

# Ion Sphere<sup>™</sup> Quality Control Kit

## USER GUIDE

for use with:

Qubit<sup>™</sup> 4 Fluorometer

Qubit<sup>™</sup> 3.0 Fluorometer

Qubit<sup>™</sup> 2.0 Fluorometer

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Revision	Date	Description
A.0	16 February 2018	New user guide for using the Ion Sphere™ Quality Control Kit with the Qubit™ 4, Qubit™ 3.0, or Qubit™ 2.0 Fluorometer.

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# Product information

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

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## Product description

The Ion Sphere™ Quality Control Kit (Cat. No. 4468656) provides reagents for performing 20 Ion Sphere™ Assays to determine the percentage of template-positive Ion Sphere™ Particles (ISPs) in ISP samples, using one of the following Qubit™ Fluorometers:

- Qubit™ 4 Fluorometer
- Qubit™ 3.0 Fluorometer
- Qubit™ 2.0 Fluorometer

Templated ISP samples with an acceptable percentage of template-positive ISPs are appropriate for loading on sequencing chips and running on Ion GeneStudio S5 Series, Ion S5™/Ion S5™ XL, Ion PGM™, and Ion Proton™ Systems.

**Note:** The Qubit™ 2.0 Fluorometer and Qubit™ 3.0 Fluorometer are discontinued and no longer available for purchase, but continue to be supported by Thermo Fisher Scientific.

## Kit contents and storage

The Ion Sphere™ Quality Control Kit provides reagents for performing templated ISP quality control with the Ion Sphere™ Assay.

Component	Amount	Storage
Ion Probes (blue cap)	20 µL	–30°C to –10°C
Alexa Fluor™ 488 Calibration Standard (green cap)	400 µL	
Alexa Fluor™ 647 Calibration Standard (red cap)	400 µL	
Annealing Buffer (white cap)	400 µL	
Quality Control Wash Buffer	20 mL	–30°C to –10°C or 2°C to 8°C

## Required materials not supplied

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

Item	Source
Qubit™ Fluorometer: <ul style="list-style-type: none"> <li>Qubit™ 4 Fluorometer</li> <li>Qubit™ 3.0 Fluorometer<sup>[1]</sup></li> <li>Qubit™ 2.0 Fluorometer<sup>[1]</sup> with v3.11 firmware and the Ion Sphere™ Quality Control assay plugin</li> </ul>	Q33226
Qubit™ Assay Tubes	Q32856
PCR tubes, 0.2 mL (Axygen™ Maxymum Recovery PCR Tube, or equivalent)	Fisher Scientific 14-222-283, or MLS
Qubit™ Easy Calculator Microsoft™ Excel™ Spreadsheet file	Download at Ion Sphere™ Quality Control Kit product page ( <b>thermofisher.com/4468656</b> )
SimpliAmp™ Thermal Cycler, or equivalent	A24811
Microcentrifuge	MLS
Unenriched template-positive ISPs	Provided by user

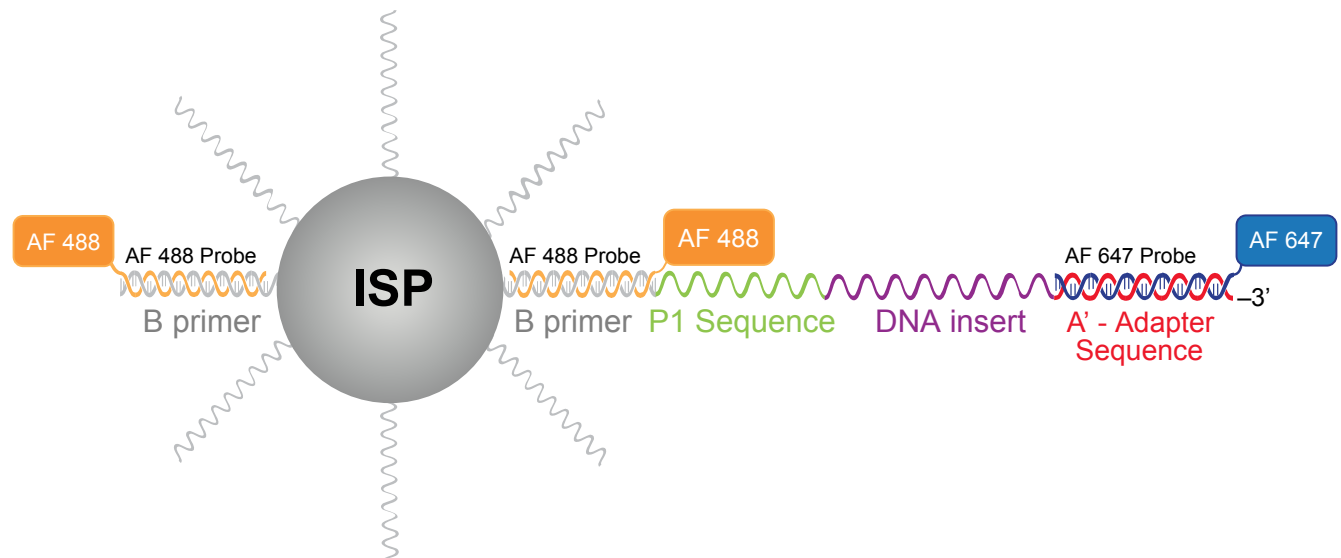
<sup>[1]</sup> Supported but no longer available for purchase

## Ion Sphere™ Assay overview

The Ion Sphere™ Assay on the Qubit™ Fluorometer measures the fluorescence of template-positive ISPs labeled with two fluorophores: Alexa Fluor™ 488 and Alexa Fluor™ 647.

