Ion 540™ Kit – OT2

USER GUIDE

Instructions for template preparation, chip loading, and sequencing

for use with:
Ion 540™ Kit – OT2
Ion OneTouch™ 2 System
Ion S5™ System
Ion S5™ XL System

Catalog Number A27753
Publication Number MAN0010850
Revision D.0
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**Revision history:** Pub. No. MAN0010850

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Description</th>
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<tr>
<td>D.0</td>
<td>21 January 2017</td>
<td>• “Perform an instrument reset run with an initialized, unused Sequencing Reagents cartridge” topic added to Supplemental procedures</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ion OneTouch™ ES Instrument setup clarified</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• ISP quality control sections updated and moved to new user guides:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide (Pub. No. MAN0016387)</td>
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<tr>
<td></td>
<td></td>
<td>- Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide (Pub. No. MAN0016388)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Web links updated</td>
</tr>
<tr>
<td>C.0</td>
<td>27 July 2016</td>
<td>Updated for Torrent Suite™ Software v5.2.</td>
</tr>
<tr>
<td>B.0</td>
<td>16 November 2015</td>
<td>Update to Ion S5™ Sequencing Reagents Kit [Cat. No. A27768] shipping and storage conditions.</td>
</tr>
<tr>
<td>A.0</td>
<td>27 August 2015</td>
<td>User guide, includes instructions for using the Ion 540™ Kit – OT2 to prepare and enrich ISPs on the Ion OneTouch™ 2 System and sequencing with Ion S5™ or Ion S5™ XL Sequencers.</td>
</tr>
</tbody>
</table>

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About this guide

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Purpose

This user guide describes how to use the Ion OneTouch™ 2 System to prepare enriched, template-positive Ion 540™ Ion Sphere™ Particles with 200 base-pair average insert size libraries for sequencing on the Ion S5™ or Ion S5™ XL Sequencers. The Ion OneTouch™ 2 System includes the Ion OneTouch™ 2 Instrument and the Ion OneTouch™ ES Instrument.

The user guide is organized as follows:

• Prepare template-positive ISPs containing clonally amplified DNA, using the Ion 540™ Kit – OT2 (for up to 200 base-read libraries) with the Ion OneTouch™ 2 Instrument (see Chapter 4, “Prepare template-positive Ion 540™ ISPs”).
• Enrich the template-positive ISPs with the Ion OneTouch™ ES (see Chapter 5, “Enrich the template-positive Ion 540™ ISPs”).
• Load the enriched template-positive ISPs onto an Ion 540™ Chip (see Chapter 7, “Load the Ion 540™ Chip”).
• Clean and initialize the Ion S5™ or Ion S5™ XL Sequencer in preparation for sequencing (see Chapter 6, “Initialize the sequencer”).
• Sequence the loaded Ion 540™ Chip on an Ion S5™ or Ion S5™ XL Sequencer (see Chapter 8, “Start the sequencing run”).

IMPORTANT! Use only the Ion 540™ Kit – OT2 (Cat. No. A27753) with this user guide and with the Ion OneTouch™ 2 Instrument. Do not use with the Ion OneTouch™ Instrument. Do not mix reactions or disposables including plates, solutions, and kit reagents from other template preparation kits. Template-positive Ion Sphere™ Particles prepared with these kits should only be used in conjunction with the Ion S5™ Sequencing Solutions Kit (Cat. No. A27767) and Ion S5™ Sequencing Reagents Kit (Cat. No. A27768).
Product description

The Ion 540™ Kit – OT2 includes the reagents and materials required to use the Ion OneTouch™ 2 Instrument to prepare 8 reactions of template-positive Ion Sphere™ Particles (ISPs) and load Ion 540™ Chips. The kit also includes reagents and materials to sequence the loaded chips on the Ion S5™ or Ion S5™ XL Sequencer.

IMPORTANT! Use only the Ion 540™ Kit – OT2 (Cat. No. A27753) with this user guide and with the Ion OneTouch™ 2 System. Do not use with the Ion OneTouch™ System. Do not mix reactions or disposables including plates, solutions, and kit reagents from these or other template preparation kits.

Library compatibility

The Ion 540™ Kit – OT2 can be used with up to 200-base-read libraries of any type prepared using any available Ion library kit.

Software compatibility

The Ion 540™ Kit – OT2 is compatible with Torrent Suite™ Software v5.2 and later. Be sure to update your Torrent Server to the latest available version of Torrent Suite™ Software before using this kit.
Kit contents and storage conditions

**Ion 540™ Kit – OT2 configuration**

The Ion 540™ Kit – OT2 (Cat. No. A27753) consist of the following components. This protocol has been verified using these specific materials. Substitution may adversely affect performance.

**IMPORTANT!** Follow these special reagent handling instructions:
- Ion S5™ Reagent Mix in the Ion 540™ Kit – OT2 is shipped at –30°C to –10°C and should be thawed before use. Store the thawed Master Mix at 2°C to 8°C.
- Use only the supplied screw cap tubes to store the reagents. Do not seal the reagents with any plastic paraffin film such as Parafilm™ M film.

### Contents

<table>
<thead>
<tr>
<th>Contents</th>
<th>Part No.</th>
<th>Quantity per kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion S5™ OT2 Solutions</td>
<td>A27747</td>
<td>1 box</td>
</tr>
<tr>
<td>Ion S5™ OT2 Supplies</td>
<td>A27748</td>
<td>1 box</td>
</tr>
<tr>
<td>Ion 540™ OT2 Reagents</td>
<td>A27752</td>
<td>1 box</td>
</tr>
<tr>
<td>Ion 540™ Loading Reagents OT2</td>
<td>A27897</td>
<td>1 box</td>
</tr>
<tr>
<td>Ion S5™ Sequencing Solutions Kit</td>
<td>A27767</td>
<td>2 boxes</td>
</tr>
<tr>
<td>Ion S5™ Sequencing Reagents Kit</td>
<td>A27768</td>
<td>2 × 4 cartridges</td>
</tr>
</tbody>
</table>

### Ion 540™ Kit – OT2 (Cat. No. A27753) kit contents

<table>
<thead>
<tr>
<th>Contents</th>
<th>Amount / box</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion OneTouch™ Oil</td>
<td>450 mL</td>
<td>15°C to 30°C</td>
</tr>
<tr>
<td>Ion PGM™ OT2 Recovery Solution</td>
<td>350 mL</td>
<td></td>
</tr>
<tr>
<td>Ion OneTouch™ Reaction Oil</td>
<td>25 mL</td>
<td></td>
</tr>
<tr>
<td>Ion OneTouch™ Wash Solution</td>
<td>16 mL</td>
<td></td>
</tr>
<tr>
<td>Tween™ Solution</td>
<td>6 mL</td>
<td></td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>30 mL</td>
<td></td>
</tr>
<tr>
<td>Ion OneTouch™ Breaking Solution (black cap)</td>
<td>2 × 1.2 mL</td>
<td></td>
</tr>
<tr>
<td>MyOne™ Beads Capture Solution (green cap)</td>
<td>2 × 1.5 mL</td>
<td></td>
</tr>
<tr>
<td>ISP Resuspension Solution (red cap)</td>
<td>1.25 mL</td>
<td></td>
</tr>
<tr>
<td>Ion OneTouch™ ES Wash Solution</td>
<td>7.2 mL</td>
<td></td>
</tr>
<tr>
<td>Contents</td>
<td>Amount / box</td>
<td>Storage</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>Ion S5™ OT2 Supplies (Part No. A27748)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion OneTouch™ Reagent Tubes</td>
<td>2</td>
<td>15°C to 30°C</td>
</tr>
<tr>
<td>Ion OneTouch™ Recovery Tubes</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Ion OneTouch™ Recovery Routers</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Ion OneTouch™ Sipper Tubes</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Ion OneTouch™ Amplification Plate</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Ion OneTouch™ ES Supplies</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>[12 pipette tips and 1 box of ES 8-well strips]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion OneTouch™ Cleaning Adapter</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>[use each adapter for one cleaning only]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion OneTouch™ Reaction Filter</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Ion 540™ OT2 Reagents (Part No. A27752)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion S5™ Enzyme Mix (brown cap)</td>
<td>960 µL</td>
<td>−30°C to −10°C</td>
</tr>
<tr>
<td>Ion S5™ Reagent Mix (violet cap)</td>
<td>8 × 2 mL</td>
<td></td>
</tr>
<tr>
<td>Ion 540™ ISPs (black cap)</td>
<td>800 µL</td>
<td></td>
</tr>
<tr>
<td><strong>Ion 540™ Loading Reagents OT2 (Part No. A27897)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion S5™ Annealing Buffer</td>
<td>30 mL</td>
<td>−30°C to −10°C</td>
</tr>
<tr>
<td>Ion S5™ Foaming Solution (violet cap)</td>
<td>1 mL</td>
<td></td>
</tr>
<tr>
<td>Ion S5™ Loading Buffer (brown cap)</td>
<td>80 µL</td>
<td></td>
</tr>
<tr>
<td>Ion S5™ Sequencing Polymerase (yellow cap)</td>
<td>48 µL</td>
<td>−30°C to −10°C</td>
</tr>
<tr>
<td>Ion S5™ Sequencing Primer (white cap)</td>
<td>160 µL</td>
<td></td>
</tr>
<tr>
<td>Ion 540™ Control Ion Spheres (clear cap)</td>
<td>40 µL</td>
<td></td>
</tr>
<tr>
<td><strong>Ion S5™ Sequencing Solutions (Part No. A27767)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion S5™ Wash Solution</td>
<td>4 × 1.5 L</td>
<td>15°C to 30°C</td>
</tr>
<tr>
<td>Ion S5™ Cleaning Solution</td>
<td>250 mL</td>
<td></td>
</tr>
<tr>
<td><strong>Ion S5™ Sequencing Reagents (Part No. A27768)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion S5™ Sequencing Reagents</td>
<td>4 cartridges</td>
<td>−30°C to −10°C</td>
</tr>
<tr>
<td>[Cartridges ship at 2°C to 8°C. Store as indicated, do not store on dry ice.]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
IMPORTANT! Do not store the Ion S5™ Sequencing Reagents Kit (Part No. A27768) on dry ice or in a closed environment containing dry ice.

