

# Qubit™ 4 Fluorometer

Catalog Number Q33226

Publication Number MAN0017209

Revision D.0

## Contents

<b>About this guide</b> .....	<b>3</b>
<b>1. Product information</b> .....	<b>5</b>
Product contents .....	5
Product description .....	6
Instrument exterior components .....	7
Product specifications .....	8
<b>2. Getting started</b> .....	<b>9</b>
Set up the Qubit™ 4 Fluorometer .....	9
<i>Optional:</i> Configure instrument settings .....	11
Guidelines for using the Qubit™ 4 Fluorometer .....	16
<b>3. Perform assays</b> .....	<b>17</b>
Overview .....	17
<i>Optional:</i> Use Reagent Calculator to prepare Qubit™ Working Solution .....	18
Calibrate assays on the Qubit™ 4 Fluorometer .....	20
Read samples .....	24
Results .....	26
Fluorometer mode .....	29
Ion Sphere™ Assay .....	31
<b>4. Manage data</b> .....	<b>32</b>
Introduction .....	32
View detailed sample data .....	32
Rename data files .....	35
Export data .....	36
Delete data .....	40
<b>5. Manage, import, and create assays</b> .....	<b>41</b>
Overview .....	41
Manage assays .....	41
Show /Hide assays .....	42
Move assays .....	44
Import assays .....	46
Create your own assay for the Qubit™ 4 Fluorometer .....	51
<b>6. Instrument maintenance</b> .....	<b>52</b>
Maintenance and cleaning .....	52
Software updates .....	53
System verification test .....	55
<b>Appendix A: Troubleshooting</b> .....	<b>57</b>
Troubleshooting .....	57

Critical Qubit™ Assay considerations .....	59
<b>Appendix B: Ordering information .....</b>	<b>61</b>
Qubit™ 4 Fluorometer and accessories .....	61
<b>Appendix C: Safety .....</b>	<b>62</b>
Symbols on instruments .....	62
Safety labels on instruments .....	63
General instrument safety .....	64
Chemical safety .....	65
Chemical waste safety .....	66
Electrical safety .....	67
Biological hazard safety .....	68
Safety, Electromagnetic compatibility (EMC), and Environmental standards .....	69
<b>Documentation and support .....</b>	<b>71</b>
Obtaining support .....	71

# About this guide

## Overview

This user guide describes how to operate the Qubit™ 4 Fluorometer.

## User documentation

The guides listed below are available with the Qubit™ 4 Fluorometer.

Guide	Pub. No.
<i>Qubit™ 4 Fluorometer User Guide</i>	MAN0017209
<i>Qubit™ 4 Fluorometer Quick Reference Card (QRC)</i>	MAN0017210

Additional resources are available on the Qubit™ Technical Resources page. Go to [thermofisher.com/qubit](http://thermofisher.com/qubit) to access protocols, application notes, and tutorials.

## Text and keyboard conventions

Text and keyboard conventions used in the *Qubit™ 4 Fluorometer User Guide* are listed below. For safety alert words and symbols used in Thermo Fisher Scientific user documentation, see page 4.

Convention	Use
<b>Bold</b>	<b>Bold</b> text indicates user action. For example: Click <b>Run</b> .
▶	Right arrow symbol (▶) indicates a menu choice and separates successive commands you execute or select from a drop-down or shortcut menu. For example: Select <b>Settings ▶ Instrument settings</b> .

## User attention words

Two user attention words appear in Thermo Fisher Scientific user documentation. Each word implies a particular level of observation or action as described below.



**Note:** Provides information that may be of interest or help but is not critical to the use of the product.

---



**IMPORTANT!** Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

---

## Safety alert words

Four safety alert words appear in Thermo Fisher Scientific user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action, as defined below:



**IMPORTANT!** – Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

---



**CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

---



**WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

---



**DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

---

Except for **IMPORTANT!** safety alerts, each safety alert word in a Thermo Fisher Scientific document appears with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to Thermo Fisher Scientific instruments (see “**Safety symbols**” in Appendix C).

## SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Thermo Fisher Scientific are available to you free 24 hours a day. For instructions on obtaining SDSs, see “**Safety Data Sheets (SDS)**”.



**IMPORTANT!** For the SDSs of chemicals not distributed by Thermo Fisher Scientific contact the chemical manufacturer.

---

# 1. Product information

## Product contents

The Qubit™ 4 Fluorometer is shipped with the components listed below:

Component	Quantity
Qubit™ 4 Fluorometer	1 each
Universal Power Cord with 4 adaptor plugs (for U.S./E.U./U.K./Australia)	1 each
USB drive (includes the user guide)	1 each
Qubit™ 4 USB cable	1 each
Qubit™ 4 Fluorometer Quick Reference Card (QRC)	1 each
Certificate of Conformity (COC)	1 each
Qubit™ screen cleaning cloth	1 each

The complete user guide is also available for download at [thermofisher.com/qubit](http://thermofisher.com/qubit).  
See page 6 for the description and specifications of the Qubit™ 4 Fluorometer.

### Upon receiving the instrument

Examine the instrument carefully for damage incurred during transit. Ensure that all parts of the instrument, including the accessories listed above, are included with the product. Damage claims must be filed with the carrier; the warranty does not cover in-transit damage.

See page 9 for instructions to set up the Qubit™ 4 Fluorometer.

## Product description

### Qubit™ 4 Fluorometer

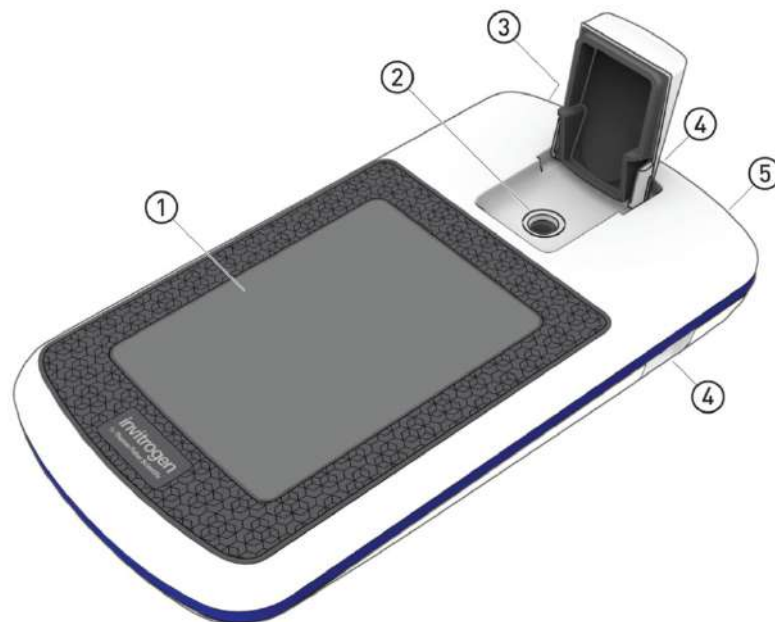
The Qubit™ 4 Fluorometer is a benchtop fluorometer that can be used for the quantitation of DNA, RNA, microRNA, and protein, as well as for the measurement of RNA integrity and quality using the highly sensitive and accurate fluorescence-based Qubit™ assays. Additionally, Ion Sphere™ Particle quality can be evaluated on the Qubit™ 4 Fluorometer using the Ion Sphere™ Quality Control Kit prior to performing a sequencing run on the Ion Personal Genome Machine™ (PGM™) Sequencer. You can also use the Qubit™ 4 Fluorometer to directly measure the fluorescence of samples or to create new assays using the MyQubit software pre-programmed into the instrument.

### Features

- Fast and highly accurate quantitation of DNA, RNA, and protein in less than 3 seconds per sample.
- Measure percentage of intact and degraded RNA in the sample in less than 5 seconds per sample.
- High levels of accuracy using only 1–20 µL of sample, even with very dilute samples.
- Use of dyes selective for dsDNA, RNA, or protein minimizes the effects of contaminants in the sample.
- Stores results from up to 1000 samples.
- Large, state-of-the-art color touchscreen for easy workflow navigation.
- Graphically displays up to 20 data points for quantitation assays.
- Instrument indicates samples that are in the extended range or out of range in the graph, as well as in the simple sample output screen.
- Saves sample data as a CSV (comma separated value) or PDF file.
- Exports data to a USB drive or directly to your computer via a USB cable.
- On-board Reagent Calculator provides instructions to prepare Qubit™ working solution using your sample and standard inputs.
- Allows easy definition and saving of assay preferences.
- Instrument user interface can be personalized to show only the frequently used assays, to add new assays, including user-defined assays created with the MyQubit software and web tool, and to display in the language of your choice including English, French, Spanish, Italian, German, simplified Chinese and Japanese.

## Instrument exterior components

### Top view



### Back view



- ① **Touchscreen** is the user interface containing the controls for all the functions needed and displays data from the assays.
- ② **Sample chamber** is used to load the assay tube containing the sample into the fluorometer for analysis.
- ③ **USB cable port (type mini-B)** allows you to transfer your data directly to your computer using the USB cable supplied with the instrument or any other similar USB cable.
- ④ **USB drive ports (Type A)** allow you to transfer and save data to your computer using the USB flash drive supplied with the instrument (or any other similar USB drive) for record keeping and printing purposes.
- ⑤ **Power inlet** connects the Qubit™ 4 Fluorometer to an electrical outlet using the supplied power cord and the appropriate plug, based on the electrical outlet configuration in your country.



## Product specifications

Physical characteristics	<b>Instrument type:</b>	Benchtop fluorometer
	<b>Instrument dimensions:</b>	5.4 in (w) × 10 in (l) × 2.2 in (h) (13.6 cm × 25 cm × 5.5 cm); rectangular shape
	<b>Weight:</b>	26.2 oz (743 g)
	<b>Operating power:</b>	100–240 VAC, 1.0 A/1.2 A
	<b>Frequency:</b>	50/60 Hz
	<b>Electrical input:</b>	12 VDC, 2.5 A
	<b>Installation site:</b>	Indoor use only
	<b>Operating temperature:</b>	10–40°C
	<b>Operating humidity:</b>	20–80% (non-condensing)
Technical specifications	<b>Dynamic range:</b>	5 orders of magnitude
	<b>Processing time:</b>	≤5 seconds/sample
	<b>Light sources:</b>	Blue LED (max ~470 nm) Red LED (max ~635 nm)
	<b>Excitation filters:</b>	Blue 430–495 nm Red 600–645 nm
	<b>Emission filters:</b>	Green 510–580 nm Red 665–720 nm
	<b>Detectors:</b>	Photodiodes; measurement capability from 300–1000 nm
	<b>Calibration type:</b>	2- or 3-point standard
	<b>Tube type:</b>	0.5-mL Real Time PCR (polypropylene) tubes
	<b>Warm-up time:</b>	<35 seconds
USB drive	<b>Capacity:</b>	4 Gigabyte

## 2. Getting started

### Set up the Qubit™ 4 Fluorometer

#### Set up the instrument

The Qubit™ 4 Fluorometer is a stand-alone instrument that does not require connection to a computer.

1. After unpacking the instrument, place the instrument on a flat, level, dry surface.
2. Plug one end of the supplied power cord into the Qubit™ 4 Fluorometer.
3. Attach the appropriate plug adaptor to the other end of the power cord.
4. Plug the power cord into the electrical outlet.



**Note:** Ensure the plug of power adaptor remains accessible to allow disconnection.

5. The instrument automatically powers on, first displaying the splash screen, and then the Home screen.
6. To power down the Qubit™ 4 Fluorometer, unplug it.



**IMPORTANT!** Use the power cord plug adaptor supplied with the instrument that is appropriate for the electrical outlet configuration in your country. Powering the instrument with an unapproved power cord may damage the instrument.

#### Home screen

When the instrument is powered on, the *Home* screen is automatically displayed.

From the Home screen, you can:

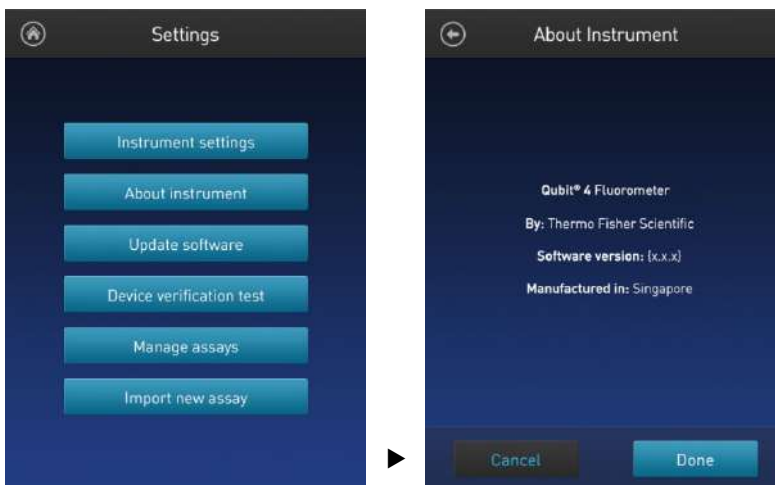
- Select one of the assays: dsDNA, RNA, oligo (ssDNA), protein, or Ion Sphere™.
- Select the Fluorometer mode (the instrument behaves like a mini-fluorometer).
- Access saved data.
- Configure instrument settings.
- Use the Reagent Calculator to determine the exact volumes of Qubit™ buffer and reagent required to prepare the Qubit™ working solution.



## About Instrument screen

The *About Instrument* screen displays information about your Qubit™ 4 Fluorometer, including the currently installed software version. To access the About Instrument screen:

1. On the Home screen, press **Settings**.
2. On the Settings screen, press **About Instrument** to display the About Instrument screen.