- A probe that is labeled with Alexa Fluor™ 488 anneals to primer B sites, or all the ISPs present.
- A probe that is labeled with Alexa Fluor™ 647 anneals to primer A sites, or only the ISPs with extended templates.

The ratio of the Alexa Fluor™ 647 fluorescence (templated ISPs) to the Alexa Fluor™ 488 fluorescence (all ISPs present) yields the percent templated ISPs.



Alexa Fluor™ 488- and Alexa Fluor™ 647-labeled probes annealed to an ISP



# Calculate the Calibration Factor

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This chapter describes the procedure to determine the instrument-specific Calibration Factor for your Qubit™ Fluorometer. Each Qubit™ Fluorometer instrument has a unique Calibration Factor that must be calculated and applied to all percent templated ISPs calculations.

It is only necessary to calculate the Calibration Factor one time for a particular instrument, unless a problem is suspected. If you have already determined Calibration Factor for your instrument, proceed to Chapter 3, “Measure the percent templated ISPs”.

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**IMPORTANT!** If you are using a Qubit™ 2.0 Fluorometer:

- Upgrade the instrument firmware and software to the latest version before performing the following procedure. See “Update the instrument software—Qubit™ 2.0 Fluorometer” on page 24 for more information.
  - Upload the Ion Sphere™ Assay plugin file, if needed. See “Upload the Ion Sphere™ Quality Control assay plugin—Qubit™ 2.0 Fluorometer only” on page 24 for more information.
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## Download the Qubit™ Easy Calculator

Download the Qubit™ Easy Calculator, a Microsoft™ Excel™ spreadsheet file, from the Ion Sphere™ Quality Control Kit product page at [thermofisher.com/4468656](http://thermofisher.com/4468656), then save the file to the computer used for Qubit™ Fluorometer data analysis.



## Prepare the calibration standard

1. From the Ion Sphere™ Quality Control Kit, thaw the Alexa Fluor™ 488 and Alexa Fluor™ 647 Calibration Standard reagents.

**Note:** Both the Alexa Fluor™ 488 and Alexa Fluor™ 647 molecules are photosensitive, so avoid exposure to direct sunlight, and to room light for long lengths of time.

2. Vortex well to mix, then pulse-centrifuge the tube to remove any liquid that is trapped in the cap.
3. Transfer 200 µL of each standard into two separate Qubit™ assay tubes, then pulse-centrifuge to bring all the liquid to the bottom of the tube.

## Measure the calibration standard—Qubit™ 2.0 Fluorometer

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**IMPORTANT!** Before using the Qubit™ 2.0 Fluorometer, ensure that the instrument is running firmware V3.10 or V3.11, and the Ion\_PluginV310\_AF.qbt file or later has been uploaded to the instrument.

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1. Press **Ion** to access Alexa Fluor™ 488 and Alexa Fluor™ 647 measurement options.
2. Press **AF 488**, insert the Alexa Fluor™ 488 Calibration Standard reagent into the Qubit™ 2.0 Fluorometer, close the lid, then press **Read**.

**Note:** The lettering on the Read () selection option changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

3. Record the RFU value, then remove the sample from the Qubit™ 2.0 Fluorometer.
4. Press **Home** ▶ **Ion** ▶ **AF 647**. Insert the Alexa Fluor™ 647 Calibration Standard into the Qubit™ 2.0 Fluorometer, close the lid, then press **Read**.

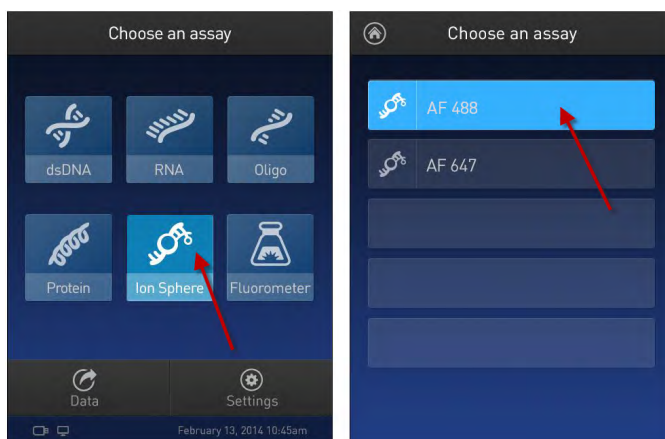
**Note:** The lettering on the Read () selection option changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