### Compatible Ion Chip™ kits

<table>
<thead>
<tr>
<th>Description</th>
<th>Catalog No.</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
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<tbody>
<tr>
<td>Ion 540™ Chip Kit [2 x 4-pack]</td>
<td>A27766</td>
<td>8 chips</td>
<td>15°C to 30°C</td>
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### Ion S5™ Controls

Human CEPH Genomic DNA Control is sufficient for 3 library preparations (1 µg each). The Ion S5™ Controls Kit (Cat. No. A27760) is ordered separately.

<table>
<thead>
<tr>
<th>Contents</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human CEPH Genomic DNA Control (red cap)</td>
<td>30 µL</td>
<td>−30°C to −10°C</td>
</tr>
<tr>
<td>Human CEPH Control 200 Library (yellow cap)</td>
<td>12 µL</td>
<td></td>
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### Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. MLS: Fisher Scientific (fisherscientific.com) or other major laboratory supplier.

<table>
<thead>
<tr>
<th>Description</th>
<th>Source</th>
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<tbody>
<tr>
<td>Ion OneTouch™ 2 System</td>
<td>4474779</td>
</tr>
<tr>
<td>The system includes:</td>
<td></td>
</tr>
<tr>
<td>• Ion OneTouch™ 2 Instrument</td>
<td></td>
</tr>
<tr>
<td>• Ion OneTouch™ ES Instrument</td>
<td></td>
</tr>
<tr>
<td>• AC Power Supply and Cords</td>
<td></td>
</tr>
<tr>
<td>• Ion S5™ Installation Kit</td>
<td></td>
</tr>
<tr>
<td>Dynabeads™ MyOne™ Streptavidin C1 Magnetic Beads</td>
<td>65001</td>
</tr>
<tr>
<td>Thermal cycler with heated lid</td>
<td>MLS</td>
</tr>
<tr>
<td>Eppendorf™ DNA LoBind™ Microcentrifuge Tubes</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td></td>
<td>13-698-791</td>
</tr>
<tr>
<td>Microcentrifuge[2]</td>
<td>MLS</td>
</tr>
<tr>
<td>Pipettes [P2, P20, P200, P1000] and appropriate low-retention tips</td>
<td>MLS</td>
</tr>
<tr>
<td>Vortexer with a rubber platform</td>
<td>MLS</td>
</tr>
<tr>
<td>Tube rack to fit 15-mL conical tube</td>
<td>MLS</td>
</tr>
<tr>
<td>Tube rack for 50-mL conical tube</td>
<td>MLS</td>
</tr>
</tbody>
</table>

[1] We have verified this protocol using this specific material. Substitution may adversely affect system performance.

[2] Must fit standard 1.5- and 0.2-mL microcentrifuge tubes; must generate 15,500 x g. To convert the RPMs of your centrifuge to RCF in units of gravity, see tools.thermofisher.com/content/sfs/brochures/TR0040-Centrifuge-speed.pdf.
Note: We recommend using a non-interruptible power supply (UPS) for laboratories that experience frequent power outages or line voltage fluctuations. The UPS must be rated for 1500 W output or higher. The 1500 VA unit from APC provides several minutes of backup power for the Ion OneTouch™ 2 Instrument, the Ion OneTouch™ ES Instrument, the Ion S5™ Sequencer, and the Torrent Server. Use a surge protector or line conditioner as needed (refer to the Ion S5™ System Site Preparation Guide, Pub. No. MAN0010810).

The Ion OneTouch™ 2 System uses common molecular biology equipment, supplies, and reagents. Unless otherwise indicated, all materials are available through thermofisher.com. MLS: Fisher Scientific (fisherscientific.com) or other major laboratory supplier.

### Recommended materials for the Ion OneTouch™ 2 System

<table>
<thead>
<tr>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion S5™ Controls Kit</td>
<td>A27760</td>
</tr>
<tr>
<td>Benchtop absorbent paper or mat</td>
<td>MLS</td>
</tr>
<tr>
<td>Bleach</td>
<td>MLS</td>
</tr>
<tr>
<td>Ethernet cable</td>
<td>MLS</td>
</tr>
<tr>
<td>1/8-inch L-wrench (hex wrench) or equivalent tool</td>
<td>MLS</td>
</tr>
</tbody>
</table>

[1] We have verified this protocol using this specific material. Substitution may adversely affect system performance.

### Additional materials required for Ion OneTouch™ ES

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>DynaMag™-2 magnet</td>
<td>12321D</td>
</tr>
<tr>
<td>0.2-mL PCR tubes (Axygen™ MAXYMum Recovery™ PCR Tube)</td>
<td>Fisher Scientific 14-222-283 or MLS</td>
</tr>
<tr>
<td>1 M NaOH</td>
<td>MLS</td>
</tr>
</tbody>
</table>

[1] We have verified this protocol using this specific material. Substitution may adversely affect system performance.
Prerequisites

The manufacturer is not liable for any damage or injury that results from use of this manual by unauthorized or untrained parties. This guide uses conventions and terminology that assume a working knowledge of the Microsoft™ Windows™ operating system, the Internet, and Internet-based browsers.

Contamination

⚠️ **CAUTION!** A primary source of contamination is DNA fragments from previous sample processing steps. Do not introduce amplified DNA into library preparation laboratory or work area.

Instrument clearances

**Ion OneTouch™ 2 Instrument and Ion OneTouch™ ES Space Requirements and Clearances:** Position the instrument so that the front is a minimum of 12 in. (30.5 cm) from the front of the laboratory bench. Place the instrument at least 40 in. (1 meter) away from major sources of electronic noise such as refrigerators or microwaves. For more information, refer to the *Ion S5™ and Ion S5™ XL Site Preparation Guide* (Pub. No. MAN0010810).
Before you begin

- Procedural guidelines ................................................. 14
- Unpack and install the Ion OneTouch™ 2 System ......................... 14
- Check the firmware ................................................... 15
- Initialize the Ion OneTouch™ 2 Instrument ............................... 15

Procedural guidelines

Preventing cross-contamination:
- Use good laboratory practice to minimize cross-contamination of products and reagents.
- When designing the laboratory layout, consider the need for space separation of pre- and post-PCR activities. Dedicate laboratory supplies and/or equipment to the appropriate space to reduce the potential for contamination.
- A primary source of contamination is DNA fragments from previous sample processing steps. Do not introduce amplified DNA into library preparation laboratory or work area.

Reagent thawing:
- Unless otherwise specified, thaw reagents on ice before use. Ensure that no ice crystals are visible in the thawed reagent.

Pipetting recommendations:
- Vortex all reagents, except for enzymes, for 5 seconds. Mix enzymes by flicking the tube with your finger 4 times. Pulse centrifuge before use.
- Pipet viscous solutions slowly and ensure complete mixing.
- Change tips between pipetting steps.

Unpack and install the Ion OneTouch™ 2 System

For detailed instructions on site preparation and installation of the Ion OneTouch™ 2 Instrument and Ion OneTouch™ ES, see:
Check the firmware

To check the firmware version, touch **Options** on the home screen, then touch **Info**. To update the firmware to the appropriate version, see the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388).

Initialize the Ion OneTouch™ 2 Instrument

**IMPORTANT!** Before operating the Ion OneTouch™ 2 Instrument for the first time, you must initialize the instrument. Initialization primes the pumps and tubing lines for reliable operation. Perform initialization at any time before the first run.

In addition, re-initialization is recommended when switching between the Ion 520™ & Ion 530™ Kit – OT2 and Ion 540™ Kit – OT2, and between kits with different lots of Ion OneTouch™ Oil.

For detailed instructions on initialization of the Ion OneTouch™ 2 Instrument, see the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388).

**Note:** To set up the Ion OneTouch™ 2 Instrument when switching between sequencing platforms, see the *Ion OneTouch™ 2 System User Guide*, Chapter 3 "Demonstrated Protocol: Setting up the Ion OneTouch™ 2 Instrument when switching between template preparation kits.”
Create a Planned Run

About Planned Runs

Planned Runs contain all the settings used in a sequencing run, including number of flows, kit types, barcodes used (if any), run type (e.g., DNA, RNA, amplicons), and reference file (if any). They provide a fast and convenient way to set up and organize your runs.

You create Planned Runs using the Torrent Browser and then select the appropriate plan in the Select Planned Run screen of the Ion S5™ or Ion S5™ XL Sequencer when you are ready to perform the run (see “Start the sequencing run” on page 67).

Use the Torrent Browser Help menu to access the Torrent Suite™ Software Help Guide for complete descriptions of the fields in the Torrent Browser application. Click Help ➔ Software Help ➔ Torrent Suite™ Software Help System.

Note: The Torrent Suite™ Software Help Guide is also available for download at thermofisher.com/ion-s5-docs.
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. Key fields are described in “Planned Run wizard key fields” on page 19.

![Planned Run wizard key fields](image)

**Note:** For a complete description of each field, see the Torrent Suite™ Software Help Guide.

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.

You can then select the appropriate plan when you are setting up the run on the Ion S5™ or Ion S5™ XL Sequencer.

