion OneTouch™ 2 System User Guide</i> (Pub. No. MAN0014388).
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

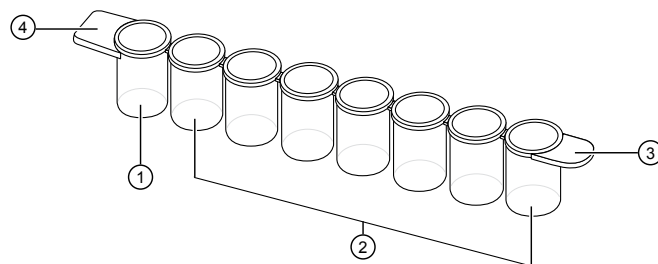
IMPORTANT! Prepare Melt-Off Solution as needed, but appropriately dispose of the solution after 1 day.

Wash and resuspend the Dynabeads™ MyOne™ Streptavidin C1 Beads

1. Vortex the tube 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 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 MyOne™ Beads Capture Solution to Well 2 of 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 ISP Resuspension Solution. If the template-positive ISPs were stored at 2°C to 8°C, follow the washing procedure for ISPs (see “Wash the template-positive ISPs” on page 3).
2. Ensure that the square-shaped tab of the 8-well strip is on the left:



- ① Well 1
- ② Wells 2–8
- ③ Rounded tab
- ④ Square-shaped tab

3. Pipet the ISPs up and down 10 times to mix, then transfer the entire volume (100 µL) of resuspended ISPs in ISP Resuspension Solution (step 1 of this procedure) into Well 1 of the 8-well strip.
4. Fill the remaining wells in the 8-well strip as follows:

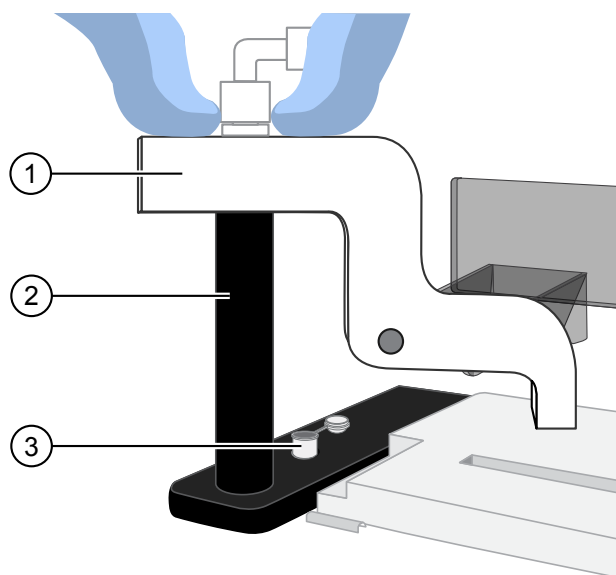
Well number	Reagent to dispense in well
Well 1 ^[1]	Entire template-positive ISP sample (100 µL; prepared in step 1 of this procedure)
Well 2	130 µL of Dynabeads™ MyOne™ Streptavidin C1 Beads resuspended in MyOne™ Beads Capture Solution (prepared in “Wash and resuspend the Dynabeads™ MyOne™ Streptavidin C1 Beads” on page 5)
Well 3	300 µL of Ion OneTouch™ ES Wash Solution
Well 4	300 µL of Ion OneTouch™ ES Wash Solution
Well 5	300 µL of Ion OneTouch™ ES Wash Solution
Well 6	Empty
Well 7	300 µL of freshly-prepared Melt-Off Solution [prepared in “Prepare Melt-Off Solution” on page 5]
Well 8	Empty

^[1] Well closest to the square-shaped tab

5. 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 Ion OneTouch™ ES Instrument tray.