5. Record the RFU value, then remove the sample from the Qubit™ 2.0 Fluorometer.

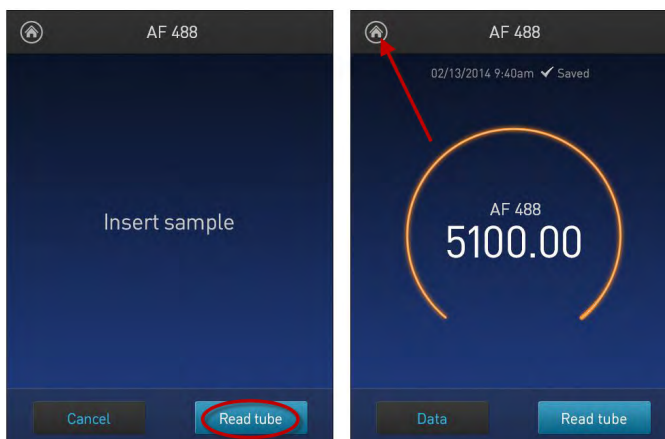
Proceed to “Calculate the Calibration Factor” on page 12.


## Measure the calibration standard—Qubit™ 3.0 or Qubit™ 4 Fluorometer

1. Power on the Qubit™ 3.0 or Qubit™ 4 Fluorometer.
2. Press **Ion Sphere** on the home screen to open the Ion Sphere™ Assay, then press **AF 488**.

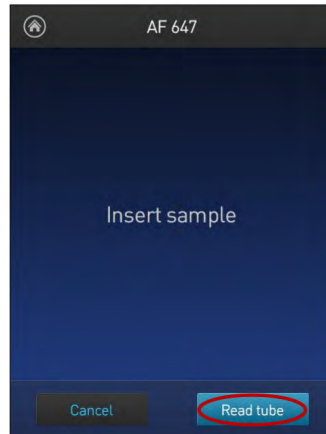


3. Insert the Alexa Fluor™ 488 Calibration Standard reagent into the fluorometer, close the lid, then press **Read tube**.

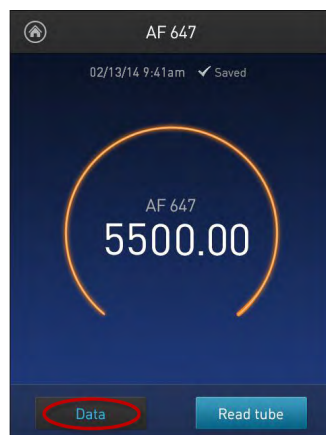


4. Record the RFU value, remove the assay tube from the fluorometer, then press the **Home**  icon in the upper left corner of the screen.

5. On the home screen, press **Ion Sphere ▶ AF 647**. Insert the Alexa Fluor™ 647 Calibration Standard into the fluorometer, close the lid, then press **Read tube**.



6. Record the RFU value, remove the assay tube from the fluorometer, then press **Data**.



7. Press **Export** to export data to a USB storage drive or to a USB-connected computer. Press **Done** to return to the home screen.



## Calculate the Calibration Factor

1. In the Qubit™ Easy Calculator, enter each recorded RFU value in the appropriately labeled green cell to display the Calibration Factor specific for your Qubit™ Fluorometer.
2. Save a copy of the Qubit™ Easy Calculator containing the Calibration Factor for use as a template for future Percent Templated ISPs calculations:

**Note:** Affix a sticker with the instrument-specific Calibration Factor to the fluorometer.

A	B	C	D	E	F	G	H	I	J
Qubit Calibration Factor Calculation									
Calibration Standard	RFU	Calibration Factor							
Alexa Fluor® 488 Calibration Standard		#DIV/0!							
Alexa Fluor® 647 Calibration Standard									
Percent Templated ISPs									
	Raw RFU Value		Background RFU (Negative Control Tube)						
Sample ID	AF 488	AF 647	AF 488	AF 647	Conversion Factor*	Percent Templated ISPs			
						#DIV/0!			
						#DIV/0!			
						#DIV/0!			
						#DIV/0!			
						#DIV/0!			
						#DIV/0!			
						#DIV/0!			
Green Cells = Raw RFU values of Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standards supplied in the Ion Sphere Quality Control Kit									
Red Cells = Raw RFU values measured in "Measure the templated unenriched sample"									
Purple Cells = Raw RFU values measured for negative control in "Measure the templated unenriched sample"									
Blue Cells= Template kit lot specific conversion factor									

**IMPORTANT!** For each fluorometer used, save a separate Qubit™ Easy Calculator Microsoft™ Excel™ Spreadsheet file containing the Calibration Factor specifically calculated for that particular instrument.

# 3

## Measure the percent templated ISPs

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This chapter describes the procedure for determining the percent templated ISPs for unenriched samples.