3. Press **Done**, **Cancel**, or **Back** (⏪) to return to the Settings screen.

## Optional: Configure instrument settings

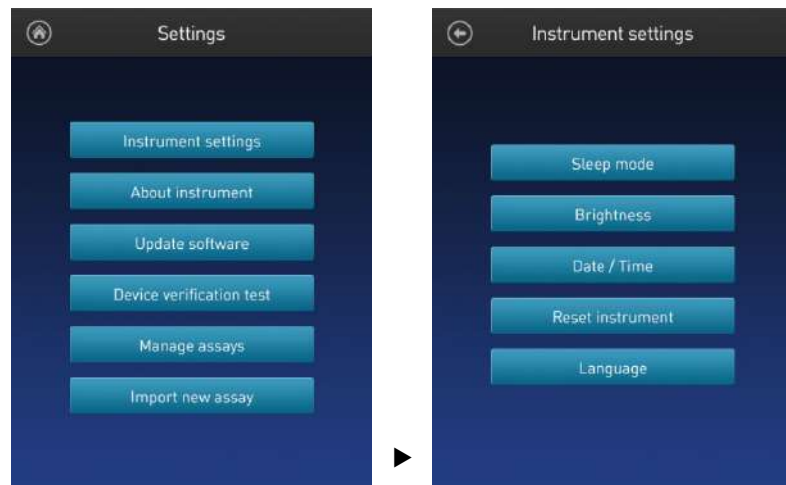
### Introduction

You can configure the following instrument settings for the Qubit™ 4 Fluorometer:

- Sleep mode (page 12)
- Brightness (page 12)
- Date/Time (page 13)
- Reset instrument (page 14)
- Language (page 15)

### Access the Instrument settings screen

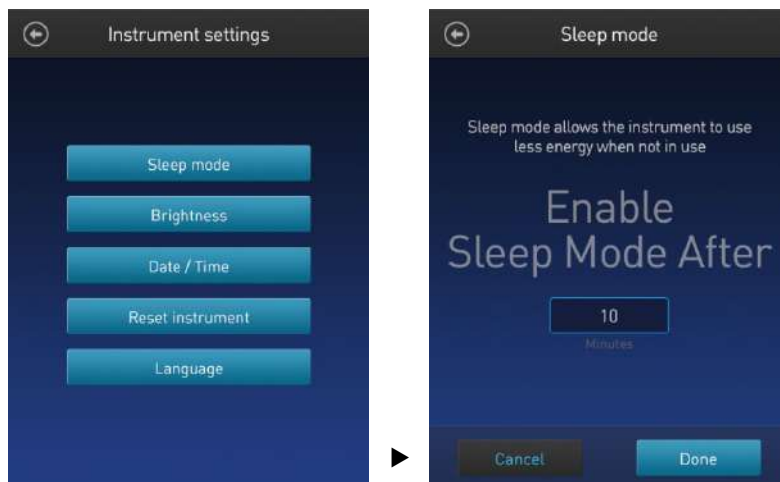
1. On the Home screen, press **Settings**.
2. On the *Settings* screen, press **Instrument settings** to display the *Instrument settings* screen.



## Adjust the sleep mode

The Qubit™ 4 Fluorometer has a sleep mode (i.e., automatic standby) that is triggered by inactivity. The system default is 10 minutes of inactivity before the instrument goes into sleep mode. To adjust this time:

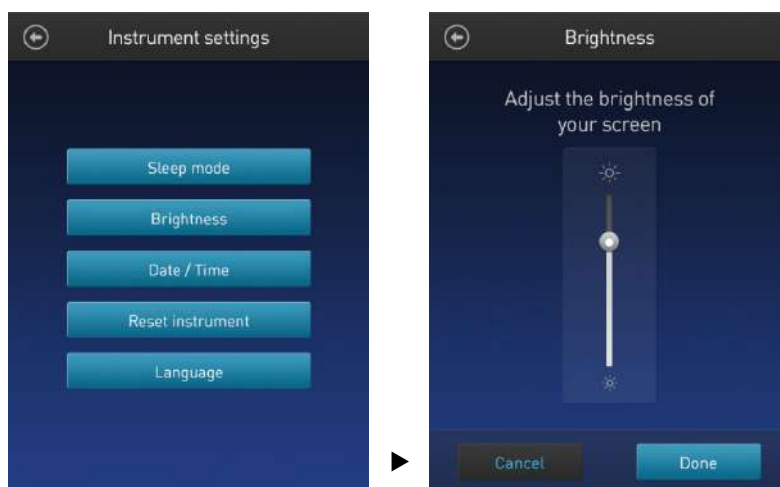
1. On the *Instrument settings* screen (page 11), press **Sleep mode** to display the *Sleep mode* screen.



2. Enter the time in minutes allowed before the instrument goes into sleep mode by pressing the **minutes** field, then using the number pad to select a value. The software requires a minimum of 1 minute and a maximum of 60 minutes.
3. Press **Done** to save the changes and return to the Instrument settings screen. Press **Cancel** or **Back** (⏪) to return to the Instrument settings screen without saving the changes.

## Adjust screen brightness

1. On the *Instrument settings* screen (page 11), press **Brightness** to display the *Brightness* screen.

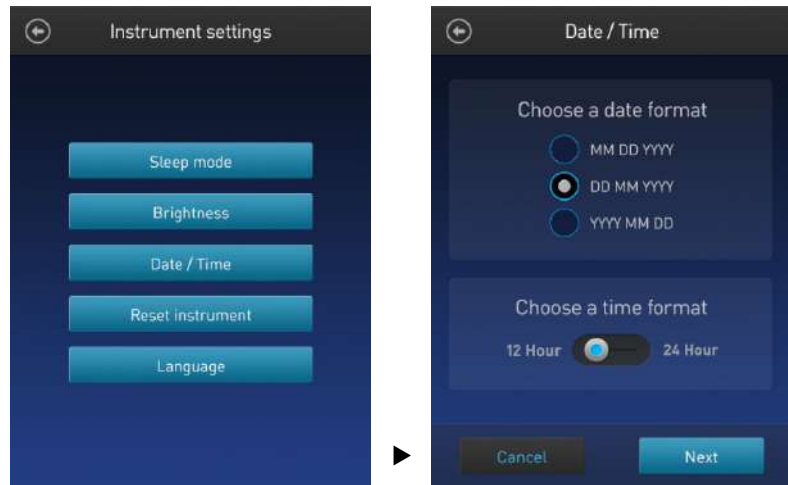


2. Move the slider button up or down to adjust the brightness of the display.
3. Press **Done** to save the changes and return to the Instrument settings screen. Press **Cancel** or **Back** (⏪) to return to the Instrument settings screen without saving the changes.

## Adjust the date and time

The date and time are preset when you receive the instrument. To adjust the date and time:

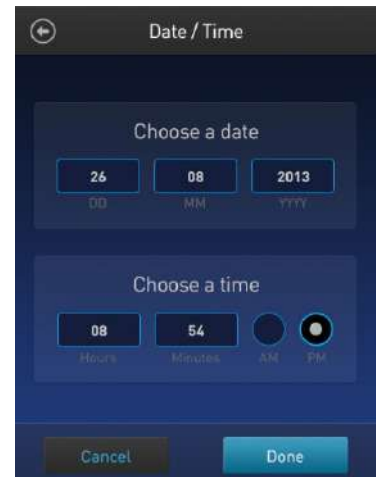
1. On the *Instrument settings* screen (page 11), press **Date/Time**.



2. Select a date format, select a time format, then press **Next**.
3. Enter the date and time:
  - a. Press a date field (**day, month, or year**), then use the number pad to select a value.
  - b. Press a time field (**hours or minutes**), then use the number pad to select a value.
  - c. Select **AM** or **PM**.

4. Press **Done** to save the changes and return to the Instrument settings screen.


Press **Cancel** or **Back** (⏪) to return to the Instrument settings screen without saving the changes.



## Reset function

The Reset function returns the Qubit™ 4 Fluorometer to its default factory settings, and **erases all saved data, user-defined instrument settings, and custom assays.**

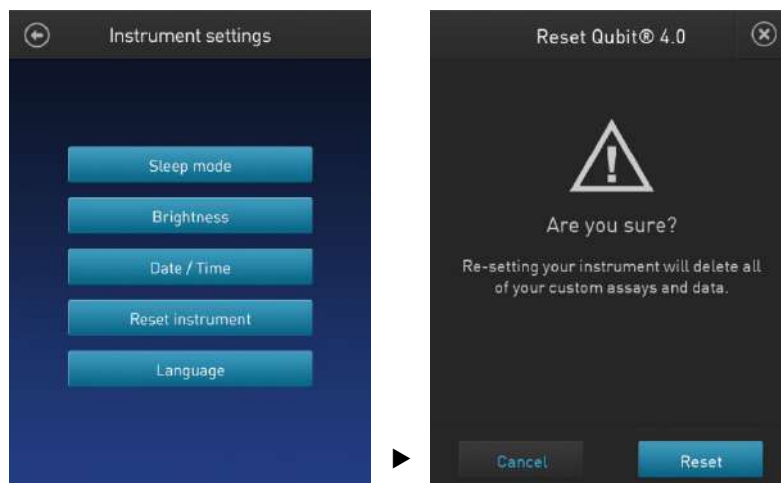
---


 **IMPORTANT!** The reset function is not reversible.

---

To return the instrument to its default factory settings:

1. On the *Instrument settings* screen (page 11), press **Reset instrument** to display the *Reset Qubit™ 4* screen.

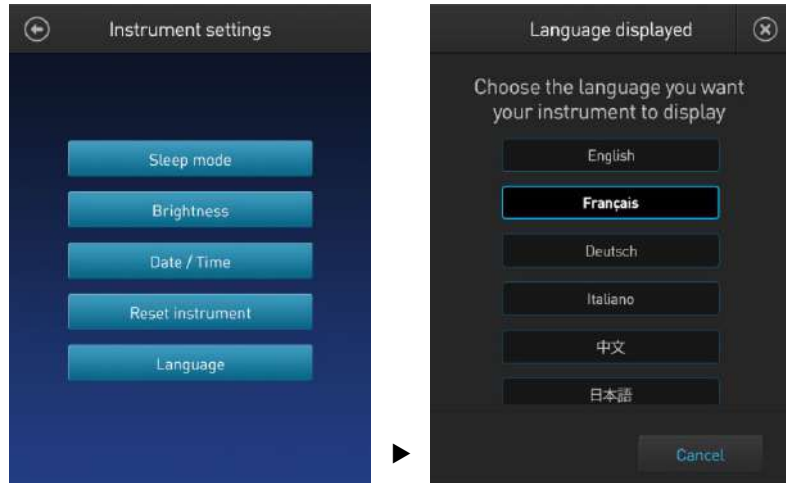


2. Press **Reset**. After the reset is complete, the Home screen is displayed. All data, user-defined instrument settings, and custom assays are removed. Press **Cancel** or **Exit** () to return to the Instrument settings screen without saving the changes.

## Change the display language

You can change the language that the Qubit™ 4 Fluorometer displays to English (default), French, German, Italian, Spanish, simplified Chinese, and Japanese.

1. On the *Instrument settings* screen (page 11), press **Language** to display the *Language* screen.



2. Select a language, then press **Next**.
3. Press **Yes** to confirm the change and return to the *Instrument settings* screen. Press **Cancel** or **Exit** (⊗) to return to the *Instrument settings* screen without saving the changes.





## Guidelines for using the Qubit™ 4 Fluorometer

- Recommendations** To obtain the best results, follow the recommendations below. For more information, see “Critical Qubit™ Assay considerations”, page 59.
- Do **not** operate the instrument in direct sunlight.
  - Wear gloves during sample handling.
  - Use the instrument at room temperature only (22–28°C).
  - Bring all kit reagents to room temperature and insert all assay tubes into the instrument only for as much time as it takes for the instrument to measure the fluorescence.
  - Do not hold the assay tubes in your hand before performing a measurement.
  - Make sure that you have calibrated the Qubit™ 4 Fluorometer using the appropriate standards.
  - Incubate the tubes for the Qubit™ DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution.
  - Incubate the tubes for the Qubit™ protein assays for 15 minutes after mixing the sample or standard with the working solution.
  - Incubate the tubes for the Qubit™ Protein Broad Range Assay for 10 minutes after mixing the sample or standard with the Protein BR Assay Reagent.
  - If you are performing multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.
- Note:** Multiple readings of RNA samples is not recommended.
- Visit [thermofisher.com/qubit](https://www.thermofisher.com/qubit) for additional application notes, technical notes, citations, software updates, and a list of validated Qubit™ assays that have been tested using the Qubit™ 4 Fluorometer.

**Assay tubes for the Qubit™ 4 Fluorometer** Only thin-wall, clear 0.5-mL PCR tubes are appropriate for use in the Qubit™ 4 Fluorometer. Acceptable tubes include Qubit™ assay tubes (Cat. No. Q32856, 500 tubes). The minimum assay volume must be 200 µL for an accurate read.

## 3. Perform assays

### Overview

This section provides instructions for using the Reagent Calculator, running new calibration standards or for using the last calibration, and for reading the samples using a Qubit™ assay kit.

#### Materials needed

- A Qubit™ assay kit appropriate for quantifying your samples (see page 61 for available Qubit™ assay kits and ordering information)
- DNA, RNA, or protein samples
- Qubit™ assay tubes or other appropriate 0.5-mL assay tubes
- Appropriate standards for the assay you are performing



**Note:** For instructions on the preparation of these standards, see the instructions that accompany the assay you are using or the *Qubit™ 4 Fluorometer Quick Reference Card (QRC)* (Pub. No. MAN0017210).