Prepare the Ion OneTouch™ ES Instrument

1. Place a new tip in the Tip Loader.
2. Install a new tip by pressing the Tip Arm down onto the tip in the Tip Loader.



- ① Tip Arm
- ② Tip Loader
- ③ 0.2-mL PCR collection tube

3. Lift the Tip Arm straight up to pull the installed tip from the Tip Loader tube.
4. Return the Tip Arm to the cradle.

IMPORTANT! 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.

Perform the run

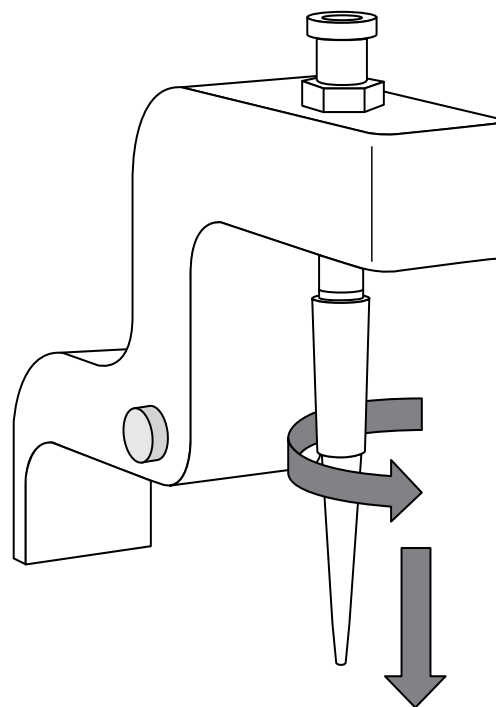
Before starting the run:

- Confirm that a new tip and open 0.2-mL PCR tube have been loaded in the Ion OneTouch™ ES Instrument, and that the 8-well strip is correctly loaded.
 - Ensure that Well 1 (ISP sample) is the left-most well and the 8-well strip is pushed to the right-most position in the slot.
1. Pipet the contents of Well 2 up and down to resuspend the beads before starting the run. Do not introduce bubbles into the solution.
 2. If needed, power on the Ion OneTouch™ ES Instrument, then 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).
 3. Press **Start/Stop**. The screen displays "run" during the run. The run takes ~35 minutes.
 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, then reset the Ion OneTouch™ ES Instrument for the next run. The instrument can be left on between runs.

5. Immediately after the run, securely close, 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. After a successful run on the instrument, the sample is in ~220 µL of Melt-Off Solution and Ion OneTouch™ Wash Solution. If the tube has <<200 µL of solution containing the enriched ISPs, contact Technical Support.

6. Remove the used tip: with the Tip Arm in its cradle, twist the tip counterclockwise (as viewed from above), then 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.

7. Remove, then discard the used 8-well strip.

Proceed immediately to "Remove and wash the enriched ISPs".

Remove and wash the enriched ISPs

1. Centrifuge the 0.2-mL PCR tube containing the enriched ISPs at 15,500 × 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 can be difficult to see.
4. Centrifuge 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:

Are Dynabeads™ MyOne™ Streptavidin C1 Beads (brown pellet) present?	Action
No	<ol style="list-style-type: none"> 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.
Yes	<ol style="list-style-type: none"> 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 Magnetic 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.

(Optional) Perform enriched 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. For more information, see the *Ion PI™ Ion Sphere™ Particles Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. No. MAN0007496), available at thermofisher.com.

When a manual cleaning of the sequencer is required

The Ion S5™ Sequencer and Ion S5™ XL Sequencer require that a cleaning be performed before initialization. This is normally performed automatically at the completion of the previous sequencing run. However, if the "Enable post-run clean" checkbox is unchecked to allow a second run, and a second run is not performed, the instrument will not allow the subsequent initialization to proceed until a manual cleaning has been performed. For more information on how to perform a manual cleaning, see the *Ion 540™ Kit – OT2 User Guide* (Pub. No. MAN0010850).