### Prepare the sample: Ion OneTouch™ 2 users

1. From the Ion Sphere™ Quality Control Kit, thaw the Ion Probes tube, Annealing Buffer, and Quality Control Wash Buffer.
2. If needed, adjust the sample volume of unenriched ISPs to 100 µL with Ion OneTouch™ Wash Solution (Ion PGM™ System users) or ISP Resuspension Solution (Ion Proton™ System, Ion S5™/Ion S5™ XL System, and Ion GeneStudio S5 Systems users) from the OT2 Solutions box, then transfer 2 µL to a 0.2-mL PCR tube.
3. Add Ion Probes to the sample.
  - If processing one sample, add 19 µL Annealing Buffer and 1 µL Ion Probes directly to the 0.2-mL PCR tube containing the ISPs, then mix well by pipetting up and down.
  - If processing more than one sample, make an Ion Probe master mix:
    - a.  $(19 \text{ µL Annealing Buffer} \times \# \text{ samples}) + (1 \text{ µL Ion Probes} \times \# \text{ samples}) = \text{total volume required}$

**Note:** To compensate for pipetting error, prepare an extra 5–10% overage.

    - b. Add 20 µL of Ion Probe master mix to the 0.2-mL PCR tubes containing the ISPs, then mix well by pipetting up and down.
4. Load one or more tubes into a thermal cycler, then perform the following protocol to anneal the Ion Probes:

Stage	Temperature	Time
Hold	95 °C	2 minutes
Hold	37 °C	2 minutes

5. Remove unbound probes by washing the samples three times with 180 µL of Quality Control Wash Buffer.
  - a. Add 180 µL of Quality Control Wash Buffer to the 0.2-mL tube.
  - b. Vortex to mix, then centrifuge at:
    - 15,500 × *g* for 1.5 minutes (Ion PGM™ System users), *or*
    - Maximum speed for 3 minutes (Ion Proton™ System, Ion S5™/Ion S5™ XL System, and Ion GeneStudio S5 Systems users).
  - c. Remove the supernatant leaving 10 µL behind. Be careful not to disturb the pelleted ISPs.
- Note:** Visually compare the supernatant to a 10-µL standard for reference.
- d. Repeat steps a – c two times for a total of three washes.
6. After the final wash, add 190 µL of Quality Control Wash Buffer for a total volume of 200 µL, mix by pipetting up and down five times, then transfer the entire sample to a Qubit™ Assay Tube.

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**IMPORTANT!** Ensure that you measure the volumes accurately.

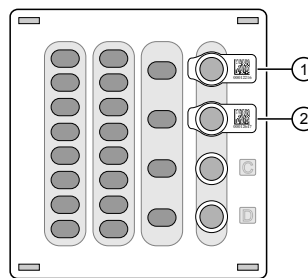
---

7. To generate a negative control, add 200 µL of Quality Control Wash Buffer to a new Qubit™ Assay Tube.

Proceed to “Measure the sample—Qubit™ 2.0 Fluorometer” on page 16, or “Measure the sample—Qubit™ 3.0 or Qubit™ 4 Fluorometer” on page 17.

## Prepare the samples: Ion Chef™ users

1. From the Ion Sphere™ Quality Control Kit, thaw the Ion Probes tube, Annealing Buffer, and Quality Control Wash Buffer.
2. Centrifuge the ISP samples taken from Positions A and B on the Reagents cartridge at:



- ① Position A (QC sample)  
 ② Position B (QC sample)

- 15,000 × *g* for 2 minutes (Ion PGM™ System users), *or*
- Maximum speed for 3 minutes (Ion Proton™ System, Ion S5™/Ion S5™ XL System, and Ion GeneStudio S5 Systems users)

**Note:**

- If you want to use both the Guava™ easyCyte 5 Benchtop Flow Cytometer and Qubit™ fluorometry for quality assessment, remove 2 µL of the sample before centrifugation for flow cytometric analysis before processing the remainder for Qubit™ analysis.
- If you are evaluating quality after completion of the Ion Chef™ run, you can remove the Library Sample Tubes from the Reagents cartridge and centrifuge the QC samples in these tubes.

3. Remove supernatant to reduce the total volume per sample to approximately 10 µL.
4. Pipet each sample up and down to mix, then transfer each sample to a new labeled 0.2-mL PCR tube.
5. Add 10 µL Annealing Buffer and 1 µL Ion Probes directly to each 0.2-mL PCR tube containing the ISPs, then mix well by pipetting up and down.

**Note:** If processing multiple samples, make an Ion Probe master mix:

(10 µL Annealing Buffer × # samples) + (1 µL Ion Probes × # samples) = total volume required

6. Load the tubes into a thermal cycler, then perform the following protocol to anneal the Ion Probes:

Stage	Temperature	Time
Hold	95 °C	2 minutes
Hold	37 °C	2 minutes

7. Remove unbound probes by washing the samples three times with 180 µL of Quality Control Wash Buffer.
  - a. Add 180 µL of Quality Control Wash Buffer to the 0.2-mL tubes.
  - b. Vortex properly to mix, then centrifuge at:
    - 15,000 × g for 2 minutes (Ion PGM™ System users), or
    - Maximum speed for 3 minutes (Ion Proton™ System, Ion S5™/Ion S5™ XL System, and Ion GeneStudio S5 Systems users).
  - c. Remove the supernatant leaving behind 10 µL. Be careful not to disturb the pelleted ISPs.
 

**Note:** Visually compare the supernatant to a 10-µL standard for reference.
  - d. Repeat steps a – c two times for a total of three Quality Control Wash Buffer washes.

8. After the final wash, add 190 µL of Quality Control Wash Buffer for a total volume of 200 µL, mix by pipetting up and down five times, then transfer the entire sample to a Qubit™ Assay Tube.