![Run Selection](image)
### Planned Run wizard key fields

<table>
<thead>
<tr>
<th>Field name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IonReporter</td>
<td>If Ion Reporter™ Software is installed and enabled and you want to analyze the run data using the software, select the account and workflow.</td>
</tr>
<tr>
<td>Application</td>
<td>Select the sequencing application you are performing.</td>
</tr>
<tr>
<td>Instrument</td>
<td>Select the sequencing system you are using.</td>
</tr>
<tr>
<td>Chip Type</td>
<td>Select the chip type you are using.</td>
</tr>
<tr>
<td>Library Kit Type</td>
<td>Select the kit used to prepare the library.</td>
</tr>
<tr>
<td>Template Kit</td>
<td>Select the instrument and kit used to prepare the templated Ion 540™ ISPs.</td>
</tr>
<tr>
<td>Sequencing Kit</td>
<td>Select the Ion S5™ Sequencing Kit.</td>
</tr>
<tr>
<td>Barcode Set (optional)</td>
<td>If you are using barcodes with:</td>
</tr>
<tr>
<td></td>
<td>- <strong>DNA libraries</strong>: Select the IonXpress barcode set, which includes all barcodes in the Ion Xpress™ Barcode Adapters 1-96 Kits.</td>
</tr>
<tr>
<td></td>
<td>- <strong>RNA libraries prepared using the Ion Total RNA-Seq Kit v2</strong>: Select the IonXpressRNA barcode set, which contains all 16 barcodes in the Ion Xpress™ RNA BC01-16 Kit.</td>
</tr>
<tr>
<td></td>
<td>If you are <em>not</em> using barcodes with:</td>
</tr>
<tr>
<td></td>
<td>- <strong>DNA libraries</strong>: Leave the Barcode field blank.</td>
</tr>
<tr>
<td></td>
<td>- <strong>RNA libraries prepared using the Ion Total RNA-Seq Kit v2</strong>: Select RNA_Barcode_None from the drop-down list. This ensures that the proper trimming is performed on the resulting sequence when the RNA library does not have a barcode.</td>
</tr>
<tr>
<td>Flows</td>
<td>Enter the appropriate number of flows for the sequencing kit and read length.</td>
</tr>
<tr>
<td>Plugins</td>
<td>Select and configure the appropriate plugins for your application.</td>
</tr>
<tr>
<td>Projects</td>
<td>Select or add a project within which to group your run data. You can include runs in multiple projects, and remove runs from a project at any time.</td>
</tr>
<tr>
<td>Planned Run Name</td>
<td>Enter a name for the run.</td>
</tr>
<tr>
<td>Reference Library</td>
<td>Select a reference library uploaded to the Torrent Server, if any.</td>
</tr>
<tr>
<td>Target Regions and Hotspot Regions</td>
<td>Select the <strong>Target Regions</strong> and/or <strong>HotSpot Regions</strong> BED file on the Torrent Server, if any.</td>
</tr>
</tbody>
</table>
**Chapter 3 Create a Planned Run**

*Planned Run wizard key fields*

<table>
<thead>
<tr>
<th>Field name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enter a sample name...</td>
<td>Specify the Sample Name, ID, and Description for each sample in the run (number of samples will change based on the number of barcodes and/or chips selected).</td>
</tr>
<tr>
<td>Monitoring Thresholds</td>
<td>Set thresholds for Bead Loading, Key Signal, and Usable Sequence. In the Torrent Browser <strong>Monitor &gt; Runs in Progress</strong> tab, an alert is displayed if the values for a run fall below the selected thresholds.</td>
</tr>
</tbody>
</table>
Prepare template-positive Ion 540™ ISPs

- Ion OneTouch™ 2 Instrument layout .............................................. 22
- Run the OT2 Instrument ................................................................. 23
- Set up the Ion OneTouch™ 2 Instrument .................................... 26
- Prepare and install the amplification solution ............................ 32
- Start the run .................................................................................. 38
- Recover the template-positive Ion Sphere™ Particles ...................... 39
- Maintain the Ion OneTouch™ 2 Instrument ............................... 42
Ion OneTouch™ 2 Instrument layout

1. Ion OneTouch™ Reaction Filter
2. Clamp handle to access the Amplification Plate in the heat block
3. Pinch valve to hold disposable tubing
4. Ion OneTouch™ Recovery Solution
5. Ion OneTouch™ Oil
6. Waste Container
7. Oil waste tray (pull out)
8. Centrifuge to spin the Recovery Tubes and Recovery Router
9. Ion OneTouch™ DL Injector Hub
10. Instrument display

Note: The line drawing does not show the disposable tubing.
Run the OT2 Instrument

**Note:** Ensure that you have the correct firmware update for your system (see “Check the firmware” on page 15).

1. Ensure that the centrifuge lid of the Ion OneTouch™ 2 Instrument is closed.

   **IMPORTANT!** If you raise the centrifuge lid, do not hit the disposable injector against the instrument. You can damage the disposable injector. If you damage the disposable injector, appropriately dispose of the injector, tubing, and Amplification Plate. Use a new disposable injector and Ion OneTouch™ Amplification Plate.

2. On the home screen, touch **Run**:

   ![Image of the home screen with Run highlighted](image)

3. Touch the Protocol drop-down menu, then select the appropriate sequencing length protocol (for example, **Ion S5™: Ion 540™ Kit – OT2**):

   ![Image of the Protocol menu](image)
4. Touch Next.

5. Touch Assisted or Expert:

- **Assisted** run. Complete each task, then touch Next. After you touch Next on the last task, you see a progress bar, and the run begins. After the runs starts, you hear clicks from the instrument. This is normal.

- **Expert** run. Empty the waste container and oil waste tray, if necessary, then touch Next. You see a progress bar, and the run begins without the list of task screens.

**IMPORTANT!** Remember to add 150 µL of Ion OneTouch™ Breaking Solution to each Recovery Tube before starting the run. On the reminder screen, confirm and touch Yes to advance:
IMPORTANT! To cancel a run, touch **Abort**, then touch **Yes** to confirm cancellation. If there is a high-pressure event on the instrument, the instrument aborts the run **automatically**, and you do **not** have to touch **Abort**.

After a run is aborted, follow these steps in this order:

- Download the log files for troubleshooting by Technical Support [refer to the *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388)].
- Turn OFF the instrument.
- If necessary, retain all consumables on the instrument for troubleshooting.
- After successful troubleshooting, appropriately dispose of all used consumables and turn ON the instrument.
- Set up the instrument with new kit components (see “Set up the Ion OneTouch™ 2 Instrument” on page 26).
- Prepare a new amplification solution (see “Prepare and install the amplification solution” on page 32).
- Start a new run.

6. Remove the samples ≤16 hours after starting the run. If you touched **Next** on the Centrifuge screen to centrifuge samples at the end of the run, proceed **immediately** to “Recover the template-positive Ion Sphere™ Particles” on page 39.
Set up the Ion OneTouch™ 2 Instrument

Note:
- To set up the Ion OneTouch™ System when switching between sequencing platforms, see the Ion OneTouch™ 2 System User Guide (Pub. No. MAN0014388).
- If this is the first run after instrument initialization, proceed to “Prepare and install the amplification solution” on page 32. The instrument consumables are already installed and ready for the run.

Materials required

Provided in Ion S5™ OT2 Supplies (Part No. A27748):
- 2 Ion OneTouch™ Reagent Tubes
- Ion OneTouch™ Recovery Router
- 2 Ion OneTouch™ Recovery Tubes
- Ion OneTouch™ Amplification Plate
- 2 Ion OneTouch™ Sipper Tubes

Provided in Ion S5™ OT2 Solutions (Part No. A27747):
- Ion OneTouch™ Oil (450 mL size)
- Ion OneTouch™ Breaking Solution
- Ion OneTouch™ Recovery Solution

Note: We have verified this protocol using only the material specified. Substitution may adversely affect performance and safety.

IMPORTANT! Follow these special reagent handling instructions:
- Use only the Ion 540™ Kit – OT2 (Cat. No. A27753) with this user guide and with the Ion OneTouch™ 2 System. Do not mix reactions or disposables including plates, solutions, and kit reagents from other template preparation kits.
- Use only the supplied screw cap tubes in the Ion 540™ Kit – OT2 to store the reagents. Do not seal the reagents with any plastic paraffin film such as Parafilm™ M film.

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

1. On the instrument display, touch **Open Lid**, wait until the lid clicks open, then lift and hold the side of the centrifuge lid.

   **IMPORTANT!** Do not lift the lid by the tubing attached to the Ion OneTouch™ DL Injector Hub. Do not force the lid open.

2. Dispense 150 µL Ion OneTouch™ Breaking Solution into each of two Recovery Tubes. The Recovery Tubes have rounded collars at their openings.

   **Note:** Breaking Solution is viscous. Draw and dispense slowly.

   **Note:** Ensure that the Ion OneTouch™ Breaking Solution is stored and used at room temperature. If a white precipitate is present in the tube, warm the solution at 30°C until the precipitate re-dissolves.
3. Insert a Recovery Tube containing Ion OneTouch™ Breaking Solution into each slot of the centrifuge:

4. Slide the Recovery Router in position around each Recovery Tube extension. Pinch the sides of the Recovery Router and push it down into the center slot of the centrifuge. The Recovery Router must be seated flat and secure in the center of the rotor:

5. Close the lid of the centrifuge.

---

Install the Ion OneTouch™ Amplification Plate

1. If there is a used Ion OneTouch™ Cleaning Adapter on the instrument, remove and appropriately discard it.

   **Note:** The Cleaning Adapter can be filled with Ion OneTouch™ Oil.

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 Ion OneTouch™ Amplification Plate to ensure that the plate port is straight and perpendicular to the plate.

   **IMPORTANT!** The disposable tubing and disposable injector are attached to the Amplification Plate. Do not disconnect tubing from the top plate port. If you have questions about the plate, contact Technical Support.

   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:

   **Note:** The disposable tubing is under the handle.

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.
Chapter 4 Prepare template-positive Ion 540™ ISPs
Set up the Ion OneTouch™ 2 Instrument

b. Gently pull the disposable tubing upwards on both sides of the pinch valve until the disposable tubing is in the slot (see figure) and secured in the round notch on each side of the pinch valve:

![Image of pinched valve with tubing]

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” on page 29):

1. Ensure that the needle of the injector is screwed tightly onto the rubber tubing. Hold the centrifuge lid down with one hand, and with other hand install the disposable injector by inserting it straight down into the injector hub. Push down until it just touches the router.

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

Note: The color of the injector may vary.
2. The spring-loaded top of the injector hub will click upon release, automatically adjusting the tip to the correct distance from the router surface. You can test this by gently pushing the injector down again and releasing. You should hear a click from the hub.

**Up position**

**Down position**

**IMPORTANT!** If the Injector Hub remains in the down position, see Appendix A, “Troubleshooting”.

**IMPORTANT!** If you raise the centrifuge lid, do not hit the injector against the instrument. If you damage the disposable injector, appropriately dispose of the injector, tubing, and Amplification Plate. Use a new injector and Ion OneTouch™ Amplification Plate.

