

Ion 520™ & Ion 530™ Kit – OT2

Catalog Number A27751

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Note: For safety and biohazard guidelines, see the “Safety” appendix in the *Ion 520™ & Ion 530™ Kit – OT2 User Guide* (Pub. No. MAN0010844). 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

IMPORTANT! If you are using the Ion 520™ & Ion 530™ Kit – OT2 with libraries that were prepared using Oncomine™ panels, see the corresponding Oncomine™ Assay User Guide for assay-specific instructions on creating a Planned Run in Torrent Suite™ Software.

For more information on creating a Planned Run in Torrent Suite™ Software, including a complete description of each field in the **Create Plan** workflow bar, see the *Torrent Suite™ Software Help*, available by clicking the **Help** button in the software.

1. Sign in to the Torrent Suite™ Software.
2. In the **Plan** tab, in the **Templates** screen, select the application that you want to run (such as AmpliSeq™ DNA) from the left navigation menu, then click either **Plan New Run** or **⚙ (Actions) ▶ Plan Run** in the row of the specific Planned Run template.
3. In the **Create Plan** workflow bar, review the **IonReporter** and **Research Application** steps, then make selections appropriate to your run. Click **Next**.
4. In the **Kits** step, make the following selections.
 - a. Select **Ion GeneStudio™ S5 System** from the **Instrument** dropdown list.
 - b. Select the appropriate chip type from the **Chip Type** dropdown list.
 - c. Select the library kit used to prepare your libraries from the **Library Kit Type** dropdown list.
 - d. (Optional) For barcoded libraries, select the barcode set used during library preparation from the **Barcode Set** dropdown list.
 - e. Select **OneTouch** for **Template Kit**, then select **Ion 520 & Ion 530 Kit - OT2** from the **Template Kit** dropdown list.
 - f. Select **Ion S5 Sequencing Kit** from the **Sequencing Kit** dropdown list.
 - g. Enter the appropriate number of flows in the **Flows** field.
 - h. Select or edit the remaining optional information fields appropriately for your run.
 - i. Click **Next**.
5. Review the **Plugins** and **Projects** steps, then make selections appropriate to your run.
6. In the **Plan** step, enter or make the following selections.
 - a. Enter a Run Plan Name, then select Reference and BED files appropriate to your run.
 - b. Enter the number of barcodes you are using in your combined library in the **Number of barcodes** field, then click **✓** to the right of this field. Edit the auto-populated list of barcodes that appears, if needed.
 - c. Scan or enter the chip barcode into the **Chip Barcode** field.

- d. Enter a sample name for each barcode in the appropriate **Sample Name (required)** fields.

7. After you have completed your selections, click **Plan Run** at the bottom right of the **Plan** step screen to save the run.

The run is listed in the **Planned Runs** screen under the name that you specified. You can select the appropriate Planned Run when you are setting up the run on an Ion S5™ Sequencer, Ion S5™ XL Sequencer, or Ion GeneStudio™ S5 Series Sequencer.

Select the Ion OneTouch™ protocol

Note: 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. Ensure that the centrifuge lid of the Ion OneTouch™ 2 Instrument is closed.
2. On the home screen, tap **Run**.
3. Tap the **Protocol** dropdown list, then select the appropriate sequencing length protocol (for example, **Ion S5™ : Ion 520™ & Ion 530™ Kit – OT2 200 bp**).
4. Tap **Next**.
5. Tap **Assisted**, then complete the setup tasks listed on screen, or tap **Expert**, then set up the instrument as described in “Set up the Ion OneTouch™ 2 Instrument”.