---

**IMPORTANT!** Ensure that you measure the volumes accurately.

---

9. To generate a negative control, add 200 µL of Quality Control Wash Buffer to a new Qubit™ Assay Tube.

Proceed to “Measure the sample—Qubit™ 2.0 Fluorometer” on page 16, or “Measure the sample—Qubit™ 3.0 or Qubit™ 4 Fluorometer” on page 17.


## Measure the sample—Qubit™ 2.0 Fluorometer

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**IMPORTANT!** Before using the Qubit™ 2.0 Fluorometer, ensure that the instrument is running firmware V3.10 or V3.11, and the Ion\_PluginV310\_AF.qbt file has been uploaded to the instrument.

---

1. Power on the Qubit™ 2.0 Fluorometer.
2. Press **Ion** to access Alexa Fluor™ 488 and Alexa Fluor™ 647 measurement options.
3. Press **AF 488**, insert the sample into the Qubit™ 2.0 Fluorometer, close the lid, then touch **Read**.


**Note:** The lettering on the Read  selection option changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample finishes (approximately 5 seconds).

**Note:** If more than one sample is being processed, all samples can be read with the AF 488 setting before moving on to the AF 647 setting.

4. Record the RFU value(s).

**Note:** The data retained on the Qubit™ 2.0 Fluorometer can be transferred to a USB drive. See “(Optional) Transfer data to a USB drive” on page 17 for more information. If more than one sample is being processed, all samples can be read with the AF 488 setting before moving on to the AF 647 setting.

5. Press **Home** ▶ **Ion** ▶ **AF 647**. Insert the sample into the Qubit™ 2.0 Fluorometer, close the lid, then press **Read**.

**Note:** The lettering on the Read  selection option changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample finishes (approximately 5 seconds).

6. Record the RFU value(s).

---

**IMPORTANT!** Ensure that you read the negative control (Quality Control Wash Buffer only) in both the Alexa Fluor™ 488 and Alexa Fluor™ 647 settings and record the RFU values.

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Proceed to “Evaluate the templated ISPs” on page 18.



## Measure the sample—Qubit™ 3.0 or Qubit™ 4 Fluorometer

1. Power on the Qubit™ 3.0 or Qubit™ 4 Fluorometer.
2. Press **Ion Sphere** to access Alexa Fluor™ 488 and Alexa Fluor™ 647 measurement options.
3. Press **AF 488**, insert the sample into the fluorometer, close the lid, then press **Read tube**.

**Note:** If more than one sample is being processed, all samples can be read with the AF 488 setting before moving on to the AF 647 setting.

4. Record the RFU value(s).

**Note:** The data retained on the fluorometer can be transferred to a USB drive. See “(Optional) Transfer data to a USB drive” on page 17 for details. If more than one sample is being processed, all samples can be read with the AF 488 setting before moving on to the AF 647 setting.

5. Press **Home** ▶ **Ion Sphere** ▶ **AF 647**. Insert the sample into the fluorometer, close the lid, then touch **Read tube**.
6. Record the RFU value(s).

---

**IMPORTANT!** Ensure that you read the negative control (Quality Control Wash Buffer only) in both the Alexa Fluor™ 488 and Alexa Fluor™ 647 settings, then record the RFU values.

---

Proceed to “Evaluate the templated ISPs” on page 18.

## (Optional) Transfer data to a USB drive

### Qubit™ 2.0 Fluorometer

1. Insert a USB drive into the instrument.
  2. In the main menu, press **Data** (at the bottom-right corner of the screen).
  3. In the **Data** screen, press **USB drive**, then wait for the instrument to download the data to the USB drive.
- Note:** The download creates a “.csv” file that can be opened on your computer using any spreadsheet software, such as Microsoft™ Excel™ software.

### Qubit™ 3.0 or Qubit™ 4 Fluorometer

1. Insert a USB drive into the instrument, or connect to a computer with a USB cable.
  2. On the home screen, press **Data** (at the bottom-left of the screen).
  3. On the **Data** screen, press **Export**, then wait for the instrument to download the data to the USB drive or computer.
- Note:** The download creates a “.csv” file that can be opened on your computer using any spreadsheet software, such as Microsoft™ Excel™ software.

## Evaluate the templated ISPs

1. Open the saved Qubit™ Easy Calculator containing the Calibration Factor specifically calculated for the Qubit™ fluorometer used.
2. Enter the raw RFU values from Alexa Fluor™ 488 and Alexa Fluor™ 647 Calibration Standards measurements in the appropriate fields for both the ISPs containing samples (red cells) and negative control sample (purple cells).

---

**IMPORTANT!** The Alexa Fluor™ 488 value must be >100 counts to produce a valid % Templated ISPs value.

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Fluorophore	Acceptable RFU Range
Alexa Fluor™ 488	>100 counts; no upper limit Samples with <100 counts usually correlate with no or very few ISPs in the assay.
Alexa Fluor™ 647	Any value, with the condition that the Alexa Fluor™ 488 RFU value is >100 counts.

3. In the appropriate field (blue cells), enter the template kit- and lot-specific conversion factor for unenriched ISPs, available at: [thermofisher.com/qubit-conversion-factors-guide](https://www.thermofisher.com/qubit-conversion-factors-guide), or downloaded from the Ion Sphere™ Quality Control Kit product page ([thermofisher.com/4468656](https://www.thermofisher.com/4468656)).