---

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

<table>
<thead>
<tr>
<th>If you are</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using a new Template Kit</td>
<td>1. Use a new Reagent Tube from the kit.</td>
</tr>
<tr>
<td></td>
<td>2. Discard the used Reagent Tube and Sipper Tube. Appropriately discard the residual Oil.</td>
</tr>
<tr>
<td></td>
<td>3. Use fresh gloves to attach the Luer-Lok™ end of a new Ion OneTouch™ Sipper Tube to the left front port. Do not let the Sipper Tube touch any surface.</td>
</tr>
<tr>
<td></td>
<td>4. Invert the Ion OneTouch™ Oil bottle (450-mL size) 3 times to mix, then fill the Reagent Tube half-full with Oil. Minimize bubbles.</td>
</tr>
<tr>
<td></td>
<td>5. Insert the filled Reagent Tube into the left front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument.</td>
</tr>
<tr>
<td>Refilling the Reagent Tube between runs</td>
<td>1. Remove the Reagent Tube from the instrument.</td>
</tr>
<tr>
<td></td>
<td>2. Invert Ion OneTouch™ Oil bottle (450-mL size) 3 times to mix.</td>
</tr>
<tr>
<td></td>
<td>3. Fill the Reagent Tube half-full with Oil. Minimize bubbles.</td>
</tr>
<tr>
<td></td>
<td><strong>Note:</strong> It is not necessary to re-mix the Oil.</td>
</tr>
<tr>
<td></td>
<td>4. Insert the filled Reagent Tube into the left front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument.</td>
</tr>
</tbody>
</table>
Install the Ion OneTouch™ Recovery Solution

**IMPORTANT!** Use only the Ion OneTouch™ Recovery Solution provided as part of the Ion 540™ Kit – OT2 for the Ion OneTouch™ 2 Instrument. Do not use a different recovery solution from another kit.

1. Ensure that the Recovery Solution is clear. If it is clear, proceed to the next step. If the Recovery Solution is not clear, heat the bottle of Recovery Solution in a 30°C bath until the Recovery Solution is clear.

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

<table>
<thead>
<tr>
<th>If you are</th>
<th>Action</th>
</tr>
</thead>
</table>
| Using a new Template Kit | 1. Use a new Reagent Tube from the kit.  
  2. Discard the used Reagent Tube and Sipper Tube. Appropriately discard the Recovery Solution.  
  3. Use fresh gloves to attach the Luer-Lok™ end of a new Ion OneTouch™ Sipper Tube to the right front port. Do not let the Sipper Tube touch any surfaces.  
  4. Invert the Recovery Solution 3 times to mix, then fill the Reagent Tube one-third-full with Recovery Solution. Minimize bubbles.  
  5. Insert the filled Reagent Tube into the right front port, and screw the Reagent Tube firmly into place, one-quarter turn on the instrument. |
| Refilling the Reagent Tube between runs | 1. Remove the Reagent Tube from the instrument.  
  2. Invert the bottle of Recovery Solution 3 times.  
  3. Add more Recovery Solution to the solution in the Reagent Tube until the tube is one-third-full. Minimize bubbles.  
  4. Insert the filled Reagent Tube into the right front port, and 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.

Inspect the oil waste tray

1. Slowly pull out the oil waste tray but do not remove it completely from the slot underneath the center of the instrument.

2. Check for oil in the oil waste tray:
   - If there is little or no oil, push the tray back fully into the instrument.
   - If there is excessive oil, remove the tray, then appropriately dispose of the oil. Reinsert the oil waste tray into the slot, then push the tray back fully into the instrument. Contact Technical Support.
Prepare and install the amplification solution

Materials required for this procedure

Provided in Ion S5™ OT2 Solutions (Part No. A27747):
- Ion OneTouch™ Reaction Oil
- Nuclease-free Water

Provided in Ion S5™ OT2 Supplies (Part No. A27748):
- Ion OneTouch™ Reaction Filter

Provided in Ion 540™ OT2 Reagents (Part No. A27752):
- Ion S5™ Reagent Mix
- Ion S5™ Enzyme Mix
- Ion 540™ Ion Sphere™ Particles

(Optional) Provided in the Ion S5™ Controls Kit (Cat No. A27760):
- Human CEPH Control 200 Library

Other Materials and Equipment:
- Diluted library
- Microcentrifuge
- 1.5-mL Eppendorf LoBind™ Tubes
- Tube rack for 15-mL conical tube
- Pipettes
- Vortexer
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. Do not mix reactions or disposables including plates, solutions, and kit reagents from other template preparation kits.

IMPORTANT! We recommend preparing the amplification solution in a room dedicated to pre-PCR activities or in a controlled pre-PCR hood.

1. Prepare the reagents as follows:

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion S5™ Reagent Mix</td>
<td>1. Allow the tube of Ion S5™ Reagent Mix (2 mL) to come to room temperature before use.</td>
</tr>
<tr>
<td></td>
<td>2. Vortex the solution for 30 seconds, then centrifuge the solution for 2 seconds.</td>
</tr>
<tr>
<td></td>
<td>Note: Visually inspect the solution at the bottom of the tube and verify that there is no residual precipitate. If precipitate is visible, see Appendix A, “Troubleshooting”.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>Ion S5™ Enzyme Mix</td>
<td>1. Flick the tube with your finger 4 times to mix.</td>
</tr>
<tr>
<td></td>
<td>2. Centrifuge the enzyme for 2 seconds.</td>
</tr>
<tr>
<td></td>
<td>3. Place on ice.</td>
</tr>
<tr>
<td>Ion Sphere™ Particles</td>
<td>Place the suspension at room temperature.</td>
</tr>
</tbody>
</table>

IMPORTANT! Use only Ion Sphere™ Particles (ISPs) from the Ion 540™ Kit – OT2 with the Ion OneTouch™ 2 System. Do not use ISPs from other or previously used kits.
2. Dilute the library as shown in the table below. Use the library dilution within 48 hours of preparation.

<table>
<thead>
<tr>
<th></th>
<th>Ion AmpliSeq™ DNA Library</th>
<th>Ion AmpliSeq™ RNA Library</th>
<th>gDNA Fragment or Amplicon Library</th>
<th>Ion TargetSeq™ Exome-Enriched Library</th>
<th>Ion Total RNA-Seq Library</th>
</tr>
</thead>
<tbody>
<tr>
<td>Library concentration</td>
<td>100 pM</td>
<td>100 pM</td>
<td>100 pM</td>
<td>100 pM</td>
<td>100 pM</td>
</tr>
<tr>
<td>Volume of library</td>
<td>6–8 µL</td>
<td>6–8 µL</td>
<td>6–8 µL</td>
<td>6–8 µL</td>
<td>6–8 µL</td>
</tr>
<tr>
<td>Total volume of diluted library to add to the amplification solution</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

**Note:** If you are troubleshooting the amplification process, to 260 µL of Nuclease-free Water, add 1 µL of the Human CEPH Control 200 Library from the Ion S5™ Controls Kit (Cat. No. A27760), use 100 µL of this diluted control library in place of your library in the amplification solution.

a. Vortex the diluted library for 5 seconds, then centrifuge for 2 seconds.

b. Place the diluted library on ice.

3. Prepare the ISPs:
   a. Vortex the ISPs at maximum speed for 1 minute to resuspend the particles.
   b. Centrifuge the ISPs for 2 seconds.
   c. Pipet the ISPs up and down to mix.
   d. *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:

<table>
<thead>
<tr>
<th>Order</th>
<th>Reagent</th>
<th>Cap color</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nuclease-free Water</td>
<td>—</td>
<td>80 µL</td>
</tr>
<tr>
<td>2</td>
<td>Ion S5™ Enzyme Mix</td>
<td>Brown</td>
<td>120 µL</td>
</tr>
<tr>
<td>3</td>
<td>Ion Sphere™ Particles</td>
<td>Black</td>
<td>100 µL</td>
</tr>
<tr>
<td>4</td>
<td>Diluted library (not stock library)</td>
<td>—</td>
<td>100 µL</td>
</tr>
<tr>
<td>—</td>
<td>Total volume (including Master Mix)</td>
<td>—</td>
<td>2400 µL</td>
</tr>
</tbody>
</table>
5. Proceed immediately to “Fill the reaction filter assembly”.

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

**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. Obtain an Ion OneTouch™ Reaction Filter from the Ion 540™ Kit – OT2.

   ![Illustration of reaction filter assembly]

   - **1.** Sample port
   - **2.** Ion OneTouch™ Reaction Filter
   - **3.** Ion OneTouch™ Reaction Tube
   - **4.** Short tubing from sample port to Ion OneTouch™ Reaction Tube

2. Place the Ion OneTouch™ Reaction Filter into a tube rack so that the 3 ports of the Ion OneTouch™ Reaction Filter face up.

3. Identify the sample port on the Ion OneTouch™ Reaction Filter. The short tubing in the Reaction Tube is connected to the sample port:

   **Note:** The color of the short tubing attached to the sample port may vary.

4. Add the amplification solution through the sample port:
   a. Set a P1000 pipettor to 800 µL, and attach a new 1000-µL tip.
   b. 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.
   c. Pipet the amplification solution up and down to mix, then fill the tip with 800 µL of the amplification solution.
d. Insert the tip firmly into the sample port so that the tip is perpendicular to the Ion OneTouch™ Reaction Filter Assembly and fully inserted into the sample port to form a tight seal:

![Image of a tip inserted into a sample port]

e. Slowly pipet 800 µL of the 2.4 mL amplification solution through the sample port. Keep the plunger of the pipette depressed to avoid aspirating solution from the Ion OneTouch™ Reaction Filter. With the plunger still depressed, remove the tip from the sample port.

f. Repeat steps c–e two more times (for a total of 3 times) to load the entire amplification solution volume into the Ion OneTouch™ Reaction Filter.

g. If necessary, gently dab a Kimwipes™ disposable wipe around the ports to remove any liquid.

5. Add Ion OneTouch™ Reaction Oil through the sample port:
   a. Set a P1000 pipettor to 200 µL, and attach a new 1000-µL tip.
   b. Draw up 200 µL of Ion OneTouch™ Reaction Oil into the pipette tip.
   c. Insert the tip firmly into the sample port so that the tip is perpendicular to the Ion OneTouch™ Reaction Filter and fully inserted into the sample port to form a tight seal.
   d. Slowly pipet 200 µL of the Reaction Oil through the sample port, then keep the plunger of the pipette depressed.
   e. With the plunger depressed, remove the tip from the sample port, then appropriately discard the tip.
   f. If necessary, gently dab a Kimwipes™ disposable wipe around the ports to remove any liquid.
**Install the filled Ion OneTouch™ Reaction Filter**

**IMPORTANT!** Follow the steps exactly to minimize contact of the short tubing in the Reaction Tube with the amplification solution.