---

- (Optional) USB drive or USB cable for data transfer, supplied with the instrument or available separately

## Optional: Use Reagent Calculator to prepare Qubit™ Working Solution

### Introduction

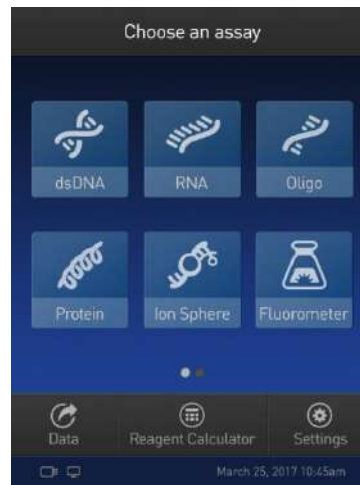
Use the on-board Reagent Calculator to quickly determine the correct amount of Qubit™ dye and buffer required to make the appropriate amount of Qubit™ Working Solution required to prepare your samples and standards.



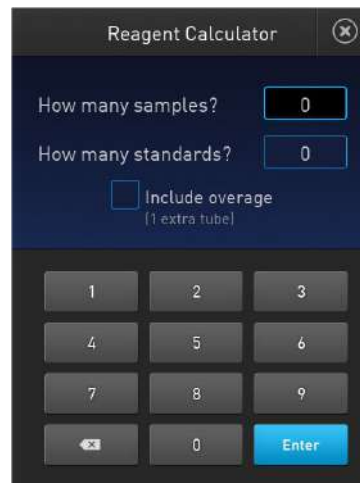
**Note:** The Reagent Calculator is not intended for use with the Qubit™ Protein Broad Range Assay.

### Use the Reagent Calculator

1. On the **Home** screen, press **Reagent Calculator**.



2. Enter the total number of samples and standards that you will be running on the Qubit™ 4 Fluorometer.



3. *Optional:* Select **Include overage** if you want to include an additional tube in the total calculated volume.

4. Press **Enter** to calculate the amount of Qubit™ dye and buffer required to prepare the Qubit™ Working Solution with these inputs.

The screenshot shows a 'Reagent Calculator' window with the following elements:

- Header: Reagent Calculator (with a close button)
- Input field: 'How many samples?' with the value '10'.
- Input field: 'How many standards?' with the value '3'.
- Checkbox: 'Include overage (1 extra tube)' which is checked.
- Results section: A box containing the text 'Add 14µL dye to 2786µL buffer for a total volume of 2800µL'.
- Bottom button: 'Done'.

**Note:** You can change the total number of tubes that you plan to run or the overage selection on this screen.

5. Press **Done** to return to the **Home** screen to run your Qubit™ assay.

# Calibrate assays on the Qubit™ 4 Fluorometer

## Introduction

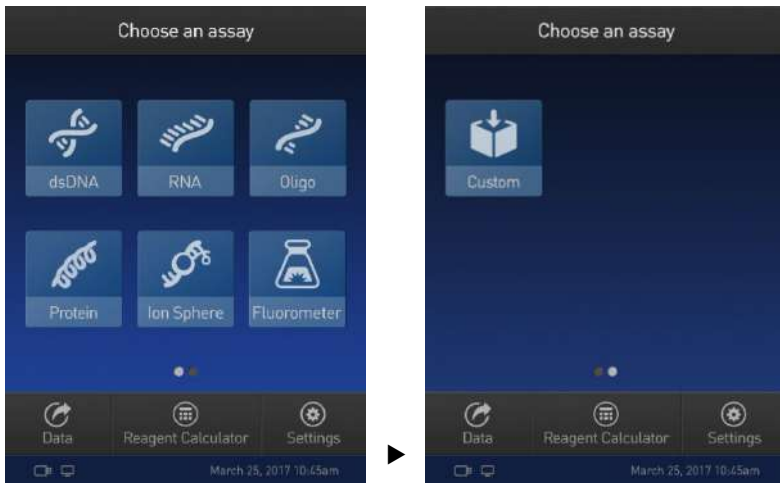
For each assay, you have the choice to run new standards for calibrating the assay on the Qubit™ 4 Fluorometer or to use the values from the previous calibration (for more information, see “Qubit™ 4 Fluorometer”, page 60).

**Note:** The Reagent Calculator is not intended for use with the Qubit™ Protein Broad Range Assay.

## Read new standards for calibration

1. On the **Home** screen, select the **assay type** for which you wish to read new standards.

To go to the second page of available assays, swipe the screen to the right. To return to the first page, swipe the screen to the left.

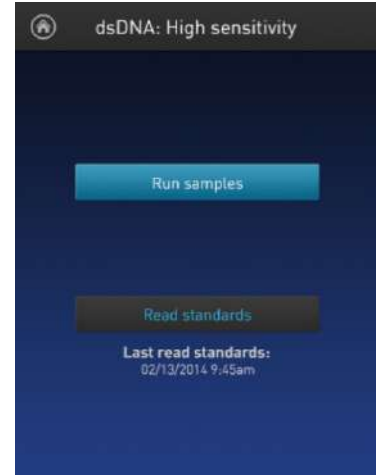


2. Select the desired **assay**.



**Note:** The software displays the available assays for the assay type you selected in step 1. The example in step 2 shows the available assays for the dsDNA assay type.

3. If you have already performed a calibration for the selected assay, the software prompts you to choose between reading new standards and running samples using the previous calibration. Press **Read standards**.



**Note:** To apply the previous calibration to your sample readings, press **Run samples**. The software prompts you to insert the assay tube containing sample. See "Read samples", page 24.

4. At the prompt, insert Standard #1 into the sample chamber and press **Read standard**. Be sure to use the Standard #1 appropriate for the assay you are performing. The reading takes approximately 3 seconds.



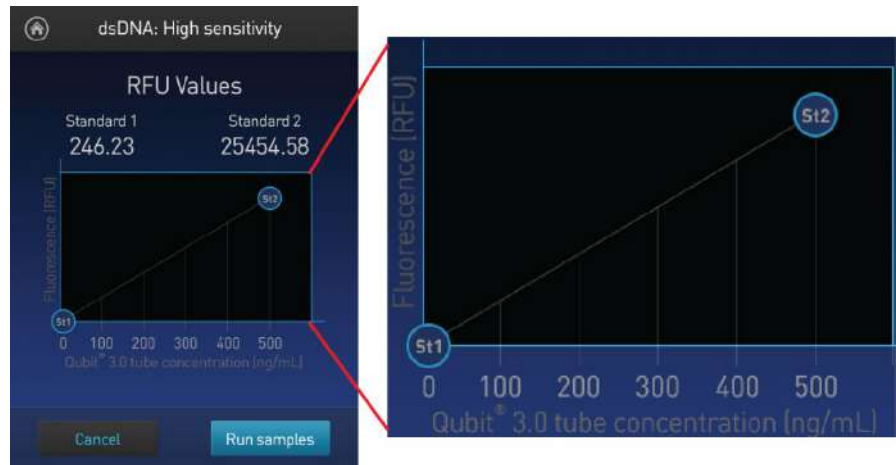
5. At the prompt, insert Standard #2 into the sample chamber and press **Read standard**. Be sure to use the Standard #2 appropriate for the assay you are performing.
6. *For Qubit™ protein and RNA IQ assays only:* At the prompt, insert Standard #3 into the sample chamber and press **Read standard**.

- The calibration is complete after Standard #2 is read (or after Standard #3 is read for the Qubit™ protein or RNA IQ assay). The software displays the results.



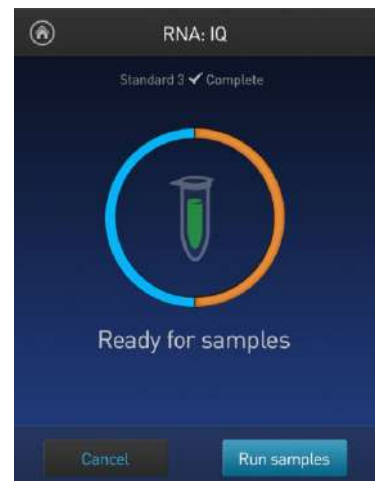
### Calibration results

- For Qubit™ quantitation assays:** If the calibration is successful, the software displays the *Read standard* screen with the *Fluorescence vs. Concentration* graph.



In the Fluorescence vs. Concentration graph, the standard data points are connected by a line and open circles represent correct standards.

- For the RNA IQ assay:** If the calibration is successful, the software displays the *Ready for samples* message.



- If the calibration is not successful, the software displays the *Calibration error* message (page 23).



**Note:** The Read standard screen displays the raw fluorescence values for Standard #1 and Standard #2 (and Standard #3, if applicable). These values can assist you in making a judgment regarding the calibration results.

For the Qubit™ dsDNA BR, Qubit™ dsDNA HS, Qubit™ ssDNA, Qubit™ microRNA, Qubit™ RNA HS, Qubit™ RNA BR, Qubit™ RNA XR, and Qubit™ protein assays, the reading given by Standard #2 should be at least ten times higher than that of Standard #1.

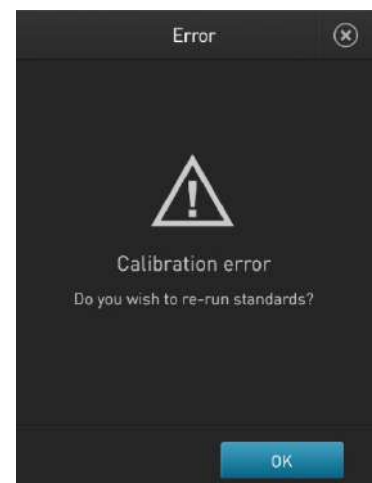
For the Qubit™ protein assay, the reading given by Standard #3 should be at least 40% higher than that of Standard #2.

For the Qubit™ RNA IQ assay, the instrument verifies the calibration parameters at each stage of the three point calibration process, confirming the appropriate response of the assay to the standards provided in the kit. If any issues arise during calibration, the instrument will guide you to appropriate actions to correct the issue.

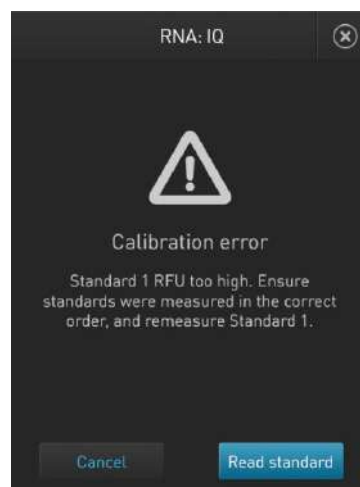
## Calibration error

If you receive the “Calibration error” message, you can re-run the standards:

1. In the Error screen, press **OK**.
2. Review the *Read standard* screen (see **Note** on page 23).
3. If you wish to re-run the standards, or run new standards, press **Read standards**, then repeat the calibration procedure (beginning on page 18).



**Note:** For the RNA IQ assay, specific standards can be re-run depending on the calibration error. The error message provides guidance on how to correct the calibration error.





## Read samples

### Before you begin

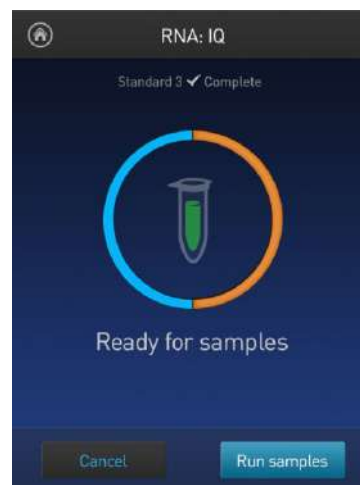
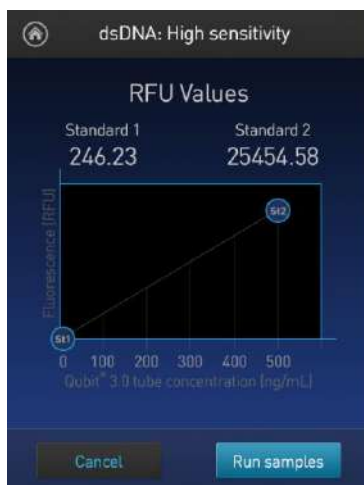
- Calibrate the Qubit™ 4 Fluorometer as described on page 18. (Run the appropriate standards or accept the values from the previous calibration.)
- Prepare the samples. Refer to the instructions provided with the assay.



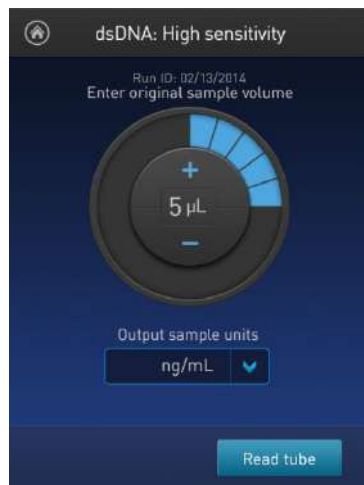
**Note:** Incubate the samples for the appropriate amount of time after mixing them with the working solution (2 minutes for the Qubit™ DNA and RNA assays, 15 minutes for the Qubit™ protein assay, or 10 minutes for the Qubit™ Protein Broad Range Assay).

### Read samples

1. In the *Read standard* (for Qubit™ quantitation assays) or *Ready for samples* screen (for Qubit™ RNA IQ assay), press **Run samples**.