If an Ion S5™ Sequencer or an Ion S5™ XL Sequencer is initialized and a sequencing run is not started within 24 hours, or a run is not started or completed due to a power failure or an abort, do not perform a manual cleaning. An instrument reset run is required before reinitialization. For more information on how to perform an instrument reset run, see the *Ion 540™ Kit – OT2 User Guide*.

Initialize the sequencer

1. In the instrument touchscreen main menu, touch **Initialize**. The door, chip, and Reagent cartridge clamps unlock.
2. When prompted, remove the Ion S5™ Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.
3. Reinstall the empty waste reservoir.
4. Replace the expended Ion S5™ Sequencing Reagents cartridge with a new cartridge equilibrated to room temperature.
5. Invert a new Ion S5™ Wash Solution bottle 5 times and swirl at an angle to mix thoroughly. Then remove the red cap and install.
6. Ensure that the used sequencing chip from the previous run is properly seated in the chip clamp and the chip clamp is pushed in all the way.
7. If necessary, install a new Ion S5™ Cleaning Solution bottle.
8. Close the door, then touch **Next**.
9. When initialization is complete (~30–40 minutes), touch **Home**.

The instrument is now ready for a sequencing run.

Prepare the ISPs for sequencing and load the chip

While the sequencer is initializing, prepare the ISPs for sequencing and load the chip.

Add Control Ion Sphere™ Particles to the enriched ISPs

IMPORTANT! Ensure that you use the correct Control Ion Sphere™ Particles for the chip type being used.

1. Vortex the Control Ion Sphere™ Particles for 5 seconds, then centrifuge for 2 seconds before taking aliquots.
2. Add 5 µL of Control Ion Sphere™ Particles directly to the entire volume of enriched, template-positive ISPs in a 0.2-mL PCR tube (non-polystyrene), then pipet up and down to mix.
9. Place the chip back in the minifuge with the chip notch pointing out, and centrifuge for 30 seconds.
10. Place the chip on a stable surface such as a benchtop. Remove the liquid that has accumulated in both of the chip wells.

Anneal Sequencing Primer to the enriched ISPs

1. Centrifuge the enriched, template-positive ISPs for 5 minutes at 15,500 × g.
2. Carefully remove the supernatant without disturbing the pellet, leaving 10 µL of supernatant in the tube (visually compare to 10 µL of liquid in a separate tube).
3. Add 15 µL of Ion S5™ Annealing Buffer for a total volume of 25 µL.
4. Add 20 µL of Ion S5™ Sequencing Primer, then confirm that the total volume is 45 µL. Add Ion S5™ Annealing Buffer if needed to bring the total volume to 45 µL.
5. Briefly vortex to mix, then centrifuge briefly to collect the contents at the bottom of the tube.
6. Program a thermal cycler for 95°C for 2 minutes and then 37°C for 2 minutes, using the heated lid option.
7. Place the tube in the thermal cycler, then run the program.
8. After cycling, add 10 µL of Ion S5™ Loading Buffer, briefly vortex to mix, then centrifuge briefly to collect the contents at the bottom of the tube.

Load the sample on the chip

1. Place the Ion 540™ Chip on a flat, stable surface.
2. Dispense the entire prepared sample (55 µL) into the chip loading well (not the chip loading port) of the chip.
3. Transfer the chip to a bucket in the Ion Chip™ Minifuge with the chip notch pointing **out**, away from the center of the minifuge. Place a used chip in the opposite bucket with the chip notch also pointing out.
4. Centrifuge for 10 minutes.
5. In a 1.5-mL tube, combine 49 µL of 50% Annealing Buffer with 1 µL of Foaming Solution (10% Triton™ X-100 solution).
6. Create foam by injecting air into the 50-µL mixture from the previous step using a Rainin™ SR-L200F pipette set to dispense 100 µL. Next, break the large bubbles into smaller bubbles by rapidly pipetting for ~5 seconds. Repeat this step one more time.
7. Place the chip on a stable surface such as a benchtop, then inject 100 µL of foam into the chip loading port. Remove the expelled liquid from the opposite port.
8. Dispense 55 µL of 50% Annealing buffer into the chip loading well (not the chip loading port).