Set up the Ion OneTouch™ 2 Instrument

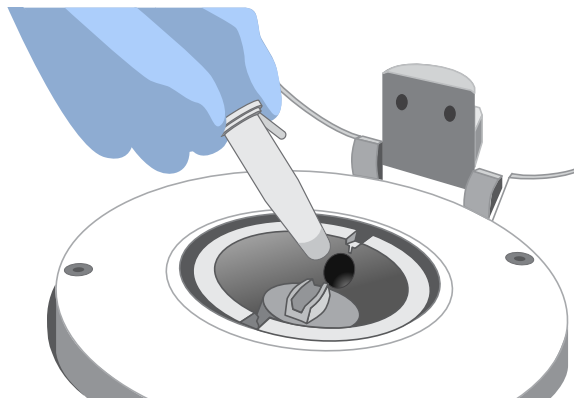
Note:

- If this is the first use of the instrument, perform the one-time initialization procedure at any time before the first run. For more information, see the *Ion 520™ & Ion 530™ Kit – OT2 User Guide* (Pub. No. MAN0010844).
- To set up the Ion OneTouch™ 2 System when switching between sequencing platforms, see the procedure in Chapter 5 of the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388). Cleaning and initialization using Ion OneTouch™ Recovery Solution and Ion OneTouch™ Oil from the new kit are required.
- If this is the first run after instrument initialization, proceed to “Prepare the amplification solution” on page 3. The instrument consumables are already installed and ready for the run.

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

1. On the instrument display, tap **Open Lid**, wait until the lid clicks open, then lift and hold the side of the centrifuge lid.
2. Dispense 150 µL Ion OneTouch™ Breaking Solution into each of the two Recovery Tubes.

3. Insert the Recovery Tubes containing Breaking Solution into the two centrifuge positions.



4. Install the Recovery Router into the center slot of the centrifuge.
5. Close the centrifuge lid.

Install the Ion OneTouch™ Amplification Plate


1. If there is a used Ion OneTouch™ Cleaning Adapter on the instrument, remove and appropriately discard it.
2. Push the handle back to open the heat block.



CAUTION! Hot Surface. Use care when working near 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.

3. Insert the Amplification Plate.
 - a. Inspect the Amplification Plate to ensure that the plate port is straight and perpendicular to the plate.
 - b. Hold the disposable injector, which is connected to the disposable tubing, in one hand and the Amplification Plate in the other hand.
- 

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.
- c. Insert the Amplification Plate into the heat block so that the single plate port aligns with the *left* hole of the Ion OneTouch™ 2 Instrument.
 4. Pull the heat block handle forward to close the block, then thread the disposable tubing through the Ion OneTouch™ DL Tubing Catch.
 5. Install the disposable tubing in the pinch valve.
 - a. Align the disposable tubing with the slot that runs along the bottom of the pinch valve.

- b. Gently pull the disposable tubing upward on both sides of the pinch valve until the disposable tubing is in the slot and fastened in the round notch on each side of the pinch valve.
- c. If needed, adjust the disposable tubing along the notches of the open pinch valve so that there is sufficient length of disposable tubing to install the disposable injector (see "Install 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.

Note: You should hear a click.



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

Install the Ion OneTouch™ Oil

Fill the appropriate Ion OneTouch™ Reagent Tube with Ion OneTouch™ Oil on the left front port .

1. Invert Oil bottle (450-mL size) 3 times to mix.
2. Fill the Reagent Tube *half*-full with Oil. Minimize bubbles.
3. Insert the filled Reagent Tube into the *left* front port, then screw the Reagent Tube clockwise until it is fastened firmly into place.

Install the Ion OneTouch™ Recovery Solution

IMPORTANT! Use only the Ion OneTouch™ Recovery Solution provided as part of the Ion 520™ & Ion 530™ Kit – OT2 for the Ion OneTouch™ 2 Instrument. Do *not* use a different recovery solution from another kit.

Fill the appropriate Ion OneTouch™ Reagent Tube with Ion OneTouch™ Recovery Solution on the right front port .

1. Invert the bottle of Recovery Solution 3 times.
2. Add more Recovery Solution to the solution in the Reagent Tube until the tube is *one-third*-full. Minimize bubbles.
3. Insert the filled Reagent Tube into the *right* front port, then screw the Reagent Tube firmly into place, one-quarter turn on the instrument.

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 520™ & Ion 530™ 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 described in the following table.