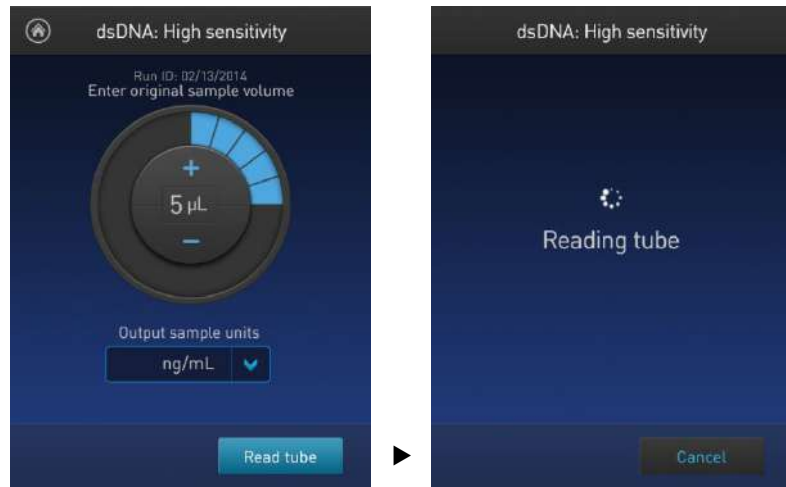


2. In the *Sample volume* screen, select the sample volume and units for the Qubit™ quantitation assays or the Qubit™ RNA IQ assay:
  - a. Press the + or – buttons on the wheel to select the sample volume added to the assay tube (between 1 and 20  $\mu\text{L}$ ).
  - b. For the Qubit™ quantitation assays, select the units for the output sample concentration from the dropdown menu.



**Note:** In the RNA IQ assay, there are no units associated with the RNA IQ value (i.e., RNA IQ#) and only sample volume is required.

3. Insert a sample tube into the sample chamber, close the lid, and then press **Read tube**. The reading takes approximately 3 seconds for the quantitation assays and approximately 5 seconds for the quality assays.



4. The software displays the results. See “Results screen” on page 26.



Results screen for quantitation assays

Results screen for quality assays

### Read multiple samples

To read multiple samples for the same assay:

1. Remove the current sample, and insert a new sample.
2. To change the sample volume, swipe **right** or press the **left arrow**.
3. Press **Read tube**.
4. Repeat step 2 (page 24) and step 3 (page 25).

## Results

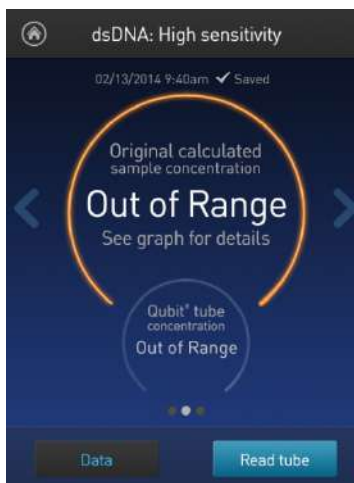
### Results screen – Sample quantitation

The Results screen displays the results of the sample run.

- If the results are within the assay's range, the concentration values are displayed. The top value (in large font) is the concentration of the original sample. The bottom value is the dilution concentration (the concentration of the sample in the tube inserted into the Qubit™ 4 fluorometer).

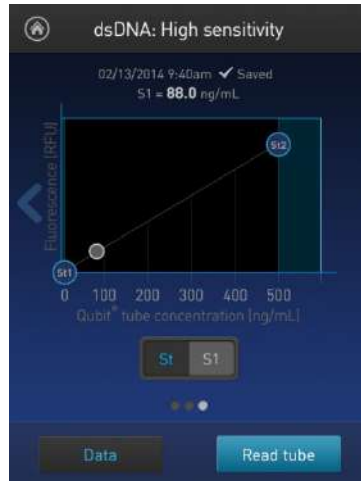


- If the results are outside of the assay's range, an "Out of Range" message is displayed.



- To return to the sample volume screen, **swipe right** or press the **left arrow**.

- To view the Fluorescence vs. Concentration graph, **swipe left** or press the **right arrow**. In the graph:
  - Open circles represent correct standards.
  - The large gray circle represents the most recent sample.
  - Blue circles represent samples that fall within the assay's core range.
  - Yellow circles represent samples that fall within the assay's extended range.
  - Red circles represent samples or standards that fall outside the assay's range.



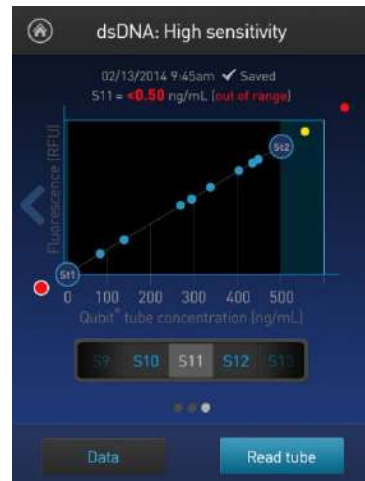
Single sample



Multiple samples



Out of range high



Out of range low

- To return to the previous screen, **swipe right** or press the **left arrow**.



**Note:** The Qubit™ 4 Fluorometer automatically stores numeric data from all reads, but does not store graphic data.

## Results screen – Sample quality

The Results screen displays the results of the sample run.

- If the results are within the assay’s range, the sample quality values are displayed. The result presented in the circle (i.e., RNA IQ#) is the total value for the RNA sample integrity and quality, which is the calculated percentage of large and small RNA in the sample.



The RNA IQ# is based on a scale of 1–10, similar to other RNA quality scores. A small RNA IQ# in the assay indicates that the sample is comprised of mainly small RNA and a larger RNA IQ# indicates that the sample consists of mainly large RNA or structured RNA (mRNA, tRNA, rRNA).

The actual percentages of small or degraded RNA and large or intact RNA in the sample are displayed below the RNA IQ#.

## Measurement error

If the results are outside of the assay’s range, a “Measurement error” message is displayed.



Measurement error – Sample concentration too low



Measurement error – Sample concentration too high

# Fluorometer mode

## Introduction

You can use the Qubit™ 4 Fluorometer as a mini-fluorometer by selecting the **Fluorometer mode**.

The Fluorometer mode allows you to choose the excitation light source (blue LED or red LED) while reading fluorescence in both the green and far-red emission channels (for the blue LED) or in the far-red emission channel only (for the red LED).

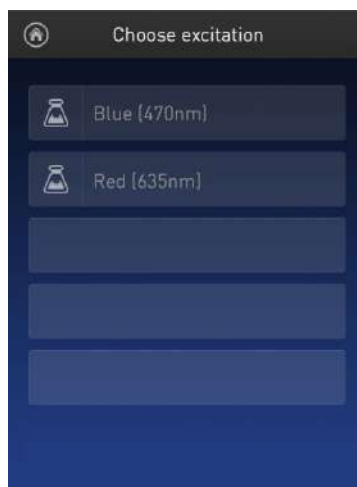
The reading is in raw fluorescence units (RFU).

## Read sample in Fluorometer mode

1. On the *Home* screen, press **Fluorometer**.



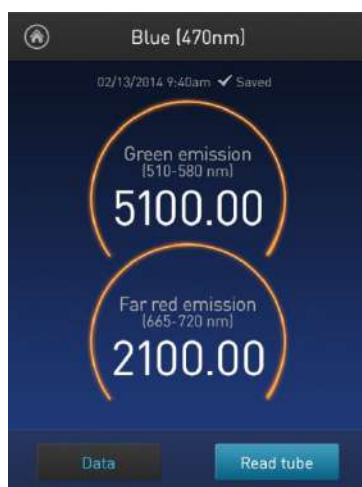
2. On the *Choose excitation* screen, select **Blue (470 nm)** or **Red (635 nm)**.



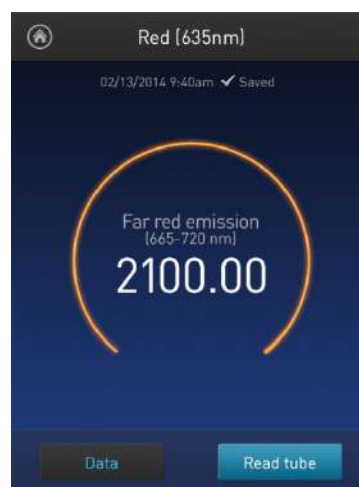
3. Insert the sample, then press **Read tube**.



4. For Blue excitation, the Qubit™ 4 Fluorometer displays both the green emission and the far red emission of your sample. For Red excitation, the Qubit™ 4 Fluorometer displays only the far red emission of your sample.



Blue (470 nm) excitation



Red (635 nm) excitation

### Read multiple samples in Fluorometer mode

To read multiple samples in the fluorometer mode:

1. Remove the current sample, and insert a new sample.
2. Press **Read tube**.

## Ion Sphere™ Assay

### Ion Sphere™ Assay

The Ion Sphere™ assay on the Qubit™ 4 Fluorometer enables quick and easy assessment of Ion Sphere™ Particles (ISPs) prior to performing a sequencing run on the Ion Personal Genome Machine™ (PGM™) Sequencer using the Ion Sphere™ Quality Control Kit.

All ISPs bind a complimentary, green-fluorescent Alexa Fluor™ 488 dye-labeled oligonucleotide, whereas only those beads that are template positive bind a complementary red-fluorescent Alexa Fluor™ 647 dye-labeled oligonucleotide. The Qubit™ 4 Fluorometer measures the fluorescence emission of each dye conjugate and a Microsoft Excel™ file template is used to calculate the percentage of template-positive ISPs from the ratio of the Alexa Fluor™ 647 dye signal to the Alexa Fluor™ 488 dye signal. This quality-control step using the Qubit™ Fluorometer determines the likelihood of a successful sequencing run, ultimately saving time and expense during subsequent sequencing.



**Note:** For more information about the Ion Sphere™ Quality Control Kit (Cat. No. 4468656), go to [thermofisher.com](http://thermofisher.com) and search for the catalog number.

---



## 4. Manage data

### Introduction

The Qubit™ 4 Fluorometer can save data for up to 1000 samples.

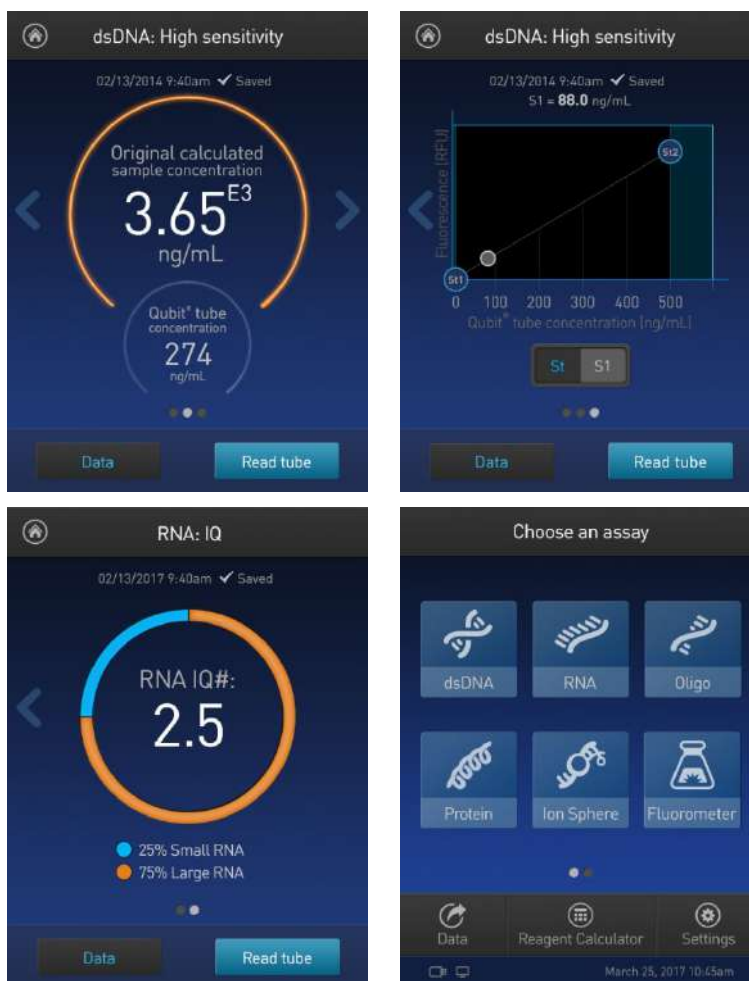
For the saved data, the instrument allows you to:

- View detailed data for each sample (page 32).
- Rename data files (page 35).
- Save data as a CSV (comma separated value) or PDF file, and export the file to a USB drive or directly to your computer (page 36).
- Delete data files (page 40)

### View detailed sample data

#### View detailed sample data

1. From the **Concentration**, **Graph**, **RNA IQ** results, or **Home** screen, press **Data**.



- On the Export data screen, press the **data set of interest**.




A *Data* screen displaying a list of data entries for that data set appears. To view additional data entries that do not fit in the screen, scroll up or down.