11. Briefly "re-foam" the foam sample by pipetting rapidly for ~5 seconds, then inject 100 µL of foam into the chip loading port. Remove the expelled liquid from the opposite port.
12. Dispense 55 µL of 50% Annealing buffer into the chip loading well (not the chip loading port).
13. Place the chip back in the minifuge with the chip notch pointing out, and centrifuge for 30 seconds. Then proceed to flushing the chip.

Flush the chip and load the Ion S5™ Sequencing Polymerase

1. Inject 100 µL of the Flushing solution into the chip loading port 2 times. After each injection, discard the solution that is expelled from the opposite port.
2. Inject 100 µL of 50% Annealing Buffer into the chip loading port 3 times. Do not introduce air bubbles. After each injection, remove the expelled liquid from the opposite port.
3. Combine 6 µL of Ion S5™ Sequencing Polymerase with 60 µL of 50% Annealing buffer.
4. Inject 65 µL of the polymerase solution into the chip loading port and remove the expelled liquid from the exit port. Be careful to avoid introducing air bubbles.
5. Allow the chip to incubate for 5 minutes, then immediately proceed to the next steps.

Start the sequencing run

We recommend that you sequence loaded chips on the Ion S5™ or Ion S5™ XL Sequencer as soon as possible after chip loading and instrument initialization are complete. However, successful sequencing runs can be started up to 24 hours after instrument initialization.

Note: Do *not* press the power button during a run. Interrupting power to the instrument during a run may result in sequencing run failure and loss of sample.

1. After completion of initialization, press **Run** in the instrument touchscreen. The door and chip clamp unlock.
2. Remove the used sequencing chip, then secure a chip loaded with template-positive Ion Sphere™ Particles in the chip clamp.
3. Push the chip clamp all the way in to engage, close the instrument door, then press **Next**.
4. In the dropdown list, select the Planned Run that you created in the Torrent Suite™ Software, then press **Review**.

Note: You can also select **Planned Run (none)**, then enter your run information on the following screen. We recommend selecting a predefined Planned Run.

5. (Optional) If this is to be the first of two sequencing runs on this initialization, **deselect** the **Enable post-run clean** checkbox, then press **Review**.

Note:

- Failure to deselect the checkbox results in a cleaning performed automatically after the first run. A second run is not available.
- When starting the second sequencing run on a single initialization, ensure the **Enable post-run clean** checkbox is selected so that the post-run cleaning is performed automatically.

6. Confirm the pre-populated settings are correct, or make changes using the buttons and dropdown lists if necessary.
7. Confirm that the instrument door is closed, then press **Start run** to begin the sequencing run.

IMPORTANT! During a run, do not open the instrument door, and avoid touching the instrument. Touching the instrument during the sequencing run may reduce the quality of the measurements.

When the sequencing run is complete, the instrument automatically performs the cleaning procedure unless the

Enable post-run clean checkbox was deselected. After cleaning, the touchscreen returns to the main menu. Use the Torrent Browser to review the results.

Limited product warranty

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Revision history: Pub. No. MAN0010852

Revision	Date	Description
D.0	21 January 2017	<ul style="list-style-type: none"> • Users referred to new ISP quality control user guides: <ul style="list-style-type: none"> - <i>Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide</i> (Pub. No. MAN0016387) - <i>Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide</i> (Pub. No. MAN0016388) • Ion OneTouch™ ES Instrument setup clarified • Web links updated
C.0	27 July 2016	Updated for Torrent Suite™ Software v5.2
B.0	16 November 2015	Update to Ion S5™ Sequencing Reagents Kit (Cat. No. A27768) shipping and storage conditions
A.0	27 August 2015	New quick reference

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