**Note: Ion PGM™ System, Ion S5™/Ion S5™ XL System, and Ion GeneStudio S5 Systems users:** Use the 200-base-read library conversion factor for libraries <300 bp in length. Use the 400-base-read library conversion factor for libraries ≥300 bp in length.

4. The **Percent Templated ISPs** calculates automatically and is displayed for each sample:

A	B	C	D	E	F	G	H	I	J
Qubit Calibration Factor Calculation									
Calibration Standard	RFU	Calibration Factor							
Alexa Fluor® 488 Calibration Standard	6548	0.58							
Alexa Fluor® 647 Calibration Standard	10265								
Percent Templated ISPs									
	Raw RFU Value		Background RFU (Negative Control Tube)						
Sample ID	AF 488	AF 647	AF 488	AF 647	Conversion Factor*	Percent Templated ISPs			
						#DIV/0!			
						#DIV/0!			
						#DIV/0!			
						#DIV/0!			
						#DIV/0!			
						#DIV/0!			
						#DIV/0!			
						#DIV/0!			
Green Cells = Raw RFU values of Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standards supplied in the Ion Sphere Quality Control Kit									
Red Cells = Raw RFU values measured in "Measure the templated unenriched sample"									
Purple Cells = Raw RFU values measured for negative control in "Measure the templated unenriched sample"									
Blue Cells= Template kit lot specific conversion factor									

- ① Previously calculated instrument-specific Calibration Factor.
- ② Enter raw RFU values for the ISP-containing samples in the red cells.
- ③ Enter raw RFU values for the negative control sample in the purple cells. Values will be the same for all samples measured at the same time.
- ④ Enter the template kit- and lot-specific Conversion Factor in the blue cells. Go to [thermofisher.com/qubit-conversion-factors-guide](https://www.thermofisher.com/qubit-conversion-factors-guide).
- ⑤ Automatically calculated percent templated ISPs value displayed in this column.

## Acceptance criteria for unenriched ISPs

The optimal amount of library corresponds to the library dilution point that gives percent templated ISPs between 10–25%, or 10–30%, depending on the Ion templating and sequencing systems used (see the following tables).

Samples that fall in the recommended range generally produce the most data. However, samples that fall outside of the recommended range can still meet the throughput specifications of the Ion chips.

The recommended optimal range is not intended to be a pass/fail criterion. 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 “Template preparation on the Ion Chef™ System” on page 21 for more information.

Template preparation on the Ion Chef™ System for sequencing on the specified instruments

Percent templated ISPs			Description
Ion S5™/Ion S5™ XL/Ion GeneStudio S5 Series Sequencers	Ion PGM™ Sequencer	Ion Proton™ Sequencer	
<10%	<10%	<10%	Sample contains an insufficient number of templated ISPs to achieve optimal loading density on the Ion Chip.
10–25% (10–30% for Ion 540™ ISPs)	10–30%	10–30%	Optimal amount of library.
>25% (>30% for Ion 540™ ISPs)	>30%	>30%	Sample yields multi-templated ISPs (mixed reads).

Template preparation on the Ion OneTouch™ 2 System for sequencing on the specified instruments

Percent templated ISPs			Description
Ion S5™/Ion S5™ XL/Ion GeneStudio S5 Series Sequencers	Ion PGM™ Sequencer	Ion Proton™ Sequencer	
<10%	<10%	<10%	Sample contains an insufficient number of templated ISPs to achieve optimal loading density on the Ion Chip.
10–25%	10–30%	10–25%	Optimal amount of library.
>25%	>30%	>25%	Sample yields multi-templated ISPs (mixed reads).



# Troubleshooting with the Ion Sphere™ Assay

The following tables provide troubleshooting information for unenriched ISPs prepared on either the Ion Chef™ System or the Ion OneTouch™ 2 System, and tested with the Ion Sphere™ Assay on a Qubit™ 2.0, Qubit™ 3.0, or Qubit™ 4 Fluorometer.

## Template preparation on the Ion Chef™ System

Template preparation on the Ion Chef™ System for sequencing on the specified instruments

Sequencer	Qubit™ Fluorometer observation	Sequencing observation	Possible cause	Recommended action
Ion GeneStudio S5 Series Sequencers Ion S5™/Ion S5™ XL Sequencers Ion PGM™ Sequencer Ion Proton™ Sequencer	<10% Templated ISPs	<ul style="list-style-type: none"> <li>Lower loading</li> <li>Lower % enriched</li> <li>Lower key signal</li> <li>Lower throughput</li> </ul>	Too little library input into template preparation	<ul style="list-style-type: none"> <li>Increase library input to target 20–25% templated ISPs.</li> </ul> <i>or</i> <ul style="list-style-type: none"> <li>Continue with sequencing—expect lower throughput.</li> </ul>
Ion GeneStudio S5 Series Sequencers Ion S5™/Ion S5™ XL Sequencers <ul style="list-style-type: none"> <li>Ion 510™/Ion 520™/Ion 530™ ISPs</li> </ul>	25%–70%	Increased number of filtered reads	Too much library input into template preparation	<ul style="list-style-type: none"> <li>Decrease library input to target 20–25% templated ISPs.</li> </ul> <i>or</i> <ul style="list-style-type: none"> <li>Continue with sequencing—expect lower throughput.</li> </ul>
<ul style="list-style-type: none"> <li>Ion 540™ ISPs</li> </ul>	30%–70%			
<ul style="list-style-type: none"> <li>Ion 550™ ISPs</li> </ul>	25%–70%			
Ion PGM™ Sequencer Ion Proton™ Sequencer	30%–70%			