Data screen for quantitation assays

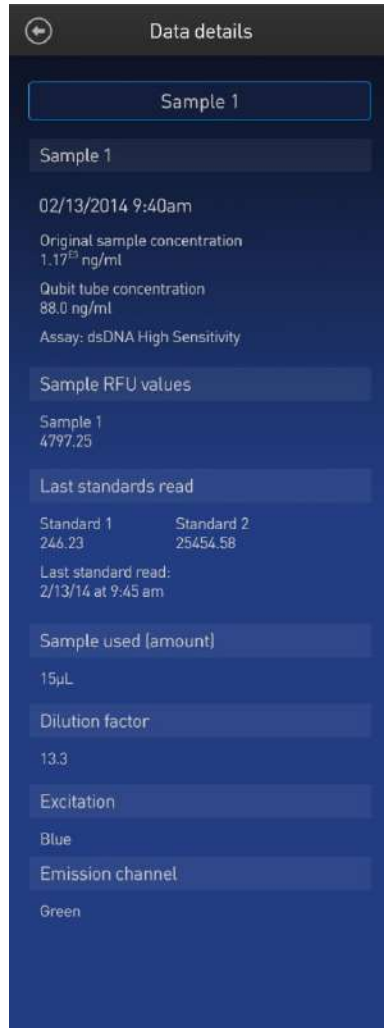


Data screen for quality assays

- To reverse the order of the data entries, press the **blue chevron** above the sample number column. 



- To view the sample details, press the **sample of interest**. A *Data details* screen opens.



Data details for quantitation assays



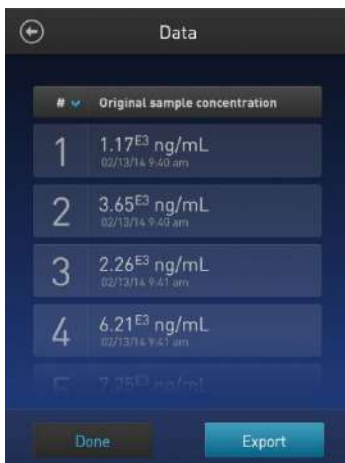
Data details for quality assays

## Rename data files

- Rename data files
1. On the **Concentration, Graph, RNA IQ results, or Home** screen, press **Data**.
  2. On the Export data screen, select the **data set of interest**.



3. From the list of data entries for the selected data set, press the **sample of interest** to open the *Data details* screen.

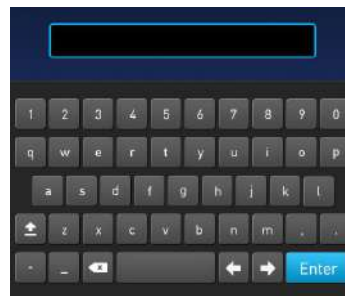


Data screen for quantitation assays



Data screen for quality assays

4. On the Data details screen, press the **Sample #** field (indicated by the red arrow), enter the new name using the keyboard that appears, then press **Enter**.



# Export data

## Introduction

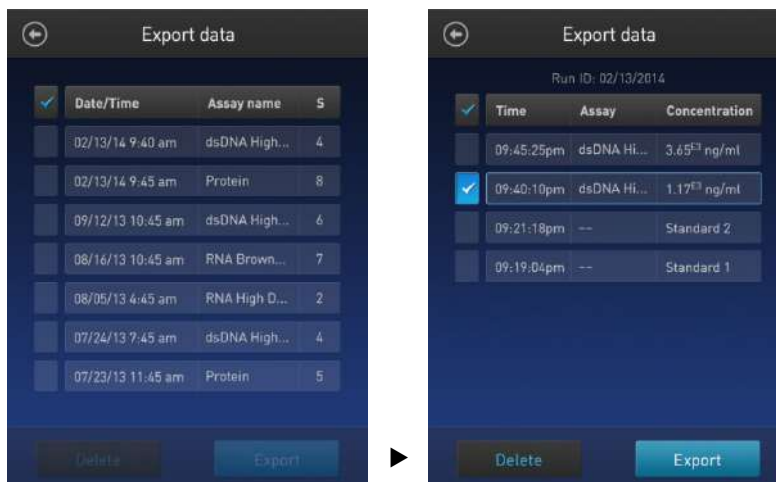
The Qubit™ 4 Fluorometer is designed for standalone use; it does not require an external computer. However, to archive data and generate reports, you can export the numeric data stored in the CSV or PDF file to a computer either by using the USB drive or by saving directly to the computer using the USB cable. You can then import the file into any spreadsheet program.

## Export data using the USB drive

1. Insert the USB drive into the Qubit™ 4 Fluorometer.
2. On the **Concentration**, **Graph**, **RNA IQ results**, or **Home** screen, press **Data**.
3. On the *Export data* screen, check the **selection box** to the left of each data set you wish to export.



4. To save only individual data entries from a data set, press the **data set of interest**, and then check the **selection box** to the left of the samples you wish to export.



5. Select CSV or PDF and press **Export** to export the data.



6. Transfer the USB drive to the USB drive port on your computer.

### Export data directly to a computer

Data files may also be exported from the Qubit™ instrument to a computer using a USB cable such as the one provided with the Qubit™ instrument. The USB cable must remain connected to both the Qubit™ instrument and the computer during this process.



**Note:** The Windows® XP operating system requires the installation of a driver. The driver and installation instructions are available for download from Qubit Technical Resources at [thermofisher.com/qubit](http://thermofisher.com/qubit).

To export data directly to a computer:

1. Connect the Qubit™ instrument to the computer using the USB cable.
2. From the computer, access the Qubit™ 4 Fluorometer as an external device.
3. Access the files saved on the Qubit™ instrument using the operating system's normal file browsing menus.
4. Copy the desired files to the computer.



**Note:** Files cannot be exported directly to an external computer using the Export workflow on the Qubit™ instrument.

Information saved to the Export file

Sample 1

Sample 1 ← Sample number

02/13/2014 9:40am ← Date and time

Original sample concentration 1.17<sup>ng</sup>/ml ← Original sample concentration and units

Qubit tube concentration 88.0 ng/ml ← Qubit<sup>®</sup> tube sample concentration and units

Assay: dsDNA High Sensitivity ← Assay name

Sample RFU values

Sample 1 4797.25 ← RFU value for the sample

Last standards read

Standard 1	Standard 2
246.23	25454.58

← RFU values for the standards

Last standard read: 2/13/14 at 9:45 am

Sample used (amount)

15µL ← Volume of original sample used

Dilution factor

13.3 ← Dilution factor

Excitation

Blue ← Excitation

Emission channel

Green ← Emission channel



**Note:** For the RNA IQ assay, there is no original sample concentration, Qubit<sup>™</sup> tube concentration or dilution factor. Instead, the results file contains % large and % small RNA information.

Sample\_#170830-204408

Sample 16

30/08/2017 08:44:08 PM

RNA IQ = 2.2

RNA

78% small RNA 22% large RNA

Sample RFU value

Green RFU = 12503.00 Far Red RFU = 826.00

Standard RFU values

Standard 1  
Green RFU = 0; Far Red RFU = 4

Standard 2  
Green RFU = 322.12; Far Red RFU = 17.12

## Export data to the cloud using Wi-Fi

Data from the Qubit™ instrument can also be exported to the cloud using a Wi-Fi connection.



**Note:** Confirm the Qubit™ 4 instrument has the latest firmware (under Settings > About Instrument) and correct date and time.

The latest firmware is available at:

<https://www.thermofisher.com/us/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/fluorometers/qubit/qubit-technical-resources.html>.

1. Insert the Wi-Fi dongle into the USB port, then power on the instrument.
2. From the **Home** screen, press **Settings > Instrument Settings**, then select **Network Connection**.
3. On the **Choose Network** screen, select the network to join.
  - a. On the **Configure & Join Network** screen, enter the password into the PSK box.
  - b. Press **Join**.

The network will be highlighted in a brighter blue color once connected.
4. On the **Home** screen, press **Data**.
5. On the **Data** screen, check the selection box to the left of each data set you wish to export.
  - a. To select all data sets, press the blue check icon in the header row.
  - b. To export only individual data entries from a data set, press the data set of interest, then check the selection box to the left of the samples desired for export.
6. After selecting the data sets or sample runs, press **Actions**, then **Export**.
7. In the **Export data** screen, select the **Export method**.
8. Press **Export**.

## Export data to the cloud using your Connect account

Data from the Qubit™ instrument can also be exported to the cloud using a Wi-Fi connection.

1. Register or sign in to your account at <https://www.thermofisher.com/us/en/home/digital-science.html>.
2. Click the **Instrument Connect** icon on the left side toolbar, then click on the blue **Add Instrument** icon in the upper right corner.
3. Select **Qubit\_4** from the drop-down menu and click **Next**. You are then instructed to “**Insert linking code: Get the code from your instrument.**”
4. On the Qubit™ 4 instrument **Home** screen, click on the profile (person) icon on in the top left corner to go to the **Sign In** screen.
5. Under **New to this instrument?**, select **Get Started**.
6. Under **Connect to the Thermo Fisher Cloud**, select **Connect**.
7. From the **Connect to the Cloud** screen on the Qubit™ 4 instrument, select **Dashboard on PC**.
8. On the **Home** screen, press **Data**.



9. On the **Data** screen, check the selection box to the left of each data set you wish to export.
  - a. To select all data sets, press the blue check icon in the header row.
  - b. To export only individual data entries from a data set, press the data set of interest, then check the selection box to the left of the samples desired for export.
10. After selecting the data sets or sample runs, press **Actions**, then **Export**.
11. In the **Export data** screen, select the **Export method**.
12. Press **Export**.

## Delete data

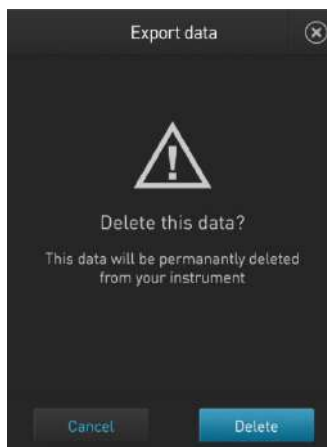
### Delete data files

1. On the **Concentration**, **Graph**, **RNA IQ results**, or **Home** screen, press **Data**.
2. On the *Export data* screen, check the **selection box** to the left of each data set you wish to delete.

To delete only individual data entries from a data set, press the **data set of interest**, and then check the **selection box** to the left of the samples you wish to delete.



3. Press **Delete**. A warning screen appears.



4. Press **Delete** to permanently delete the sample data or data set. Press **Cancel** to return to the screen previously viewed without deleting any data.

# 5. Manage, import, and create assays

## Overview

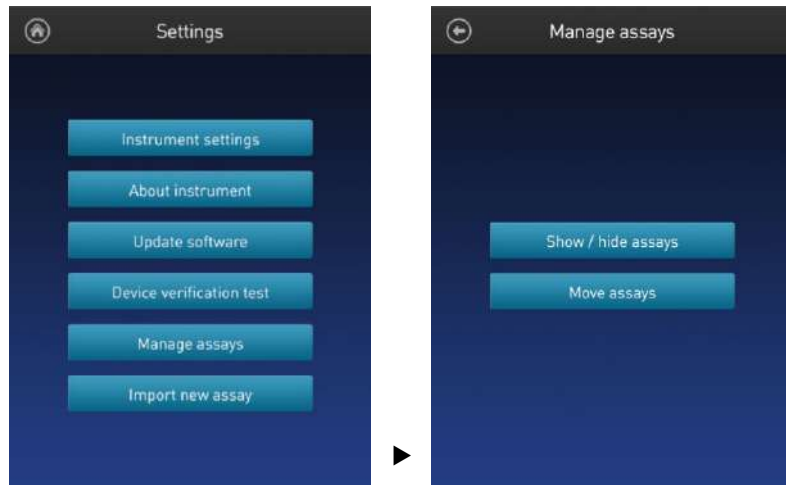
The Qubit™ 4 Fluorometer allows you to personalize the instrument’s user interface to hide and show (unhide) assays or folders and to move them to different locations. In addition, you can add custom MyQubit assays by importing them from our website at [thermofisher.com/qubit](http://thermofisher.com/qubit) or create new assays using the MyQubit software and web tool.

## Manage assays

### Manage assays function

The *Manage assays* function allows you to hide and show (unhide) assays or folders and to move them to different locations.

1. To access the Manage assays function, press **Settings** on the Home screen.
2. On the Settings screen, press **Manage assays** to open the *Manage assays* screen. From the Manage assays screen, you can select **Show/hide assays** (page 42) or **Move assays** (page 44).

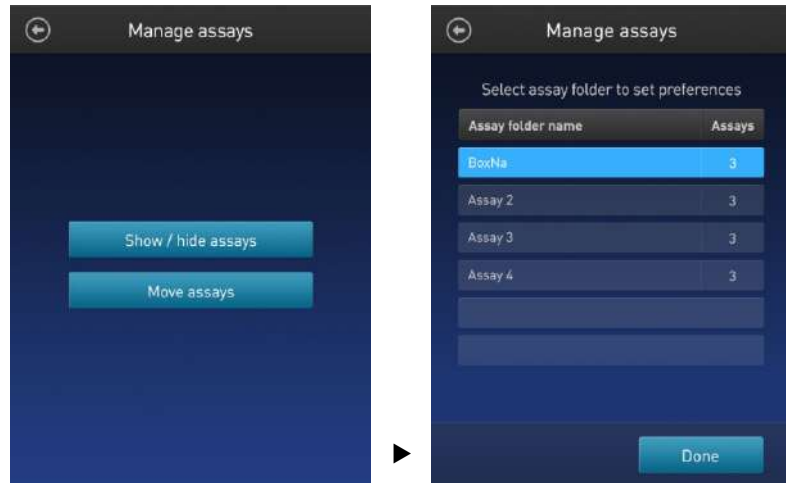


## Show/Hide assays

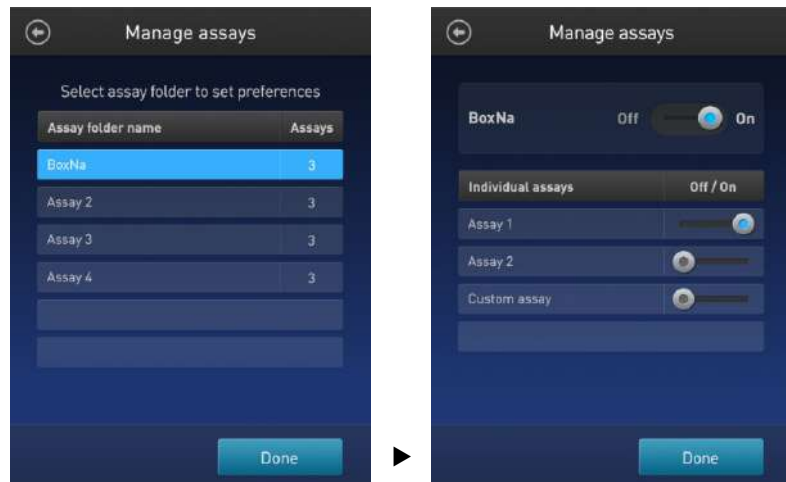
Show/Hide all assays within the assay folder

1. From the **Settings** screen, press **Manage assays**.
2. On the *Manage assays* screen, press **Show/hide assays**.

The software displays a list of the existing *assay folders* available on the instrument. Each assay folder contains individual assays.