Reagents	Preparation
Ion S5™ Reagent Mix	<ol style="list-style-type: none"> 1. Allow the tube of 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 Reagent Mix at room temperature during use. Store thawed 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, 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 Reagent Mix)	—	2,400 µL

IMPORTANT! If you are performing a *de novo* sequencing experiment that does not include a reference BAM file, add 4 µL of the Ion S5™ Calibration Standard to the amplification solution and reduce the volume of nuclease-free water to 76 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 the reaction filter assembly

IMPORTANT! We recommend filling the Ion OneTouch™ Reaction Filter in a room that is 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. Place the Ion OneTouch™ Reaction Filter into a tube rack so that the 3 ports of the Ion OneTouch™ Reaction Filter face *up*.
2. Identify the sample port on the Ion OneTouch™ Reaction Filter.
Note: The short tubing in the Reaction Tube is connected to the sample port.
3. Add 800 µL of the amplification solution through the sample port.
4. Repeat step 3 two more times (for a total of 3 times) to load the entire amplification solution volume into the Ion OneTouch™ Reaction Filter.
5. Pipet 200 µL of Ion OneTouch™ Reaction Oil through the sample port.

Install the filled Ion OneTouch™ Reaction Filter

1. Position the tube rack containing the Ion OneTouch™ Reaction Filter so that the sample port on the filter is on the left.
2. Lift the Ion OneTouch™ Reaction Filter straight out of the tube rack. With the sample port still on the left, rotate the filter assembly clockwise until the Reaction Tube is inverted and the three ports point down.
3. Wipe the three holes on the top stage of the Ion OneTouch™ 2 Instrument with a Kimwipes™ disposable wipe to remove any oil or residue from the previous run.
4. Install the inverted Ion OneTouch™ Reaction Filter by inserting the three ports into the three holes on the top stage of the Ion OneTouch™ 2 Instrument.

Start the run

1. After installing the Ion OneTouch™ Reaction Filter, tap **Next** to start the run.
2. Remove the samples ≤16 hours after starting the run. If you tapped **Next** on the centrifuge screen to centrifuge samples at the end of the run, proceed *immediately* to “Recover the template-positive ISPs”.

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 centrifuged the sample or have not processed the sample after 15 minutes, centrifuge the sample on the instrument.
 - a. On the home screen of the instrument, tap **Open Lid**, wait until the lid clicks open, then insert the two filled Ion OneTouch™ Recovery Tubes from the run into the centrifuge rotor. Close the lid until it locks.
 - b. Tap **Options ▶ Final Spin**, then follow the screen prompts until the centrifugation starts.
 - c. When the centrifuge stops, *immediately* proceed to step 3—step 5.



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

2. During the final centrifuge spin, place a 50-mL conical tube in a tube rack, then place the tube rack with the empty tube next to the instrument.
3. *Immediately* after the centrifuge stops, perform the following steps.
 - a. Gently pull the disposable tubing downward on both sides of the pinch valve until the disposable tubing is out of the valve.
 - b. Remove the disposable injector from the Injector Hub, then place the disposable injector into the empty 50-mL conical tube in the tube rack.
4. In the instrument display, tap **Open Lid**, wait until the lid clicks open, then remove and discard the Ion OneTouch™ Recovery Router.
5. *Carefully* remove both Recovery Tubes from the instrument, then put the two Recovery Tubes in a tube rack.

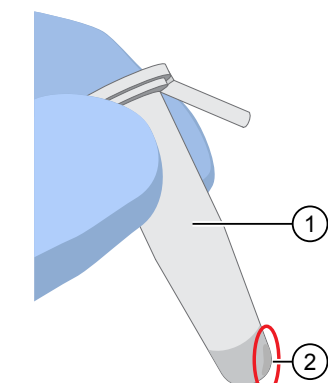


CAUTION! PHYSICAL INJURY HAZARD. The pointed end of the disposable injector can puncture your skin. Keep your hand away from the point of the disposable injector.