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 as shown below, until the Reaction Tube is inverted and the three ports point down.

   ![Rotation Diagram](image1)

   **IMPORTANT!** Correct rotation of the Ion OneTouch™ Reaction Filter ensures minimal exposure of the short tubing in the Reaction Tube with the amplification solution.

3. Insert the three ports into the three holes on the top stage of the Ion OneTouch™ 2 Instrument, so that the Ion OneTouch™ Reaction Filter is firmly seated (right) on the instrument. The tab protruding from the outer edge of the Reaction Filter fits into the front notch of the stage:

   ![Seating Diagram](image2)

   **Note:** After inserting the Ion OneTouch™ Reaction Filter, bubbles may shoot up into the Reaction Tube.
**Start the run**

1. After installing the Ion OneTouch™ Reaction Filter, touch **Next** to start the run.

   **IMPORTANT!** Remember to add 150 µL of Ion OneTouch™ Breaking Solution to each Recovery Tube before starting the run. On the reminder screen, confirm and touch **Yes** to advance:

   ![Image of reminder screen](image)

   **Note:** After the run starts, the instrument makes clicking sounds. This is normal.

2. Remove the samples ≤16 hours after starting the run. If you touched **Next** on the centrifuge screen to centrifuge samples at the end of the run, proceed *immediately* to “Recover the template-positive ISPs” on page 39.

   **Note:** Typical instrument run time is 4.8–6.5 hours.

**Abort and restart a run**

If it is necessary to abort and restart a run, follow these steps in the order listed:

1. Touch **Abort**, then touch **Yes** to confirm cancellation.

   **Note:** If there is a high-pressure event on the instrument, the instrument aborts the run *automatically*, and you do *not* have to touch **Abort**.

2. After a run is aborted, download the log files for troubleshooting by Technical Support (see the *Ion OneTouch™ 2 System User Guide* [Pub. No. MAN0014388] for more information).

3. Power off the instrument.

4. If needed, retain all consumables on the instrument for troubleshooting.

5. After successful troubleshooting, appropriately discard all used consumables, then power on the instrument.

6. Set up the instrument with new kit components (see “Set up the Ion OneTouch™ 2 Instrument” on page 26).
7. Prepare a new amplification solution (see “Prepare and install the amplification solution” on page 32).

8. Start a new run.

**Recover the template-positive Ion Sphere™ Particles**

**Materials required**

Provided in Ion S5™ OT2 Solutions (Part No. A27747):
- Ion OneTouch™ Wash Solution
- ISP Resuspension Solution
- Nuclease-free Water

**Other materials and equipment:**
- Eppendorf™ DNA LoBind™ Microcentrifuge Tubes
- Pipettes
- Vortexer
- Microcentrifuge

**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, touch **Open Lid**, wait until the lid clicks open, then insert the two filled Ion OneTouch™ Recovery Tubes from the run in the centrifuge rotor. Close the lid until it locks.
   
   b. Touch **Options ▶ Final Spin** (see the following figure), then follow the screen prompts (touch **Next** on the next 2 screens) until the centrifugation starts. Centrifugation of the samples takes 10 minutes.

   ![Centrifuge Screen](image)

   c. When the centrifuge stops, **immediately** proceed to steps 3–5.
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:
   a. Gently pull the disposable tubing downwards on both sides of the pinch valve until the disposable tubing is out of the valve.
   b. Place one hand on the centrifuge lid, then with the other hand firmly grip the rigid plastic connector at the top of the disposable injector and steadily pull the disposable injector straight up from the 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.

   c. Place the disposable injector into the empty 50-mL conical tube in the tube rack.

4. In the instrument display, touch Open Lid, wait until the lid clicks open, then remove and discard the Ion OneTouch™ Recovery Router.

5. Carefully remove both Ion OneTouch™ Recovery Tubes from the instrument, then put the two Recovery Tubes in a tube rack. You may see some cloudiness in the tube, which is normal.

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™ 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:

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:

<table>
<thead>
<tr>
<th>If you have</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qubit™ 2.0 Fluorometer or Qubit™ 3.0 Fluorometer</td>
<td>Continue to step 8. You can perform quality control on the ISPs in step 12.</td>
</tr>
<tr>
<td>(Optional) Guava™ easyCyte™ 5 Flow Cytometer</td>
<td>Perform quality control on the diluted, unenriched ISPs. 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), available at thermofisher.com.</td>
</tr>
</tbody>
</table>

8. Centrifuge the template-positive ISP suspension for 8 minutes at 15,500 × g.

**Note:** To convert the RPMs of your centrifuge to RCF in units of gravity, see tools.thermofisher.com/content/sfs/brochures/TR0040-Centrifuge-speed.pdf.

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
Proceed to “Maintain the Ion OneTouch™ 2 Instrument” and Chapter 5, “Enrich the template-positive Ion 540™ ISPs”. 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.

## Maintain the Ion OneTouch™ 2 Instrument

**IMPORTANT!** 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. Do not skip this procedure. The cleaning procedure is performed according to the steps displayed on the instrument after removing the Recovery Tubes.

**Note:** To set up the Ion OneTouch™ 2 Instrument when switching between sequencing platforms and/or template preparation kits, refer to Chapter 5 of the Ion OneTouch™ 2 System User Guide (Pub. No. MAN0014388).

### Materials required

**Provided in Ion S5™ OT2 Supplies (Part No. A27748):**
- Ion OneTouch™ Cleaning Adapter (single-use)

**Provided in Ion S5™ OT2 Solutions (Part No. A27747):**
- Ion OneTouch™ Oil

**Other materials and equipment:**
- Kimwipes™ disposable wipes
- 50-mL conical tube
- Tube rack for 50-mL conical tube

**Note:** To ensure continued safe operation, visually inspect the rotor assembly and casing periodically to ensure there are no signs of cracks or other physical damage.

### Clean the Ion OneTouch™ 2 Instrument

1. Determine the appropriate reagents to use for maintaining the Ion OneTouch™ 2 Instrument:

<table>
<thead>
<tr>
<th>If you are</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Switching to the Ion 540™ Kit – OT2 from another kit?</td>
<td>See Chapter 5 of the Ion OneTouch™ 2 System User Guide (Pub. No. MAN0014388). Use the reagents from the appropriate kit to maintain the Ion OneTouch™ 2 Instrument.</td>
</tr>
<tr>
<td>Already using the Ion 540™ Kit – OT2.</td>
<td>Proceed to step 2. Continue to use the reagents that are provided in the Ion 540™ Kit – OT2.</td>
</tr>
</tbody>
</table>

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.
   
   **Note:** The Reaction Tube is filled with Ion OneTouch™ Oil.

4. Keep the Ion OneTouch™ Amplification Plate in the heat block.

5. 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 (see the following illustration). One of the two tabs protruding from the outer edge of the Cleaning Adapter fits into the front notch of the stage:

6. Place a 50-mL conical tube in a tube rack, then place the tube rack next to the instrument.
   
   **Note:** Steps 6–9 are only necessary if you have not already removed the disposable injector before removing the Recovery Tubes from the instrument.

7. Gently pull the disposable tubing downwards on both sides of the pinch valve until the disposable tubing is out of the valve.

8. Remove the disposable injector from the Ion OneTouch™ DL Injector Hub.
   a. Place one hand on the centrifuge lid.
   b. With the other hand, firmly grip the rigid plastic connector at the top of the disposable injector.
   c. Slowly and steadily withdraw the disposable injector straight from the port of the 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.

9. Place the used, disposable injector into the empty 50-mL conical tube in the tube rack. The conical tube is used to collect waste.
Chapter 4 Prepare template-positive Ion 540™ ISPs
Maintain the Ion OneTouch™ 2 Instrument

10. On the home screen of the instrument, touch Clean.

![Clean button on the screen]

11. Complete each task that is displayed on the screen, then touch Next. After you touch Next on the last task, a progress bar appears, and the cleaning starts.

12. 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.

13. Appropriately discard the waste that was collected in the 50-mL conical tube.

14. Remove and appropriately discard the used Amplification Plate, disposable injector, and tubing.
   a. Push the handle to open the heat block.
   b. Remove the disposable tubing from the Ion OneTouch™ DL Catch.
   c. Gently pull back the Amplification Plate from the inlet and outlet holes of the instrument.
   d. Remove the Amplification Plate from the heat block, then appropriately discard the used Amplification Plate, injector, and tubing.
   e. Leave the heat block open.

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

15. On the instrument display, touch 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.

16. Touch Next to return to the home screen on the instrument.
Enrich the template-positive Ion 540™ ISPs

Materials required

- Materials required .......................................................... 45
- Residual volume test .......................................................... 46
- Prepare reagents then fill the 8-well strip ............................... 46
- Prepare the Ion OneTouch™ ES ........................................ 48
- Perform the run ............................................................... 50
- Remove and wash the enriched ISPs ..................................... 52
- (Optional) Perform enriched ISP quality control ...................... 53

Materials required

Provided in Ion S5™ OT2 Solutions (Part No. A27747):
- Ion OneTouch™ Wash Solution
- MyOne™ Beads Capture Solution
- Ion OneTouch™ ES Wash Solution
- Tween™ Solution
- Nuclease-free Water

Provided in Ion S5™ OT2 Supplies (Part No. A27748):
- 8-well strip
- Eppendorf™ LoRetention Dualfilter Tips (P300)

Other materials and equipment:
- Dynabeads™ MyOne™ Streptavidin C1 Beads
- Eppendorf™ DNA LoBind™ Microcentrifuge Tubes
- 0.2-mL PCR tubes
- 1 M NaOH
- Pipettes
- Vortexer
- DynaMag™-2 magnet
- Microcentrifuge
Residual volume test

**IMPORTANT!** Ensure that the AC line voltage module is installed correctly into the Ion OneTouch™ ES Instrument, refer to *Unpack and Install the Ion OneTouch™ 2 System for Use with the Ion Proton™ System* (Pub. No. 4478350) or the *Ion S5™ System Site Preparation Guide* (Pub. No. MAN0010810).