**Appendix A** Troubleshooting with the Ion Sphere™ Assay  
*Template preparation on the Ion Chef™ System*

Sequencer	Qubit™ Fluorometer observation	Sequencing observation	Possible cause	Recommended action
Ion GeneStudio S5 Series Sequencers Ion S5™/Ion S5™ XL Sequencers Ion PGM™ Sequencer Ion Proton™ Sequencer	>70% Templated ISPs	<ul style="list-style-type: none"><li>Increased % primer-dimer filtered reads</li><li>Lower throughput</li></ul>	Adapter dimer contaminating library, more likely in short amplicon, Ion AmpliSeq™ or miRNA libraries	<ul style="list-style-type: none"><li>Check Agilent™ 2100 Bioanalyzer™ traces for adapter dimer peak (Amplicon library or Ion AmpliSeq™ library peak approx. 70 bp; miRNA library peak approx. 60 bp).</li><li>Re-purify library using Agencourt™ AMPure™ XP Kit clean-up steps as outlined in the appropriate user guides.</li></ul>
		<ul style="list-style-type: none"><li>Low loading</li><li>Low % enriched</li><li>Lower throughput</li><li>High % filtered reads</li></ul>	Ion Chef™ System underperformance	<ul style="list-style-type: none"><li>Troubleshoot with Technical Support or a Field Application Scientist.</li></ul>



## Template preparation on the Ion OneTouch™ 2 System

Template preparation on the Ion OneTouch™ 2 System for sequencing on the specified instruments

Sequencer	Qubit™ Fluorometer observation	Sequencing observation	Possible cause	Recommended action
Ion GeneStudio S5 Series Sequencers Ion S5™/Ion S5™ XL Sequencers Ion PGM™ Sequencer Ion Proton™ Sequencer	<10% Templated ISPs	<ul style="list-style-type: none"> <li>Lower loading</li> <li>Lower % enriched</li> <li>Lower key signal</li> <li>Lower throughput</li> </ul>	Too little library input into template preparation	<ul style="list-style-type: none"> <li>Increase library input to target 20– 25% templated ISPs. <i>or</i></li> <li>Continue with sequencing— expect lower throughput.</li> </ul>
Ion GeneStudio S5 Series Sequencers Ion S5™/Ion S5™ XL Sequencers Ion Proton™ Sequencer	25%–70%	Increased number of filtered reads	Too much library input into template preparation	<ul style="list-style-type: none"> <li>Decrease library input to target 20– 25% templated ISPs. <i>or</i></li> <li>Continue with sequencing— expect lower throughput.</li> </ul>
Ion PGM™ Sequencer	30%–70%			
Ion GeneStudio S5 Series Sequencers Ion S5™/Ion S5™ XL Sequencers Ion PGM™ Sequencer Ion Proton™ Sequencer	>70% Templated ISPs	<ul style="list-style-type: none"> <li>Increased % primer-dimer filtered reads</li> <li>Lower throughput</li> </ul>	Adapter dimer contaminating library, more likely in short amplicon, Ion AmpliSeq™ or miRNA libraries	<ul style="list-style-type: none"> <li>Check Agilent™ 2100 Bioanalyzer™ traces for adapter dimer peak (Amplicon library or Ion AmpliSeq™ library peak approx. 70 bp; miRNA library peak approx. 60 bp).</li> <li>Re-purify library using Agencourt™ AMPure™ XP Kit clean-up steps as outlined in the appropriate user guides.</li> </ul>
		<ul style="list-style-type: none"> <li>Low loading</li> <li>Low % enriched</li> <li>Lower throughput</li> <li>High % filtered reads</li> </ul>	Ion OneTouch™ 2 System underperformance	<ul style="list-style-type: none"> <li>Troubleshoot with Technical Support or a Field Application Scientist.</li> </ul>



# Supplemental information

## Update the instrument software—Qubit™ 2.0 Fluorometer

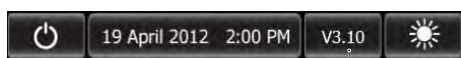
We recommend that you update the instrument firmware to the latest available version.

1. Go to [thermofisher.com/qubit](http://thermofisher.com/qubit), then navigate to **Technical Resources** ▶ **Resources for Previous Models of Qubit Fluorometer** ▶ **Qubit 2.0 Firmware Update (v3.11) and Instructions**.
2. Follow the "Step-by-step firmware instructions (if applicable)" to download v3.11 instrument firmware, then install it on your Qubit™ 2.0 Fluorometer.

**Note:** Contact Technical Support if you require more help. See the *Qubit™ 2.0 Fluorometer User Manual* (Pub. No. MAN0003231), available at [thermofisher.com/qubit/qubit-technical-resources/previous-qubit-models](http://thermofisher.com/qubit/qubit-technical-resources/previous-qubit-models), for more information on instrument maintenance and operation.