IMPORTANT! Do not store the recovered, template-positive ISPs 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™ Reagent 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™ Reagent 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 remaining 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) Evaluate the quality of the unenriched, template-positive ISPs using the Guava™ easyCyte 5 Benchtop 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 Sphere™ Particles Quality Assessment Using the Guava™ easyCyte 5 Benchtop 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. In the new labeled tube, bring the volume of combined washed suspensions to 100 µL with the ISP Resuspension Solution (in other words, add 80 µL of ISP Resuspension Solution to bring the total volume up to 100 µL).

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 Benchtop 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 using a Qubit™ Fluorometer. Transfer a 2.0-µL aliquot of the resuspended, unenriched ISPs to a 0.2-mL PCR tube, then analyze the QC sample as described in the *Ion Sphere™ Quality Control Kit User Guide* (Pub. No. MAN0017531) available at thermofisher.com.

For more information on QC sample analysis, see *Quality control of Ion 520™ & Ion 530™ ISPs in Appendix B of the Ion 520™ & Ion 530™ Kit – OT2 User Guide* (MAN0010844).

Enrich the template-positive ISPs with the Ion OneTouch™ ES. You can 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.

Clean 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 520™ & Ion 530™ 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 520™ & Ion 530™ Kit – OT2	Proceed to step 2. Continue to use the reagents that are provided in the Ion 520™ & Ion 530™ Kit – OT2.

2. Check the level of Ion OneTouch™ Oil in the Reagent Tube. If the Reagent Tube has <20 mL of Oil, pour Oil into the 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. Firmly insert the 3 ports of a new *single-use* Cleaning Adapter into the three holes on the top stage of the Ion OneTouch™ 2 Instrument.

5. Place a 50-mL conical tube in a tube rack, then place the tube rack next to the instrument.
Note: step 5 through step 8 are only necessary if you have not already removed the disposable injector before removing the Recovery Tubes from the instrument.
6. Gently pull the disposable tubing downward on both sides of the pinch valve until the disposable tubing is out of the valve.
7. Remove the disposable injector from the Ion OneTouch™ DL Injector Hub.



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.

8. Place the used, disposable injector into the empty 50-mL conical tube in the tube rack.
Note: The conical tube is used to collect waste.
9. On the home screen of the instrument, tap **Clean**.
10. Complete each task that is displayed on the screen, then tap **Next**. After you tap **Next** on the last task, a progress bar appears, and the cleaning starts.
At the end of the cleaning run, the screen displays **Time Remaining 00:00:00, Cleaning Run Complete**.
11. Tap **Next**, then ensure that the task "**Remove plate, injector, conical tube, and waste**" is displayed.
Note: Keep the used Cleaning Adapter on the instrument between runs.
12. Appropriately discard the waste that was collected in the 50-mL conical tube.
13. Remove and appropriately discard the used Amplification Plate, disposable injector, and tubing.



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

14. On the instrument display, tap **Open Lid**, wait until the lid clicks open, then open the centrifuge lid. Wipe the residue from the centrifuge lid with dry Kimwipes™ disposable wipers, then close the centrifuge lid.
15. Tap **Next** to return to the home screen on the instrument.

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

Residual volume test

IMPORTANT! Ensure that the AC line voltage module is installed correctly into the Ion OneTouch™ ES Instrument. For more information, see the *Ion S5™ and Ion S5™ XL System Site Preparation Guide* (Pub. No. MAN0010810) or the *Ion GeneStudio™ S5 System Site Preparation Guide* (Pub. No. MAN0017529).

If the condition is...	Then...
First use of the instrument and during monthly maintenance	Perform a residual volume test, refer to 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	Operate the instrument without performing the residual volume test. Proceed to "Prepare reagents then fill the 8-well strip" on page 6.

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 discard 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 resuspend the beads thoroughly, then *immediately* proceed to the next step.
2. Transfer 100 µL of Dynabeads™ MyOne™ Streptavidin C1 Beads to a new 1.5-mL Eppendorf DNA 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, then 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.