<table>
<thead>
<tr>
<th>If the condition is...</th>
<th>Then...</th>
</tr>
</thead>
<tbody>
<tr>
<td>First use of the instrument and during monthly maintenance</td>
<td>Perform a residual volume test, refer to the <em>Ion OneTouch™ 2 System User Guide</em> (Pub. No. MAN0014388).</td>
</tr>
<tr>
<td>Routine use and residual volume in Well 1 and Well 8 is (\geq 5.0 , \mu\text{L})</td>
<td>Operate the instrument without performing the residual volume test. Proceed to “Prepare reagents then fill the 8-well strip” on page 46.</td>
</tr>
<tr>
<td>Routine use and residual volume in Well 1 and Well 8 is (\leq 5.0 , \mu\text{L})</td>
<td></td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Order</th>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tween&lt;sup&gt;™&lt;/sup&gt; Solution</td>
<td>280 µL</td>
</tr>
<tr>
<td>2</td>
<td>1 M NaOH</td>
<td>40 µL</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Total</strong> 320 µL</td>
</tr>
</tbody>
</table>

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

The final composition of the Melt-Off Solution is 125 mM NaOH and 0.1% Tween<sup>™</sup> 20 detergent.

1. Vortex the tube containing the Dynabeads<sup>™</sup> MyOne<sup>™</sup> Streptavidin C1 Beads for 30 seconds to thoroughly resuspend the beads, then immediately proceed to the next step.

2. Transfer 100 µL of Dynabeads<sup>™</sup> MyOne<sup>™</sup> 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<sup>™</sup> MyOne<sup>™</sup> Streptavidin C1 Beads.

4. Add 1 mL of Ion OneTouch™ Wash Solution to the aliquot of Dynabeads<sup>™</sup> MyOne<sup>™</sup> 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 40).

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

   ![8-well strip diagram]

   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 (see the figure in step 5):

<table>
<thead>
<tr>
<th>Well number</th>
<th>Reagent to dispense in well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well 1[1]</td>
<td><em>Entire</em> template-positive ISP sample (100 µL; prepared in step 1 of this procedure)</td>
</tr>
<tr>
<td>Well 2</td>
<td>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 46)</td>
</tr>
</tbody>
</table>
### Chapter 5 Enrich the template-positive Ion 540™ ISPs

#### Prepare the Ion OneTouch™ ES

#### Table: Reagents to Dispense in Each Well

<table>
<thead>
<tr>
<th>Well number</th>
<th>Reagent to dispense in well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well 3</td>
<td>300 µL of Ion OneTouch™ ES Wash Solution</td>
</tr>
<tr>
<td>Well 4</td>
<td>300 µL of Ion OneTouch™ ES Wash Solution</td>
</tr>
<tr>
<td>Well 5</td>
<td>300 µL of Ion OneTouch™ ES Wash Solution</td>
</tr>
<tr>
<td>Well 6</td>
<td>Empty</td>
</tr>
<tr>
<td>Well 7</td>
<td>300 µL of freshly-prepared Melt-Off Solution [prepared in &quot;Prepare Melt-Off Solution&quot; on page 46]</td>
</tr>
<tr>
<td>Well 8</td>
<td>Empty</td>
</tr>
</tbody>
</table>

**Note:** 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 tray:

![](image)

**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.

![Diagram showing steps 1 to 3]

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

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.
   a. Tilt the Tip Arm back (below left), then align the pins with the round notches in the cradle (below center).
   b. Lower the Tip Arm into position (below center).
   c. Move the Tip Arm forward into the working position (below right).

![Diagram showing steps 4 and 5]

1. Tip Arm pins resting in the notches in 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.

**IMPORTANT!** Remove the enriched ISPs ≤15 minutes after 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.

*Note:* 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.

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?

<table>
<thead>
<tr>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No</strong></td>
</tr>
<tr>
<td>1. Remove all but ~10 μL of supernatant without disturbing the pellet.</td>
</tr>
<tr>
<td>2. Add sufficient Nuclease-free Water for a final volume of 100 μL.</td>
</tr>
<tr>
<td>3. Pipet up and down 10 times to resuspend the pellet.</td>
</tr>
<tr>
<td>4. Sequence or store the template-positive ISPs: Proceed to Chapter 6, “Initialize the sequencer”, or Store the enriched ISPs at 2°C to 8°C for up to 3 days.</td>
</tr>
</tbody>
</table>

| **Yes** |
| 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: Proceed to Chapter 6, “Initialize the sequencer”, or Store the enriched 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 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.
Initialize the sequencer

- Ion S5™ System component positions ........................................ 54
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- When a manual cleaning of the sequencer is required ..................... 55
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Initialization takes ~40 minutes.

**Ion S5™ System component positions**

1. Touchscreen
2. Power button
3. Ion S5™ Sequencing Reagents cartridge
4. Chip clamp
5. Ion S5™ Wash Solution bottle. Waste reservoir located behind the Wash Solution bottle (shown on the right).
6. Ion S5™ Cleaning Solution bottle
7. Waste reservoir

**Note:** The system uses RFID technology to verify that the proper reagents are loaded in positions 3, 5, and 6. Reagents that exceed their expiration date or usage count generate an error message prompting the user to replace the reagent before performing the run.

**Note:** RFID regulatory information can be found on the main screen under Options ➔ Regulatory info.
Before you begin

The Ion S5™ and Ion S5™ XL Sequencers are equipped to verify the compatibility of each chip and consumable loaded during initialization and sequencing, and that these components do not exceed their expiration date. To avoid exceptions during initialization, inspect this information for each consumable before installing onto the instrument.

- Unbox the Ion S5™ Sequencing Reagents cartridge 45 minutes before use, then allow it to equilibrate to room temperature.
  Do not remove the Ion S5™ Sequencing Reagents cartridge from its foil packaging until immediately before loading, so that you can return the unused cartridge to storage if your sequencing run is delayed.

- Unbox the Ion S5™ Wash Solution bottle. Invert the bottle 5 times, then swirl at an angle to mix thoroughly.

- Remove the red cap from the Ion S5™ Wash Solution and Ion S5™ Cleaning Solution bottles immediately before installing on the instrument.

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. When two sequencing runs are performed on a single initialization, the post-run cleaning is performed after the second sequencing run. However, if the “Enable post-run clean” checkbox (see “Start the sequencing run”) 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. See “Perform a manual cleaning of the sequencer” on page 76 for more information on how to perform a manual cleaning.

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. See “Perform an instrument reset run with an initialized, unused Sequencing Reagents cartridge” on page 77 for more information.
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. Ensure the new Ion S5™ Wash Solution bottle is thoroughly mixed. 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.
   
   Note: The Ion S5™ Cleaning Solution bottle contains sufficient reagent to complete 4 cleanings.

8. Close the door, then touch Next.
   
   The instrument confirms that the consumables and chip are properly installed and that the Ion S5™ Cleaning Solution bottle contains sufficient reagent to perform the post-run clean. Follow all on-screen recommendations to ensure proper installation of required consumables.

   IMPORTANT! If the allowed number of post-run cleans has been met, the instrument prompts the user to replace the Ion S5™ Cleaning Solution bottle.

   Note: See if a Checking Reagent: Failed warning appears.

9. When initialization is complete (~30–40 minutes), touch Home.
   
   The instrument is now ready for a sequencing run.

### Reagent consumables disposal

**IMPORTANT!** Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of Ion S5™ reagent consumables.

**CO₂ scrubber removal and disposal**

To properly dispose of the CO₂ scrubber, you must first remove it from the Ion S5™ Sequencing Reagents cartridge.

1. Invert the Ion S5™ Sequencing Reagents cartridge over an appropriate receptacle to drain all residual liquid.

2. Wearing gloves, insert the Ion S5™ Cartridge Tool firmly into the CO₂ scrubber until the flange stops on the top of the cartridge.
3. Pull straight up on the tool while holding the nucleotide reagent cartridge down.

4. Remove the scrubber from the cartridge tool, then dispose of the scrubber according to applicable hazardous waste regulations.

Recycle Ion S5™ Wash and Cleaning solution bottles

The Ion S5™ Wash solution and Ion S5™ Cleaning solution bottles are made of recyclable plastic.

1. Open the expended bottle by unscrewing the cap.

2. Remove the cap, sipper, and filter, then pour any residual liquid into an appropriate receptacle.

3. Rinse the empty bottle with water. Pour out the rinse water into the same liquid waste receptacle.

4. Recycle or dispose of the clean bottle, cap, sipper, and filter according to applicable regulations.
Load the Ion 540™ Chip

Materials required

Materials provided in the Ion 540™ Loading Reagents OT2 Kit (Part No. A27897)
- Ion S5™ Annealing Buffer
- Ion S5™ Foaming Solution
- Ion S5™ Loading Buffer
- Ion S5™ Sequencing Polymerase
- Ion S5™ Sequencing Primer
- Ion 540™ Control Ion Spheres

Other Materials and Equipment:
- Ion 540™ Chip Kit (Cat. No. A27766)
- Enriched template-positive Ion Sphere™ Particles prepared with the Ion 540™ Kit – OT2 (Cat. No. A27753)
- Rainin™ Pipet-Lite™ XLS LTS with RFID pipette and tips
- P200 and P10 pipette and filtered tips
- Vortex mixer
- Molecular-biology grade nuclease-free water
- Thermal cycler with heated lid (programmed at 95°C for 2 minutes and 37°C for 2 minutes)
- Ion Chip™ Minifuge (Cat. No. 4479672 or 4479673) equipped with Ion Proton™ Rotor and Buckets (Cat. No. 4482578)
Before you begin

**IMPORTANT!** Use enriched, template-positive Ion Sphere™ Particles prepared using the Ion 540™ Kit – OT2.

**IMPORTANT!** For best results, begin your run within 4 hours after initialization completes.

- Thaw the Sequencing Primer on ice.
- Prior to initial use, thaw the Ion S5™ Annealing Buffer, Ion S5™ Foaming Solution, and Ion S5™ Loading Buffer at room temperature.

**IMPORTANT!** Do not refreeze. After thawing store at room temperature.

- Prepare the following stock solutions fresh weekly or as needed:
  - **50% Annealing Buffer.** In a 1.5-mL tube, combine 0.5 mL Ion S5™ Annealing Buffer with 0.5 mL of nuclease-free water (you need 509 µL of 50% Annealing Buffer for each run).
  - **Flush solution.** In a 1.5-mL tube, combine 0.25 mL of 100% isopropanol with 0.25 mL of Ion S5™ Annealing Buffer (for use in “Flush the chip and load the Ion S5™ Sequencing Polymerase”; you need 200 µL of Flushing solution for each run).