3. Select the **assay folder** containing the assays you wish to show or hide. The software displays a list of existing individual assays within the selected assay folder and the controls for showing and hiding them.

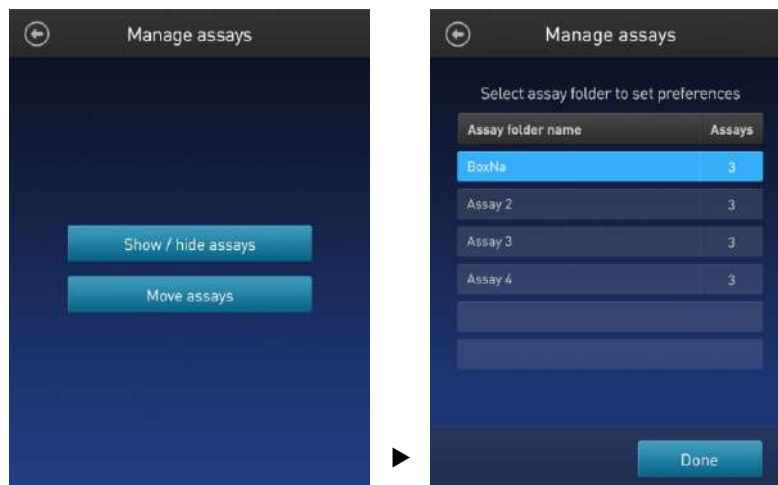


4. To hide all of the assays in the assay folder, swipe the **Assay folder On/Off** switch located to the right of the assay folder name to the **Off** position (to the **left**).  
To show all of the assays in the assay folder, swipe the **Assay folder On/Off** switch to the **On** position (to the **right**).
5. Press the **Back** button (⏪) to save the changes and return to the previous screen, or press **Done** to save the changes and return to the Settings screen.

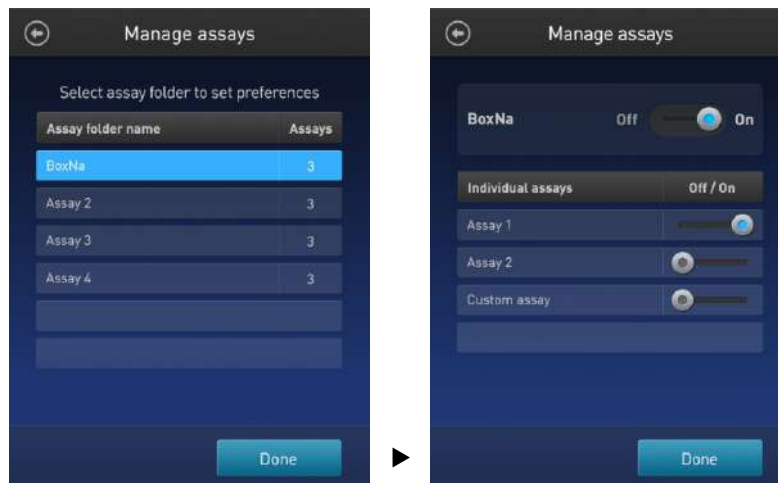
## Show/Hide individual assays

1. From the **Settings** screen, press **Manage assays**.
2. On the *Manage assays* screen, press **Show/hide assays**.

The software displays a list of the existing assay folders available on the instrument. Each assay folder contains individual assays.



3. Select the **assay folder** containing the assays you wish to show or hide. The software displays a list of existing individual assays within the selected assay folder and the controls for showing and hiding them.



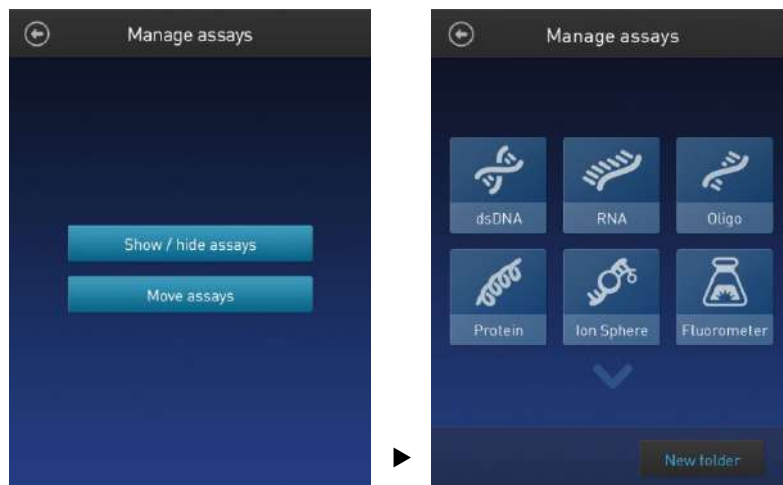
4. To hide an **individual assay**, swipe the **Assay On/Off** switch located to the right of the assay to the **Off** position while keeping the **Folder On/Off** switch in the **On** position. To show an **individual assay**, swipe the respective **Assay On/Off** switch to the **On** position while keeping the **Folder On/Off** switch in the **On** position.
5. Press the **Back** button (⏪) to save the changes and return to the previous screen, or press **Done** to save the changes and return to the Settings screen.

## Move assays

### Move assay folders/ individual assays on the Home screen

1. From the **Settings** screen, press **Manage assays**.
2. On the *Manage assays* screen, press **Move assays**.

The software displays the Manage assays screen with available assay folders and individual assays, and activates the move function.

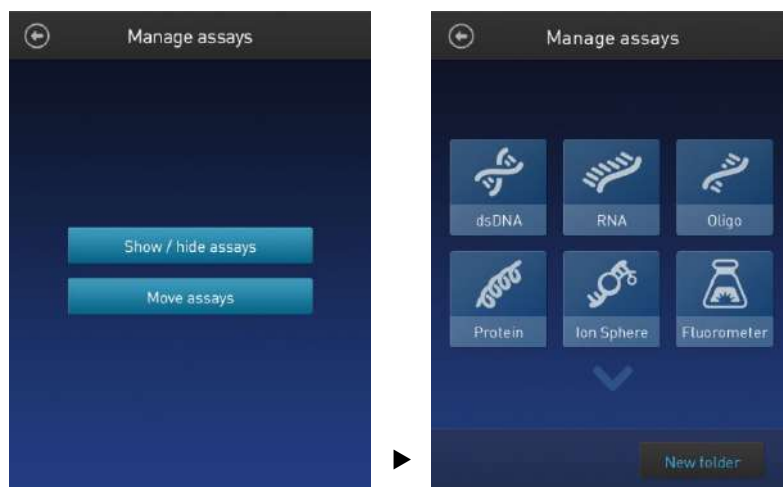


3. Press and hold on the desired assay folder or individual assay to detach it from its current position, then drag it to its new location.  
To move the selected object to a different Home screen page, drag it up or down to the desired screen.
4. Release the drag to drop the selected assay folder or individual assay in its new location.
5. Press the **Back** button (⏪) to save the changes and return to the Manage assays screen.

## Move assays between folders

1. From the **Settings** screen, press **Manage assays**.
2. On the *Manage assays* screen, press **Move assays**.

The software displays the Manage assays screen with available assay folders and individual assays, and activates the move function.



3. Press the desired **assay folder** to open the folder's assay screen.
4. Press and hold the **individual assay** to detach it and drag it over the Home screen icon (🏠).
5. Release the drag to drop the selected assay to the Home screen.
6. Press the **Home** button (🏠) to return to the Home screen.  
In the Home screen, you can press and drag the selected assay to another assay folder or location as described in "Move assay folders/ individual assays on the Home screen", page 44.
7. Press the **Back** button (⏪) to save the changes and return to the Manage assays screen.

## Move assays within folders

1. From the **Settings** screen, press **Manage assays**.
2. On the *Manage assays* screen, press **Move assays**.
3. Press the desired **assay folder** to open the folder's assay screen.
4. Press and hold the **individual assay** to detach it, and drag and drop it to its new location in the folder.
5. Press the **Back** button (⏪) to save the changes and return to the Manage assays screen.

## Import assays

### MyQubit assay files

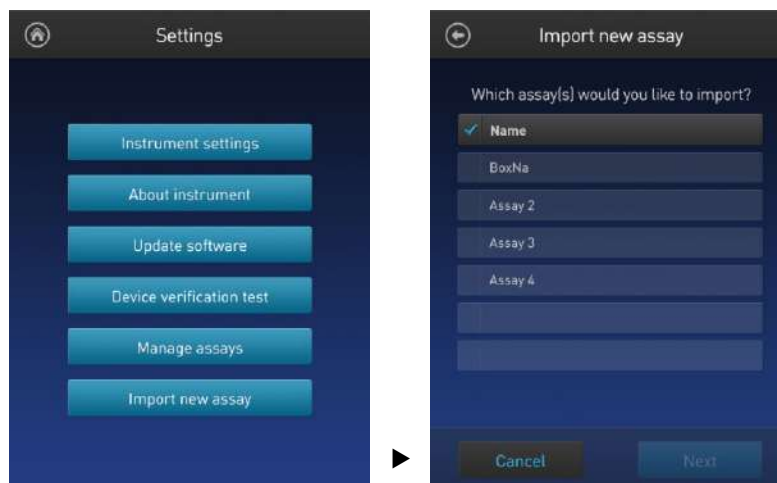
You can add pre-existing MyQubit assays to your Qubit™ 4 Fluorometer by downloading the assay files from [thermofisher.com/qubit](https://www.thermofisher.com/qubit), then importing the files to the instrument from a USB drive or from an external computer via a USB cable.



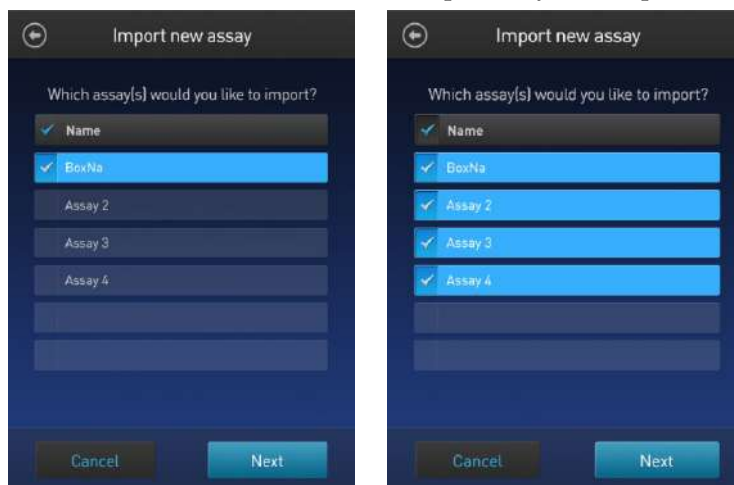
**Note:** MyQubit assay files have the file extension .qbt.

### Import assays from a USB drive

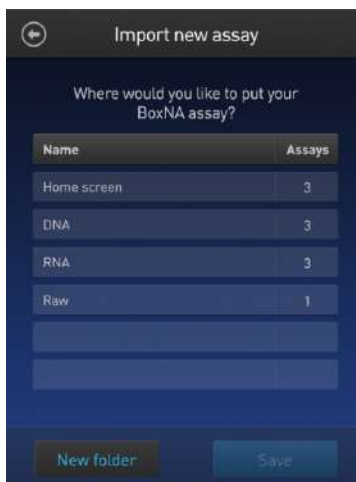
1. To import one or more new assays, go to [thermofisher.com/qubit](https://www.thermofisher.com/qubit) and download the MyQubit assay files (.qbt file) you wish to install to the **root directory** of a USB drive.  
Do **not** download the .qbt files to a folder in the USB drive.
2. Insert the USB drive containing the new assay files into the USB drive port of the Qubit™ 4 Fluorometer.
3. Go to the Settings screen and press **Import new assay**. The instrument searches the USB drive for assay files and the *Import new assay* screen displays the names of the assays found in the USB drive.



4. Press the assays you wish to import to select. Touching the selected assay a second time deselects it. You can select multiple assays for import.



5. Press **Next**. The *Import new assay* screen displays the name of the assay being imported (or the first assay, if multiple assays were selected) and lists the destination folders where you can save it.