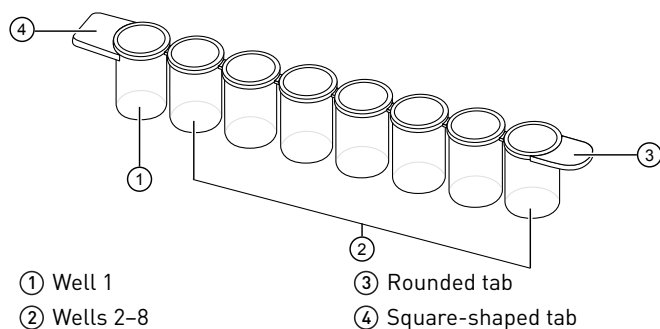
Note: You add the resuspended Dynabeads™ MyOne™ Streptavidin C1 Beads in the 130-µL MyOne™ Beads Capture Solution to Well 2 of the 8-well strip.

8. Remove the tube from the magnet, vortex the tube for 30 seconds, then 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 in 1-mL Nuclease-free Water at 2°C to 8°C, follow the washing procedure for ISPs (see “Wash the template-positive ISPs” on page 5).

2. Ensure that the square-shaped tab of the 8-well strip is on the left.



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 (see step 1) into Well 1 of the 8-well strip.

4. Fill the remaining wells in the 8-well strip as described in the following table.

Well number	Reagent to dispense in well
Well 1 ^[1]	Entire template-positive ISP sample (100 µL; prepared in step 1)
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 6)
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 6]
Well 8	Empty

^[1] Well nearest 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

Before every enrichment performed on the Ion OneTouch™ ES Instrument, install a new PCR collection tube and a new Eppendorf™ LoRetention Dualfilter P300 pipette tip.

1. Insert an open 0.2-mL PCR tube into the hole in the base of the Tip Loader, as shown in the figure in step 3.
2. Place a new tip in the Tip Loader. Remove the Tip Arm from the cradle, then align the metal fitting of the Tip Arm with the tip.
3. 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.
4. Lift the Tip Arm *straight* up to pull the installed tip from the Tip Loader tube.
5. 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 the following criteria are met:

- A new tip and an open 0.2-mL PCR tube are loaded in the Ion OneTouch™ ES Instrument, and the 8-well strip is correctly loaded.

- Well 1 (ISP sample) is the left-most well and the 8-well strip is pushed to the right-most position in the slot.
- Pipet the contents of Well 2 up and down to resuspend the beads before starting the run. Do not introduce bubbles into the solution.
 - 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).
 - Tap **Start/Stop**. The screen displays **run** during the run. The run takes ~35 minutes.
 - At the end of the run, the instrument displays **End** and beeps every 60 seconds. Tap 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.
 - 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.
 - 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.

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

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

Remove and wash the enriched ISPs

- Centrifuge the 0.2-mL PCR tube containing the enriched ISPs at 15,500 × g for 5 minutes.
- Remove all but ~10 µL of supernatant without disturbing the pellet, then add 200 µL of Nuclease-free Water.
- Pipet the solution up and down 10 times to resuspend the pellet. The pellet can be difficult to see.
- Centrifuge the 0.2-mL PCR tube at 15,500 × g for 5 minutes.

- Check for Dynabeads™ MyOne™ Streptavidin C1 Beads (a brown-tinted pellet) at the bottom of the centrifuged tube.

Is the brown pellet present?	Action
No	<ol style="list-style-type: none"> Remove all but ~10 µL of supernatant without disturbing the pellet. Add sufficient Nuclease-free Water for a final volume of 100 µL. Pipet up and down 10 times to resuspend the pellet. Sequence or store the template-positive ISPs at 2°C to 8°C for up to 3 days.
Yes	<ol style="list-style-type: none"> Pipet up and down 10 times to resuspend the pellet. Place the 0.2-mL PCR tube against a magnet such as a DynaMag™-2 magnet for 4 minutes. Transfer the supernatant to a new 0.2-mL PCR tube without disturbing the pellet of Dynabeads™ MyOne™ Streptavidin C1 Magnetic Beads. Centrifuge the supernatant at 15,500 × g for 5 minutes. Remove all but ~10 µL of supernatant without disturbing the pellet. Add sufficient Nuclease-free Water for a final volume of 100 µL. Pipet up and down 10 times to resuspend the pellet. Sequence or store the template-positive ISPs at 2°C to 8°C for up to 3 days.