## Upload the Ion Sphere™ Quality Control assay plugin—Qubit™ 2.0 Fluorometer only

If your Qubit™ 2.0 Fluorometer is equipped with V3.10 or V3.11 firmware, you *only* have to upload the plugin for the Ion Sphere™ Quality Control assay to your fluorometer:

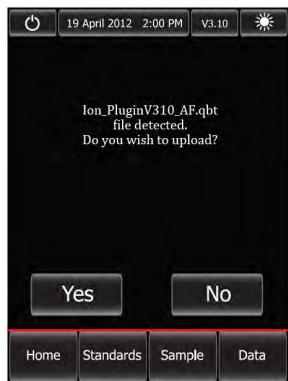


Firmware version

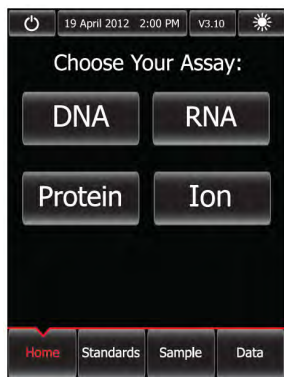


To upload the Ion Sphere™ Quality Control assay plugin to your fluorometer, follow these steps:

1. Download the Ion Sphere™ Quality Control assay (.qbt) plugin file from the Ion Sphere™ Quality Control Kit product page ([thermofisher.com/4468656](http://thermofisher.com/4468656)) to a USB drive.
2. With the USB drive (containing the Ion\_PluginV310\_AF.qbt file) inserted into the Qubit™ 2.0 Fluorometer, power-cycle the instrument by unplugging it, then plugging it back in. Press **Yes** to upload the file permanently to the instrument:

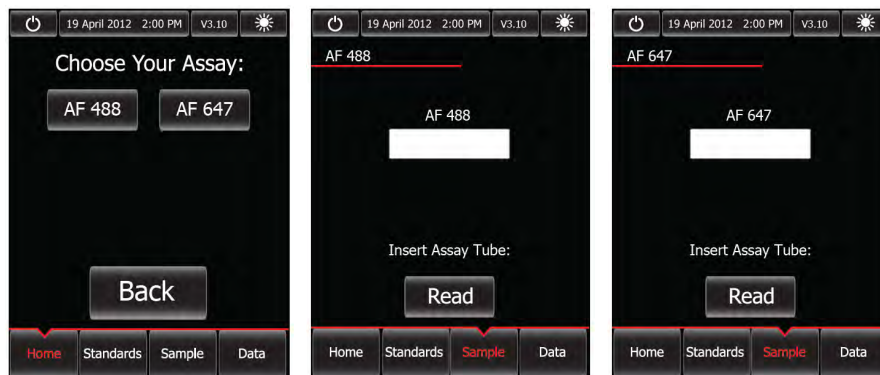


3. Ensure that the Ion Sphere™ Quality Control assay plugin is functional by checking the following screens:
  - a. In the main menu, the **Ion** selection option is present.



- b. After pressing **Ion** ( **Ion** ), **AF 488** ( **AF 488** ) and **AF 647** ( **AF 647** ) selection options appear on the screen:

**Note:** Pressing **AF 488** or **AF 647** enters the respective measurement channels.



Proceed to Chapter 2, “Calculate the Calibration Factor”.

## Update the instrument software—Qubit™ 3.0 Fluorometer or Qubit™ 4 Fluorometer

We recommend updating the instrument software of the Qubit™ 3.0 and Qubit™ 4 Fluorometer to the latest available version.

1. To verify the version of software currently on your instrument, press **Settings** on the home screen, then press **About instrument**.
2. If your instrument requires a software update, download the latest software at one of the following links, then transfer the file to a USB drive.
  - Qubit™ 3.0 Fluorometer: [thermofisher.com/qubit/qubit-technical-resources/previous-qubit-models](http://thermofisher.com/qubit/qubit-technical-resources/previous-qubit-models)
  - Qubit™ 4 Fluorometer: [thermofisher.com/qubit/qubit-fluorometer](http://thermofisher.com/qubit/qubit-fluorometer)
3. Insert the USB drive into the fluorometer, press **Settings**, then **Update software**. When the new software is detected, press **Update**.
4. When prompted, press **Restart** to complete the update.

**Note:** For more detailed instructions, see the

- *Qubit™ 3.0 Fluorometer User Guide* (Pub. No. MAN0010866), or
- *Qubit™ 4 Fluorometer User Guide* (Pub. No. MAN0017209)



# Safety



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**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.
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## Chemical safety



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**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.
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## 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.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:  
[www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf](http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf)
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:  
[www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf](http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf)

# Documentation and support

## Related documentation

Document	Publication number
<i>Qubit™ 4 Fluorometer User Guide</i>	MAN0017209
<i>Qubit™ 3.0 Fluorometer User Guide</i>	MAN0010866
<i>Qubit™ 2.0 Fluorometer User Manual</i>	MAN0003231

User guides for Qubit™ Fluorometers can be downloaded at **thermofisher.com**.

## Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- 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

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