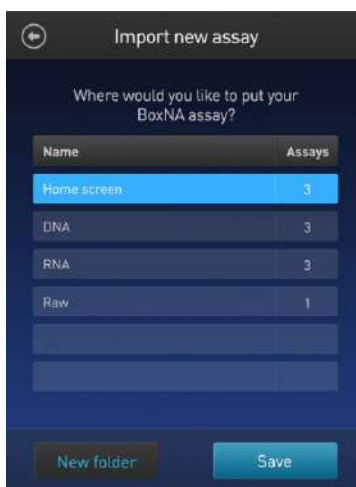


Press the **Back** button (⏪) or **Cancel** to return to the Settings screen without importing the assays.



**Note:** Each assay folder can contain up to 9 individual assays. The Home screen can contain up to 36 assays or folders.

6. Select the destination folder for the new assay and press **Save**. The *Verification* screen opens (see Step 9, page 48).  
Alternatively, press **New folder** to create a new destination folder (see Step 8, page 48).



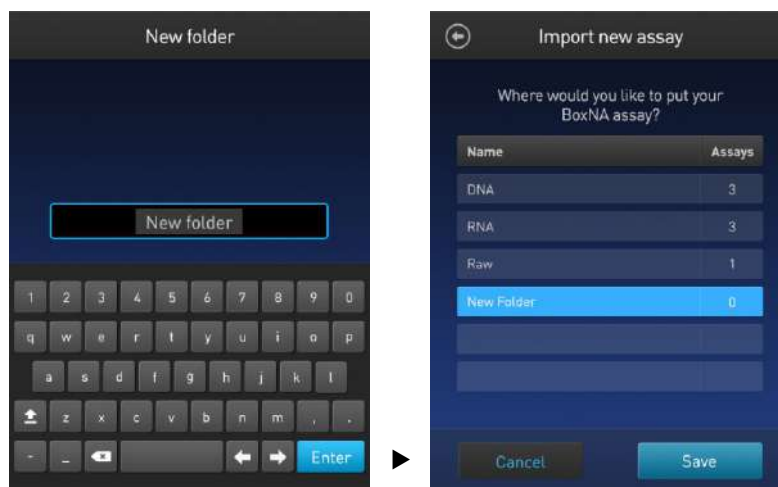
Press the **Back** button (⏪) or **Cancel** to abort the import process and return to the Settings screen.



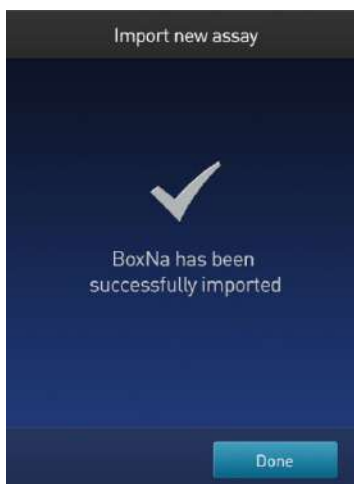
7. In the *New folder* screen, press the **New folder** text field. The keyboard opens. Using the **keyboard**, enter the name of the new destination folder. You can use up to 11 characters; spaces at the beginning or the end of the name are not recognized.

Press **Enter**, then press **Done** to return to the *Import new assay – Where* screen. The newly created folder will appear on the list of folders.

Select the new destination folder, then press **Save**. The *Verification* screen opens (see Step 9, page 49).



8. In the *Verification* screen, press **Done** to complete the import process. If importing only one assay, you will be returned to the Home screen.

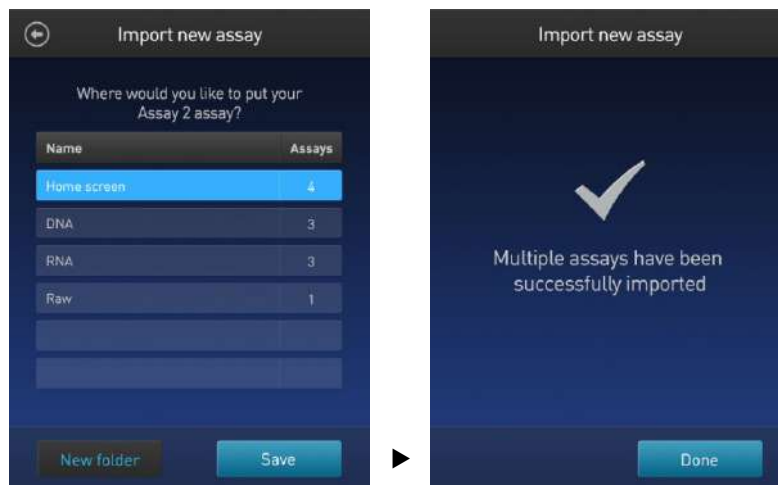


If importing multiple assays, you will be returned to the *Import new assay* screen and prompted to select the destination folder for the next assay in your import queue (see Step 10, page 49).

9. Select the destination folder for the next assay in your import queue, then press **Save**.

Repeat this process until you have imported all the assays you have selected in Step 4 (page 46).

Once all the assays you have selected are imported successfully, the *Verification* screen opens.



10. Press **Done** to complete the import process and return to the *Home screen*.

## Import assays from a computer

When the Qubit™ 4 Fluorometer is connected to a computer via a USB cable, MyQubit .qbt assay files can be transferred directly from the computer to the instrument.

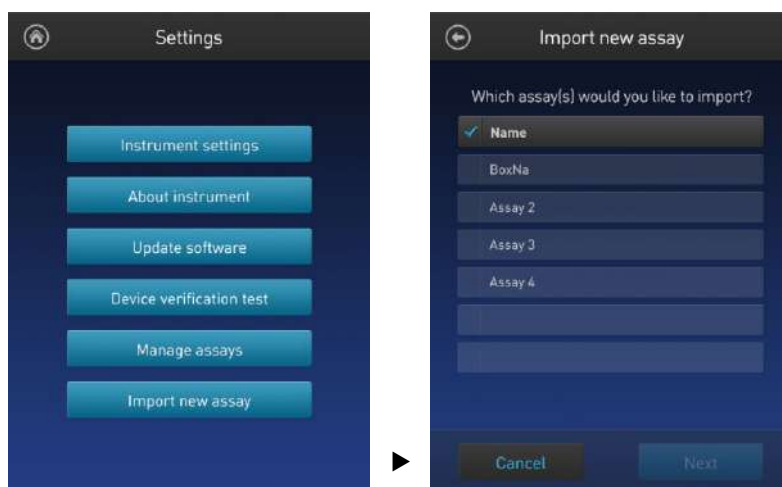
1. Go to **thermofisher.com/qubit** and download the MyQubit assay files (.qbt file) you wish to install.
2. Connect the Qubit™ 4 Fluorometer from the USB cable port to the computer using a mini-B USB cable. The Qubit™ 4 Fluorometer should be recognized as an external device by the computer.
3. **Copy and paste** or **click-drag** the .qbt files you wish to import to the Qubit™ instrument window on your computer.



**Note:** The assays will not be visible on the Qubit™ 4 Fluorometer until you perform the next step.

---

4. Go to the Settings screen on the Qubit™ 4 Fluorometer and press **Import new assay**. The *Import new assay* screen displays the names of the assays found on the computer.



5. Follow Steps 4–11 of the import procedure described in “Import assays from a USB drive”, page 46.



**Note:** The USB cable must remain connected to both the Qubit™ 4 Fluorometer and the computer for the assay to be imported.

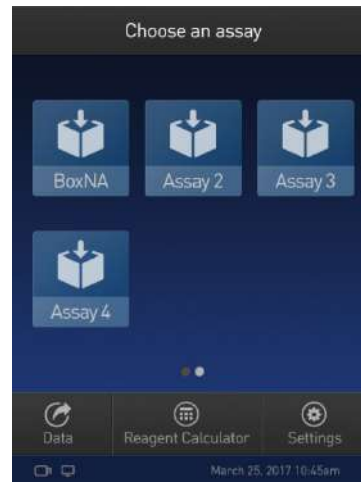
---

## Create your own assay for the Qubit™ 4 Fluorometer

### Create a MyQubit assay

All Qubit™ 4 Fluorometers are pre-loaded with MyQubit software, which allows you to create new applications for the Qubit™ 4 Fluorometer using parameters uploaded to the instrument via a USB drive, without changing the existing assays.

Fluorometer mode allows you to use the Qubit™ 4 Fluorometer with any reagent or assay compatible with the optical settings of the instrument. In this way, the device can be used as a mini-fluorometer. With the data gathered in the Fluorometer mode, personalized, custom assays can be created using the guided webtool and the MyQubit EZGuide available at [thermofisher.com/qubit](http://thermofisher.com/qubit).



**Note:** For complete instructions on creating your own assay and access to the web tool, go to [thermofisher.com/qubit](http://thermofisher.com/qubit).

## 6. Instrument maintenance

### Maintenance and cleaning

#### Maintenance

The Qubit™ 4 Fluorometer does not need regular maintenance. To troubleshoot problems with the instrument, contact Technical Support (page 71).

- **Do not** perform any repairs or service on the Qubit™ 4 Fluorometer to avoid damaging the instrument.
- **Do not expose the Qubit™ 4 Fluorometer to direct sunlight.**



**CAUTION!** Never disassemble or service the instrument yourself. Do not remove any covers or parts that require the use of a tool. Unauthorized repairs may damage the instrument or alter its functionality, which may void your warranty. Contact your local distributor to arrange for service.

---

#### Clean the Qubit™ 4 Fluorometer

We recommend that you clean the Qubit™ 4 Fluorometer periodically to prevent the buildup of dust and dirt that might reduce its performance and cause contamination.



**CAUTION!** To avoid electrical shock, always disconnect the power cable before cleaning or decontaminating the instrument.

---



**IMPORTANT!** Using a cleaning or decontaminating method other than that specified by the manufacturer may result in damage to the instrument.

---

- Clean the surface of the Qubit™ 4 Fluorometer with a damp cloth.
- To clean the touchscreen, disconnect the power cable, and clean the touchscreen with a soft cloth lightly moistened with LCD (liquid crystal display) cleansing detergent.
- Cleaning the screen with excessive force can damage the touchscreen. Wipe the screen dry immediately.
- Do not use abrasive cleaning solutions or material to prevent the touchscreen from getting scratched.
- To disinfect the instrument, disconnect the power cable from the Qubit™ 4 Fluorometer and clean the instrument, including the touchscreen, with a soft cloth lightly moistened with 70% ethanol, 70% isopropanol, or 10% bleach (0.6% sodium hypochlorite).
- The cloth included with the instrument is not recommended for use with ethanol or isopropanol.
- Ensure that the cleaning solution does not enter the power button, the power inlet, the sample port, or the USB drive ports.
- Never pour or spray any liquids directly on the instrument to avoid electrical shock when the instrument is plugged in.

## Software updates

### Update the software

To update the Qubit™ 4 Fluorometer with the latest software from Thermo Fisher Scientific:

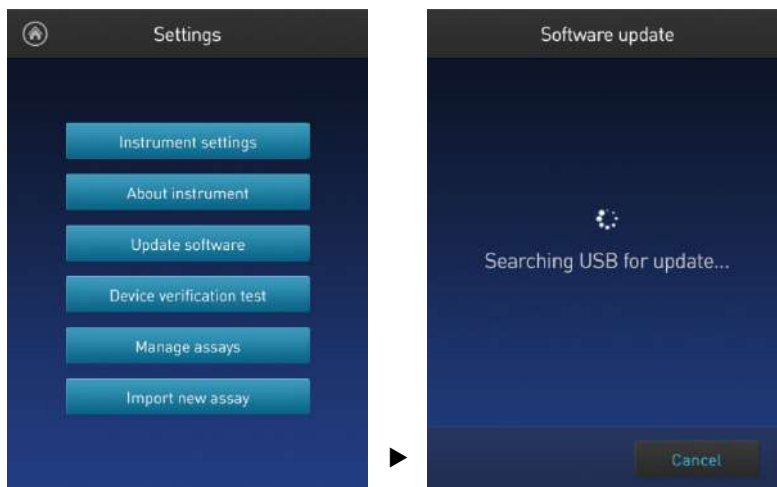
1. Download the latest software to a USB drive or to your computer from **thermofisher.com/qubit**.
2. If using a USB drive, insert the USB drive into the instrument.

If using a computer, connect the Qubit™ instrument to the computer using a USB cable. The Qubit™ Fluorometer will be recognized as an external device by the computer.



**Note:** The connection between the computer and the Qubit™ instrument must be maintained until the transfer and update (described in the next steps) are complete.

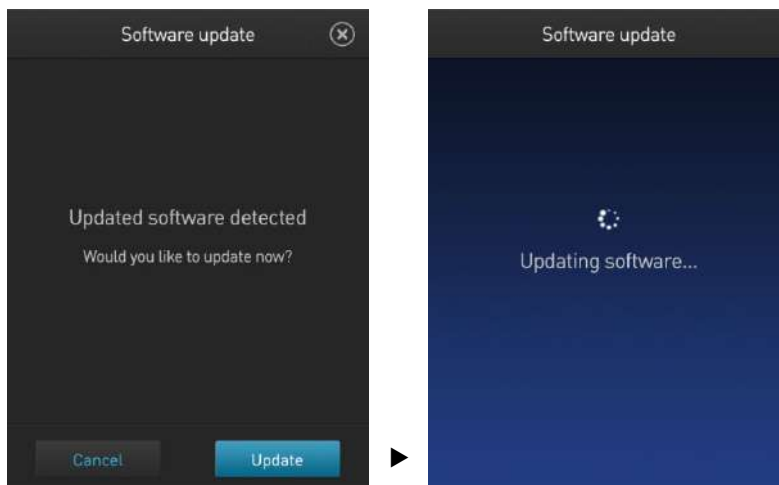
- a. On your computer, find the Qubit™ software update file that you wish to upload.
  - b. Copy and paste or click-drag the software file to the Qubit™ instrument drive. This saves the software file to the root directory of your Qubit™ instrument; however, the software file transfer will not be visible on the Qubit™ instrument.
3. On the Home screen, press **Settings**.
  4. On the Settings screen, press **Update software**. The instrument searches the USB drive or the computer for the update.