(Optional) Perform enriched ISP quality control

You can determine the appropriate library dilution and/or the enrichment efficiency by using the Guava™ easyCyte 5 Benchtop 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 Sphere™ Particles Quality Assessment Using the Guava™ easyCyte 5 Benchtop Flow Cytometer User Bulletin* (Pub. No. MAN0007496), available at thermofisher.com.

When a manual cleaning of the sequencer is required

The Ion S5™ Sequencer, Ion S5™ XL Sequencer, and Ion GeneStudio™ S5 Series 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 deselected 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 520™ & Ion 530™ Kit – OT2 User Guide* (Pub. No. MAN0010844).

If the 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 520™ & Ion 530™ Kit – OT2 User Guide*.

Initialize the sequencer

1. In the instrument touchscreen main menu, tap **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 tap **Next**.
9. When initialization is complete (~50 minutes), tap **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.

Anneal Ion S5™ 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 undiluted Ion S5™ Annealing Buffer for a total volume of 25 µL.
4. Add 20 µL of Ion S5™ Sequencing Primer and confirm that the total volume is 45 µL. Add Ion S5™ Annealing Buffer if necessary 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 and run the program.

STOPPING POINT The reaction can remain at room temperature until you are ready to proceed with chip loading and sequencing.

8. After cycling, add 6 µL of Ion S5™ Sequencing Polymerase and 10 µL of Ion S5™ Loading Buffer, briefly vortex to mix, then centrifuge briefly to collect the contents at the bottom of the tube.
9. Incubate 5 minutes at room temperature.

Load the sample on the sequencing chip

1. Place the sequencing chip on a flat, stable surface.
2. Slowly dispense 40 µL of the sample directly into the chip loading port of the chip, then dispense the remaining ~20 µL into the chip loading well.
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 5 minutes.
5. In a 1.5-mL tube, combine 900 µL of 50% Annealing Buffer with 100 µL of Foaming Solution (10% Triton™ X-100).
6. Create foam by shaking vigorously.
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 exit well.
8. Using a new pipette tip each time, repeat step 7 three more times.
9. Inject 100 µL of 50% Annealing Buffer into the chip loading port, then remove the expelled liquid from the exit well.

IMPORTANT! Do not introduce air bubbles into the chip.

10. Using a new pipette tip each time, repeat step 9 three more times.

Start the sequencing run

We recommend that you start a sequencing run 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.

1. After completion of initialization, tap **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 tap **Next**.
4. In the dropdown list, select the Planned Run that you created in the Torrent Suite™ Software. If this run is the first of two sequencing runs on this initialization, **deselect** the **Enable post-run clean** checkbox, then tap **Review**.
5. Confirm that the pre-populated settings are correct, or tap **Edit** to make changes if needed.
6. Confirm that the instrument door is closed, then tap **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 can 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 Torrent Suite™ Software to review the results.

If you are sequencing a second chip on a single initialization, start the second run within 24 hours of start of initialization.

IMPORTANT!

- 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 that the **Enable post-run clean** checkbox is selected so that the post-run cleaning is performed automatically.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision history: Pub. No. MAN0010849

Revision	Date	Description
E.0	05 December 2018	<ul style="list-style-type: none"> • Updated for Ion GeneStudio™ S5 Systems. • Clarification made to dilute barcoded library pools, if used. • Updated for the Qubit™ 4 Fluorometer. • References to ISP quality control user guides updated to the <i>Ion Sphere™ Quality Control Kit User Guide</i> (Pub. No. MAN0017531). • Updated for Torrent Suite™ Software 5.10; Planned Run creation topics reorganized for ease of use.
D.0	7 March 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 5.2
B.0	16 November 2015	Update to Ion S5™ Sequencing Reagents (Cat. No. A27768) shipping and storage conditions
A.0	27 August 2015	New quick reference

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