**Note:** You can prepare multiple 1-mL aliquots of stock solutions and store at room temperature. After opening an aliquot, use the contents within 1 week. Discard any opened, unused solution after 1 week.

- Check for updates to the Torrent Suite™ Software and Ion S5™ System software, and install the updates if available.
- Before using the Ion Chip™ Minifuge for the first time, set up and test the Ion Chip™ Minifuge (refer to the Ion S5™ and Ion S5™ XL Instrument User Guide (Pub. No. MAN0010811).
Chip loading guidelines

Ion 540™ Chip

When loading a sample:

- Place the chip on a flat, stable surface such as a benchtop.
- Pipet the sample into the chip loading well.
Chapter 7 Load the Ion 540™ Chip

Prepare the template-positive ISPs for sequencing

When injecting reagents or buffers:

- Place the chip on a flat, stable surface such as a benchtop.
- With the pipette tip at a 90° angle to the chip, press the tip firmly into the circular loading port, and apply gentle pressure between the pipette tip and chip.

- Pipet carefully to avoid introducing bubbles into the chip flow cell (see example with introduced air bubbles).

- After each injection, remove the expelled liquid from the exit well, opposite the loading well.

Add Control Ion Sphere™ Particles to the enriched ISPs

**IMPORTANT!** If you are performing an installation or troubleshooting run, do not use enriched ISPs. Refer to “Troubleshooting using Control Ion Sphere™ Particles” in Ion OneTouch™ 2 System User Guide (Pub. No. MAN0014388) to prepare the Ion 540™ Control Ion Spheres for the installation or troubleshooting run.

**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.
**Annot Sequencing Primer to the enriched ISPs**

**Note:** The Ion 540™ ISPs are difficult to see. To avoid aspirating the particles in the following steps, orient the PCR tube the same way each time when centrifuging so that it is easy to know where the pellet has formed, and remove the supernatant from the top down.

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 sequencing chip**

**Load the sample on the chip**

We recommend performing chip loading while the sequencer is initializing. By loading the chip while the sequencer initializes, you ensure that the chip can be sequenced as soon as possible after loading is complete. See “Initialize the sequencer” on page 56 for more information.

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.
   
   **Note:** Some sample will enter the flow cell at this point by capillary action; the remaining sample will be loaded into the flow cell by centrifugation.

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).

**Note:** You can prepare a bulk mixture by combining 4.9 mL of 50% Annealing Buffer with 100 µL of Foaming Solution. The mix can be stored at 4°C and used for up to 6 months.

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.

**Note:** Do not over-inject the air; the final volume of foam should be approximately 250 µL.

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).

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.

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 “Start the sequencing run”.
Start the sequencing run

- Chip handling guidelines .............................................. 66
- Start the sequencing run .............................................. 67
- Maintain the sequencer ............................................... 69

Chip handling guidelines

To place a chip in the chip clamp:

1. Slide the chip clamp out.
2. Remove the chip currently in the clamp.
3. Place the appropriate loaded chip in the chip clamp with the chip notch in the bottom-front corner.

Note: Do not force the chip into the clamp. If the chip does not fit easily in the clamp, confirm that the notch is oriented as shown in the drawing.

4. Slide the metal tab in fully to engage the clamp, then close the instrument door.
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.

![Select Run](image)

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.
Maintain the sequencer

Required materials
• Lint-free wipes
• 70% isopropanol
• (optional)– 10% bleach solution

Clean or decontaminate the sequencer

In the event of a spill or leak on or inside the instrument, perform the following steps.

Note: Dispose of all waste in appropriate liquid or solid waste containers.

1. Remove the Ion S5™ Wash Solution bottle, then remove and empty the waste reservoir.

2. Remove the Ion S5™ Sequencing Reagents cartridge.

3. Inspect the waste and nucleotide reagent bays for liquid.

4. Using absorbent paper soak up as much liquid as possible, then wash the affected area with 10% bleach solution.

5. Wipe the affected surfaces with 70% isopropanol, then allow to air-dry.
### Troubleshooting

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- **Ion OneTouch™ ES** .......................................................... 72
- **Ion S5™ System setup and operation** ................................. 74
- **Ion Sphere™ Assay troubleshooting table** .......................... 75

## Ion OneTouch™ 2 Instrument

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disposable injector remains in “down” position in the Ion OneTouch™ DL Injector Hub</td>
<td>Reagent has built up around Injector Hub</td>
<td>Clean any excess reagent from Injector Hub with moistened Kimwipes™ wipe.</td>
</tr>
</tbody>
</table>
| Newly installed Injector Hub does not move freely. | | 1. *Gently* pull from the top of the disposable injector until the disposable injector just returns to the “up” position in the Injector Hub.  
2. Briefly press then release the spring-loaded top of the Injector Hub 5–10 times at the point indicated by the arrow. You should hear a click.  
3. If the Injector Hub remains in the “down” position, repeat step 2 once (up to 10 more clicks).  

**Note:** If the Injector Hub still does not move freely and click up into place, contact Technical Support. |
<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifuge lid does not open</td>
<td>• A power failure has occurred.</td>
<td>1. Slide a 1/8-inch L-wrench (hex wrench) or equivalent tool into the right hole and along the top edge of the centrifuge hinge:</td>
</tr>
<tr>
<td></td>
<td>• A software crash has occurred.</td>
<td>2. Push the tool into the hole until there is a slight compression of the tool against the instrument and the centrifuge lid unlocks and opens.</td>
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<tr>
<td></td>
<td></td>
<td>3. Remove the tool from the hole, then open the lid.</td>
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<tr>
<td></td>
<td></td>
<td>4. If necessary, troubleshoot the lid lock, then use the instrument normally. Do not force the lid open.</td>
</tr>
<tr>
<td>The run fails</td>
<td>Various causes are possible.</td>
<td>Retrieve the log files.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IMPORTANT! Do not turn off or power cycle the instrument until the log files are downloaded. See the Ion S5™ and Ion S5™ XL Instrument User Guide (Pub. No. MAN0010811). If a run fails, contact Technical Support.</td>
</tr>
<tr>
<td>Precipitate is visible in the Ion S5™ Reagent Mix after vortexing</td>
<td>Thawed Reagent Mix has been stored at &lt;2°C.</td>
<td>1. Ensure that the solution is fully thawed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Vortex the solution for 30 seconds, then leave the tube at room temperature for 15 minutes.</td>
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<tr>
<td></td>
<td></td>
<td>3. Vortex the solution again at maximum speed for 1 minute.</td>
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<td></td>
<td>4. Centrifuge the tube for 30 seconds.</td>
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<tr>
<td></td>
<td></td>
<td>5. Visually inspect the solution at the bottom of the tube and verify that there is no residual precipitate. If precipitate is visible, then repeat steps 1–4.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Note: If precipitate is still visible, then contact Technical Support.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6. Keep the Reagent Mix at room temperature during use.</td>
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<tr>
<td></td>
<td></td>
<td>7. After use, store the solution at 2°C to 8°C.</td>
</tr>
<tr>
<td>Observation</td>
<td>Possible cause</td>
<td>Recommended action</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>---------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Recovery Tubes filled with gel</td>
<td>Breaking Solution was not added to Recovery Tubes prior to start of run.</td>
<td>1. Add 150 μL Ion OneTouch™ Breaking Solution to each Recovery Tube.</td>
</tr>
<tr>
<td>after run.</td>
<td></td>
<td>2. Seal the Recovery Tubes with Parafilm™ M film and vigorously invert and vortex the tubes for 10 seconds to break the emulsion.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Follow the Final Spin procedure [see “Recover the template-positive Ion Sphere™ Particles” on page 39] to recover the ISPs. Alternatively, transfer samples to two 1.5-mL tubes and spin in an Eppendorf™ centrifuge for 10 minutes at 15,500 × g to pellet the ISPs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Proceed to the ISP washing steps [see “Wash the template-positive ISPs” on page 40]. Perform the ISP wash in 1 mL Nuclease-free Water (steps 5–8) twice.</td>
</tr>
</tbody>
</table>

### Ion OneTouch™ ES

For Ion OneTouch™ ES vertical and horizontal axis calibration and residual volume test procedures, see Chapter 3 of the Ion OneTouch™ 2 System User Guide (Pub. No. MAN0014388).

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excessive foaming occurs</td>
<td>• Instrument is improperly calibrated resulting in inadequate volume in one or more wells. • Fitting is loose. • Pipette tip is cracked.</td>
<td>1. Use the recommended volumes for all wells. 2. Ensure that fittings are tight, especially at the elbow fitting, and the pipette tip is not cracked. 3. If necessary, perform the residual volume test. If the residual volume test fails, then calibrate the instrument.</td>
</tr>
<tr>
<td>Solution overflows during run</td>
<td>Reagent volumes are overloaded.</td>
<td>Repeat with reagent volumes described in enrichment procedure.</td>
</tr>
<tr>
<td>Tip is causing 8-well strip to lift out of Tray slot during run</td>
<td>Tip is not aligned vertically</td>
<td>Perform vertical calibration procedure, refer to the Ion OneTouch™ 2 System User Guide (Pub. No. MAN0014388).</td>
</tr>
<tr>
<td>Percent template-positive ISPs after enrichment is &lt;50% as measured by flow cytometry</td>
<td>Multiple causes are possible.</td>
<td>Contact Technical Support.</td>
</tr>
<tr>
<td>Observation</td>
<td>Possible cause</td>
<td>Recommended action</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>One of the following:</td>
<td>Instrument is not calibrated properly</td>
<td>• Perform horizontal position calibration.</td>
</tr>
<tr>
<td>• Strip lifts up during strip push</td>
<td></td>
<td>• Perform vertical calibration.</td>
</tr>
<tr>
<td>• Strip lifts up when tip is raised from well</td>
<td></td>
<td>• Perform horizontal position calibration.</td>
</tr>
<tr>
<td>• Immediately after strip push, the strip is not in contact with the magnet</td>
<td></td>
<td>Refer to the <em>Ion OneTouch™ 2 System User Guide</em> (Pub. No. MAN0014388) for more information.</td>
</tr>
<tr>
<td>Tip grinds into base of instrument and Code “1999” displays</td>
<td>• Unit not calibrated properly</td>
<td>1. Erase the memory on the instrument:</td>
</tr>
<tr>
<td></td>
<td>• Vertical calibration setting too low or out-of-range</td>
<td>Hold down the vertical adjust button while powering ON the instrument. The</td>
</tr>
<tr>
<td></td>
<td></td>
<td>instrument beeps several times.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Recalibrate the instrument.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Perform a residual volume test.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Refer to the <em>Ion OneTouch™ 2 System User Guide</em> (Pub. No. MAN0014388) for more</td>
</tr>
<tr>
<td></td>
<td></td>
<td>information.</td>
</tr>
<tr>
<td>Tip hits the top of the tray at start of run</td>
<td>Tray is not properly seated in the instrument.</td>
<td>Check for debris between the tray and the instrument, then reinstall the tray.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Press down firmly to ensure that tray is fully seated in the instrument.</td>
</tr>
<tr>
<td>Instrument does not aspirate or dispense liquids</td>
<td>Fitting[s] are loose.</td>
<td>• Ensure that the Luer-Lok™ connections at the elbow on the Tip Arm and at the</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tubing on the rear syringe pump are finger-tight.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ensure that the metal tip adapter fitting on the Tip Arm is finger-tight.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>IMPORTANT!</strong> After any adjustments to the metal tip adapter, recalibrate the</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Ion OneTouch™ ES</em>.</td>
</tr>
</tbody>
</table>
Ion S5™ System setup and operation