**Note:** If the USB drive is not inserted into the USB drive port or the instrument does not recognize the USB drive, a warning message is displayed.

To proceed with the software update, insert the correct USB drive into the instrument, then press **Close**.

5. When the appropriate files are detected, the Software update screen is displayed. Press **Update** to update the software.



6. When prompted, press **Restart** to complete the software update.



## System verification test

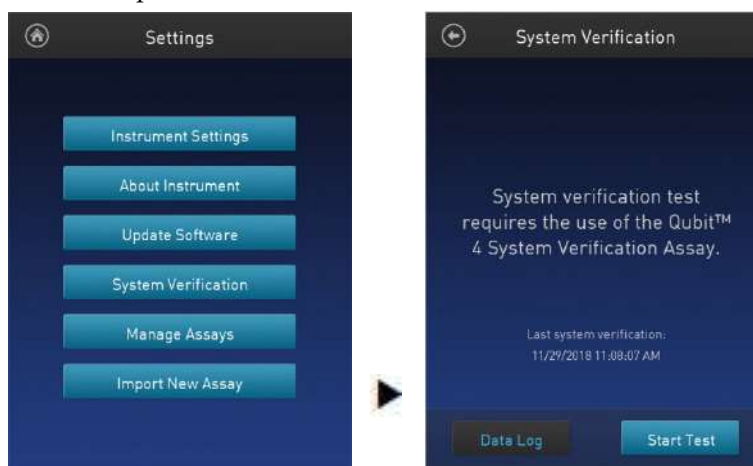
### Perform System verification test

The system verification test checks the internal components of the Qubit™ 4 Fluorometer and requires the use of the Qubit™ 4 System Verification Assay Kit (Cat. No. Q33237). Perform the system verification when a problem with the instrument is suspected. It is not necessary to regularly perform the verification.

Required materials not provided:

- Qubit™ 4 System Verification Assay Kit (Cat. No. Q33237)
- Qubit™ assay tubes (500 tubes, Cat. No. Q32856)

1. On the Home screen, press **Settings** then **System Verification Test**. Press **Start Test** to proceed.



2. Set up 3 Qubit™ assay tubes (Cat. No. Q32856).
3. Label the tube lids 1, 2, and 3.



**Note:** Do not label the side of the tube as this could interfere with the sample read.

4. Add 200  $\mu$ L of the Blank Reagent (Component A) to Tube 1.
5. Add 200  $\mu$ L of the Green Fluorescence Reagent (Component B) solution to Tube 2.
6. Add 200  $\mu$ L of the Far Red Fluorescence Reagent (Component C) solution to Tube 3.
7. Insert Tube 1 containing the Blank Reagent (Component A) into the sample chamber, close the lid, then press **Read tube**. When the reading is complete (~3 seconds), remove Tube 1.
8. Insert Tube 2 containing the Green Fluorescence Reagent (Component B) into the Qubit™ sample chamber, close the lid, then press **Read tube**. When the reading is complete (~3 seconds), remove Tube 2.



9. Insert Tube 3 containing the Far Red Fluorescence Reagent (Component C) into the sample chamber, close the lid, then press **Read tube**. When the reading is complete (~3 seconds), remove Tube 3.



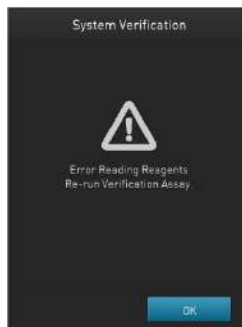
**Note:** Always insert tubes in the order 1, 2, 3.

10. Upon read completion, the instrument displays the overall result of the system verification.

- If no errors are found, the System Verification Pass message appears. Press **Close** to return to the Settings screen or press **Next** to view the System Verification Report.



- If errors are found, the Error Reading Reagents message appears. Verify that the test was run with the lid closed, then press **OK** to re-run the test with the tube strips in the correct order.



- If the System Verification Failed message persists after re-running the tube strips with the lid closed, contact Technical Support for help (page 70).

- Press **Next** to view system verification test results details or press **Data Log** to access and export system verification test results.



# Appendix A: Troubleshooting

## Troubleshooting

### Handling samples

- The calibration standards included in the Qubit™ microRNA, Qubit™ RNA HS, Qubit™ RNA XR, Qubit™ RNA IQ, and Qubit™ RNA BR Assay Kits are high-quality RNA standards. The integrity and concentration of these standards is critical to the optimal performance of the Qubit™ RNA assays. We highly recommend treating the rRNA standards as you would any other precious RNA. Use appropriate RNase-free handling techniques, including RNase-free gloves, pipette tips, and tubes. Keep the tube lids closed whenever possible; do not press the pipet to the inside wall of the tube when withdrawing a sample. Return the RNA standards to  $-80^{\circ}\text{C}$  as soon as possible after use.
- Ensure that the assay tubes are at room temperature at the time the reading is taken. Do not hold assay tubes in your hand and do not leave assay tubes in the Qubit™ 4 Fluorometer for longer than it takes to read the fluorescence. See “Assay temperature”, page 60.
- Be careful not to spill sample into the sample chamber. Promptly wipe any spills.
- The Qubit™ assays are very sensitive and even small amounts of material from a previous sample may result in errors. Use a clean 0.5-mL PCR tube for each reading.
- The tube **must be clean and dry** on the outside when taking readings. Moisture and condensation on the tube surface can lead to reading errors.
- Minute bubbles in samples will cause errors in readings. Be sure not to introduce bubbles into samples. Slight tapping on the tube wall or brief centrifugation will often help dissipate bubbles.

### High reading

- The sample is out of range. Use a sample that is less concentrated.
- For Qubit™ quantitation assays, view the Fluorescence vs. Concentration graph in the Results screen to confirm that the values for the samples fall between the values of the standards (page 27).
- Ensure that the lid is closed while reading standards and samples.
- Prepare samples and standards according to the instructions in the Qubit™ assay kit you are using.
- Ensure that the assay is performed entirely at room temperature.



## Low reading

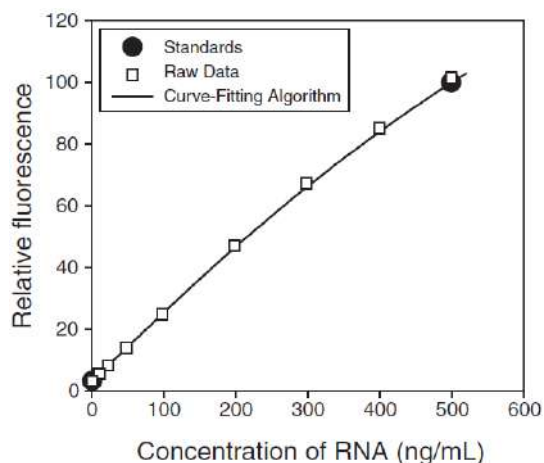
- The sample is out of range. Use a sample that is more concentrated or use a lower dilution (for example, 20  $\mu\text{L}$  in 180  $\mu\text{L}$  instead of 10  $\mu\text{L}$  in 190  $\mu\text{L}$ ).
- For Qubit™ quantitation assays, view the Fluorescence vs. Concentration graph in the Results screen to confirm that the values for the samples fall between the values of the standards (page 27).
- Ensure that you have prepared the Qubit™ working solution correctly (1:200 dilution using the buffer provided in the kit).
- Ensure that you have prepared the standard tubes correctly (10  $\mu\text{L}$  of each standard in 190  $\mu\text{L}$  of Qubit™ working solution).
- Ensure that the standard and sample tubes are filled to 200  $\mu\text{L}$ .
- Protect the Qubit™ reagent and working solutions from light.
- Select the correct Qubit™ 4 Fluorometer assay for the Qubit™ assay you are performing and calibrate the fluorometer correctly. Standards must be used in the correct order.
- Ensure that the assay is performed entirely at room temperature.



## Critical Qubit™ Assay considerations

### How the Qubit™ 4 Fluorometer calculates concentration

The Qubit™ 4 Fluorometer generates concentration data based on the relationship between the two standards used in calibration (three for the Qubit™ protein assay). The plot below shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ RNA HS assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line. This plot demonstrates that the curve-fitting algorithm gives accurate values for quantitation.



**Figure 1.** The curve-fitting algorithm used to determine concentration in the Qubit™ RNA HS assay. Data for other Qubit™ quantitation assays are generated by similar algorithms.

### Incubation time

To allow the Qubit™ assay to reach maximum fluorescence, incubate the tubes for 2 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature for all nucleic acid assays except the Qubit™ ssDNA assay, which is stable for up to 30 minutes.

The Qubit™ protein assay requires 15 minutes of incubation for a stable signal. For greatest accuracy in the protein assay, the incubation time of the samples should be within 10 minutes of the incubation time of the standards. The Qubit™ Protein Broad Range Assay requires 10 minutes of incubation time for best results.

### Photobleaching of Qubit™ reagents

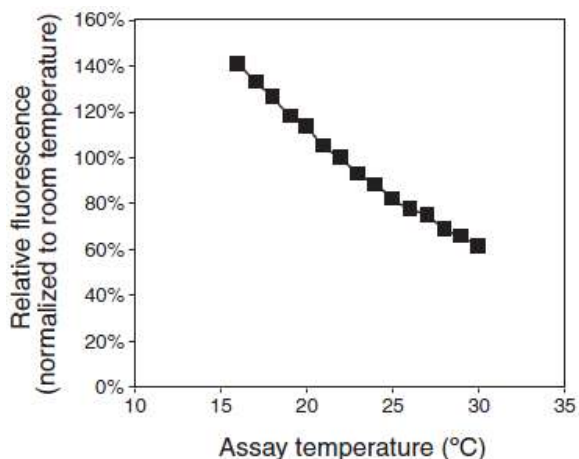
The Qubit™ DNA and protein exhibit high photostability in the Qubit™ 4 Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit™ 4 Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (see Figure 2 in “Assay temperature”, page 60). The RNA assays including the RNA IQ assay should only be read once.

Note that the temperature inside the Qubit™ 4 Fluorometer may be as much as 3°C above room temperature after 1 hour. For this reason, if you want to perform multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

## Assay temperature

The Qubit™ assays were designed to be performed at room temperature (22–28°C), and temperature fluctuations can influence the accuracy of the assay.

To minimize temperature fluctuations, store all kit reagents at room temperature and insert all assay tubes into the Qubit™ 4 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, because the Qubit™ 4 Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before a measurement, because holding the tubes warms the solution and results in a low reading.



**Figure 2.** Effect of temperature on the Qubit™ dsDNA BR assay. Qubit™ dsDNA HS, Qubit™ ssDNA, Qubit™ RNA HS, Qubit™ RNA XR, Qubit™ RNA IQ, and Qubit™ protein assays show similar sensitivities over the same range.

## Qubit™ 4 Fluorometer calibration

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, you perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you can determine the level of comfort you have using the calibration data stored from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing the standards and the samples is stable for not longer than 3 hours. See Figure 1 in “How the Qubit™ 4 Fluorometer calculates concentration” (page 59) for an example of the calibration curve used to generate the quantitation results.

## Appendix B: Ordering information

### Qubit™ 4 Fluorometer and accessories

The following products can be used with the Qubit™ 4 Fluorometer and are available separately from Thermo Fisher Scientific. For more information, visit [thermofisher.com](http://thermofisher.com) or contact Technical Support (page 71).

Product	Quantity	Cat. No.
Qubit™ 4 Fluorometer, with Wi-Fi	1 kit	Q33238
Qubit™ 4 Quantitation Starter Kit, with Wi-Fi	1 kit	Q33239
Qubit™ 4 NGS Starter Kit, with Wi-Fi	1 kit	Q33240
Qubit™ RNA IQ Starter Kit, with Wi-Fi	1 kit	Q33241
Qubit™ 4 USB Flash Drive	1 each	Q46009
Qubit™ 4 Fluorometer International Power Supply (replacement)	1 each	A36204
Qubit™ Assay Tubes	500 tubes	Q32856
Qubit™ Protein BR Assay Kit *0.1–20 mg/mL*	100 assays	A50668
	500 assays	A50669
Qubit™ Protein Assay Kit *0.25–5 µg*	100 assays	Q33211
	500 assays	Q33212
Qubit™ 1X dsDNA HS Assay Kit	100 assays	Q33230
	500 assays	Q33231
Qubit™ 1X dsDNA BR Assay Kit	100 assays	Q33265
	500 assays	Q33266
Qubit™ ssDNA Assay Kit *1–200 ng*	100 assays	Q10212
Qubit™ RNA IQ Assay Kit	75 assays	Q33221
	275 assays	Q33222
Qubit™ RNA HS Assay Kit	100 assays	Q32852
	500 assays	Q32855
Qubit™ RNA BR Assay Kit	100 assays	Q10210
	500 assays	Q10211
Qubit™ RNA XR Assay Kit	100 assays	Q33223
	500 assays	Q33224
Qubit™ microRNA Assay Kit *0.5–100 ng*	100 assays	Q32880
	500 assays	Q32881