Ion S5™
Sequencer alarms
and events

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red “Alarms” and/or “Events” message in Main Menu</td>
<td>• Software updates available&lt;br&gt; • Connectivity issues&lt;br&gt; • Instrument not detecting required files or hardware</td>
<td>Click on the red pop-up to see detailed messages.&lt;br&gt; • If a message states “Newer Software Available”:&lt;br&gt; <strong>IMPORTANT!</strong> After updates are installed, the instrument must be restarted.&lt;br&gt; a. On the home screen, select Settings ➤ Check for Updates.&lt;br&gt; b. Select the Released Updates checkbox, then press Update.&lt;br&gt; c. When installation is complete, follow the onscreen prompts to restart the instrument.&lt;br&gt; <strong>Note:</strong> In some cases, the instrument restarts automatically after software installation.&lt;br&gt; • If a message states “No Connectivity to Torrent Server”, “No Connectivity to ftp server”, or “Network Manager not connected”, disconnect and re-connect the ethernet cable, confirm that the router is operational, and verify that the network is up and running.&lt;br&gt; • For any other messages:&lt;br&gt; a. Power off the instrument: On the home screen, select Settings ➤ System Tools ➤ Shut Down ➤ Shut Down.&lt;br&gt; b. Wait 30 seconds, then press the button on the front of instrument to power on the instrument.&lt;br&gt; • If the red “Alarms” and/or “Events” message still appears in the main menu, contact Technical Support.</td>
</tr>
</tbody>
</table>
### Ion Sphere™ Assay troubleshooting table

The following table contains troubleshooting information for unenriched ISPs tested with the Ion Sphere™ Assay on the Qubit™ 2.0 or Qubit™ 3.0 Fluorometer.

<table>
<thead>
<tr>
<th>Qubit™ Fluorometer observation</th>
<th>Ion S5™ System observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| <10% Templated ISPs             | • Lower loading            | Too little library input into template preparation | • Increase library input to target 20–25% templated ISPs.  
• Lower % enriched  
• Lower key signal  
• Lower throughput | or  
• Continue with sequencing; expect lower throughput. |
|                                | Increased number of filtered reads | Too much library input into template preparation | • Decrease library input to target 20–25% templated ISPs.  
• Continue with sequencing; expect lower throughput. |
| >70% Templated ISPs             | • Increased % primer dimer filtered reads  
• Lower throughput | Adapter dimer contaminating library, more likely in short amplicon, Ion AmpliSeq™ or miRNA libraries | • Check Bioanalyzer™ traces for adapter dimer peak (Amplicon library or Ion AmpliSeq™ library peak around 70 bp; miRNA library peak around 60bp).  
• Re-purify library using Agencourt™ AMPure™ XP Kit clean-up steps as outlined in the appropriate user guides. |
|                                | • Low loading  
• Low % enriched  
• Lower throughput  
• High % filtered reads | Ion OneTouch™ 2 Instrument underperformance | Troubleshoot with Technical Support or a Field Application Scientist. |
Supplemental procedures

- Perform a manual cleaning of the sequencer ........................................... 76
- Perform an instrument reset run with an initialized, unused Sequencing Reagents cartridge .................................................... 77
- Quality control of Ion 540™ ISPs .......................................................... 78

Perform a manual cleaning of the sequencer

A cleaning protocol is normally performed automatically at the completion of each sequencing run. In the event that a cleaning is necessary, follow the listed steps:

1. On the home screen, select Settings ➤ Clean Instrument.
   The instrument door unlocks allowing access to the consumables.

2. 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 and the used Ion S5™ Wash Solution bottle.

4. Ensure the Ion S5™ Sequencing Reagents cartridge and Ion S5™ Cleaning Solution bottle are properly installed.

   IMPORTANT! Perform the cleaning with a used reagent cartridge and wash solution bottle installed. The cleaning procedure pumps cleaning solution into the wash solution bottle and reagent cartridge making them unsuitable for sequencing.

5. Place a used sequencing chip in the chip clamp, then push the chip clamp in all the way to engage.
6. Close the instrument door, then press Next.
   Cleaning takes ~35 minutes to complete. Upon completion the instrument door automatically unlocks and the chip and cartridge clamps disengage.

7. Proceed to “Initialize the sequencer” on page 56.

**Perform an instrument reset run with an initialized, unused Sequencing Reagents cartridge**

Cleaning is normally performed at completion of a sequencing run automatically. If an Ion S5™ Sequencer or an Ion S5™ XL Sequencer is initialized and
- a sequencing run is not started within 24 hours after initialization, or
- a sequencing run is not completed due to a power failure or an abort, and <200 flows occurred before the stoppage

an instrument reset run is required to ensure proper cleaning before reinitialization. Do NOT perform a manual cleaning with an unused, initialized Ion S5™ Sequencing Reagents cartridge.

**Note:**
- If a power failure or abort occurs during the second of two runs started after a single initialization, a manual cleaning (page 76) is sufficient.
- If the number of flows that occurred before a power failure or abort is unknown, perform an instrument reset run.

To perform an instrument reset run, use the following procedure before reinitialization:

1. In the instrument touchscreen main menu, press Run. The instrument door and chip clamp unlocks.

2. Ensure that a used sequencing chip is in the chip clamp, then push the chip clamp in all the way to engage.

3. Close the instrument door, then press Next.

4. When prompted, select Planned Run (none). Ensure that the Enable post-run clean checkbox is selected, then press Review.

5. In the Select Run screen, press Edit, then in the Detail screen set the number of flows to 200 manually. Ensure that the Post-Run/Clean checkbox is selected, then press Close.

6. Press Start run, then press Accept to confirm that Post-Run Clean is enabled, and to start the run.

When the instrument reset run completes, the instrument automatically performs the cleaning procedure. After cleaning, the touchscreen returns to the main menu.
Quality control of Ion 540™ ISPs

Quality control using the Ion Sphere™ Assay on the Qubit™ Fluorometer

The Qubit™ 2.0 Fluorometer or the Qubit™ 3.0 Fluorometer can be used to perform a quality assessment of unenriched Ion 540™ ISPs generated for up to 200 base-read sequencing on the Ion S5™ or Ion S5™ XL Sequencer. For detailed protocols, see
- Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide (Pub. No. MAN0016387)
- Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide (Pub. No. MAN0016388)

available at thermofisher.com/order/catalog/product/4468656.

Acceptance criteria for unenriched Ion 540™ ISPs

The optimal amount of library corresponds to the library dilution point that gives Percent Templated ISPs between 10–25%.

Samples that fall within the recommended range generally produce the most data; however, samples that fall outside of the recommended range can still meet the throughput specifications on the Ion chips.

The recommended optimal range is not intended to be a pass/fail criteria. The range provides guidance for the quality of the sample.

Note: If the results are outside the desired Percent Templated ISPs range, then increase or decrease the library input appropriately. See the “Ion Sphere™ Assay troubleshooting table” on page 75 for more information.

<table>
<thead>
<tr>
<th>Percent Templated ISPs</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10%</td>
<td>Sample contains an insufficient number of templated ISPs to achieve optimal loading density on the Ion Chip.</td>
</tr>
<tr>
<td>10–25%</td>
<td>Optimal amount of library.</td>
</tr>
<tr>
<td>&gt;25%</td>
<td>Sample will yield multi-templated ISPs (mixed reads).</td>
</tr>
</tbody>
</table>

Quality control using the Guava™ easyCyte™ 5 Flow Cytometer

The Guava™ easyCyte™ 5 Flow Cytometer can be used for quality assessment of unenriched and enriched Ion Sphere™ Particles generated for up to 200 base-read sequencing on the Ion S5™ System. For details, 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.

Quality control using the Attune™ Acoustic Focusing Cytometer

The Applied Biosystems™ Attune™ Acoustic Focusing Cytometer can be used for quality assessment of unenriched and enriched Ion Sphere™ Particles generated for up to 200 base-read sequencing on the Ion S5™ or Ion S5™ XL Sequencer. For details, see the Demonstrated Protocol: Ion Sphere™ Particles (ISPs) Quality Assessment using the Applied Biosystems™ Attune™ Acoustic Focusing Cytometer User Bulletin (Pub. No. 4477181), available at thermofisher.com.

IMPORTANT! Thermo Fisher Scientific Demonstrated Protocols have been successfully demonstrated by research and development but not verified. There are no technical specifications for demonstrated protocols. Users assume all risk when using these protocols, and recognize that support for Thermo Fisher Scientific Demonstrated Protocols occurs through community discussion.
WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
Instrument safety

For detailed information on instrument safety symbols and alerts, safety and electromagnetic compatibility standards, and general instrument safety, see the Safety appendices of the Ion OneTouch™ 2 System User Guide (Pub. No. MAN0014388) and the Ion S5™ and Ion S5™ XL Instrument User Guide (Pub. No. MAN0010811), available at thermofisher.com.

Chemical safety

**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.
Biological hazard safety

**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

Documentation and support

Customer and technical support

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- Worldwide contact telephone numbers
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- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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