invitrogen

Qubit[™] 4 Fluorometer USER GUIDE

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Revision	Date	Description
Е	5 June 2025	Conversion to CCMS and minor updates were made throughout for consistency of style and terminology.
		 Version numbering was changed to new format and reset to E in conformance with internal document control procedures.
D.0	20 October 2021	Adding various edits, including deleting "Register your instrument" topic and product edits in "Ordering Information".
C.0	9 April 2018	Add note about the requirement for driver installation in "Export data directly to computer".
B.0	30 January 2018	Update Safety and EMC standards and Results screen images for quality assays.
A.0	5 October 2017	New document for Qubit™ 4 Fluorometer User Guide.

The information in this guide is subject to change without notice.

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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.
Qubit™ 4 Fluorometer User Guide	MAN0017209
Qubit™ 4 Fluorometer Quick Reference Card (QRC)	MAN0017210

Additional resources are available on the Qubit[™] Technical Resources page. Go to 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 "Safety alert words" on page 8.

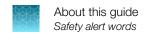
Convention	Use
Bold	Bold text indicates user action. For example: Click Run.
•	Right arrow symbol (►) indicates a menu choice and separates successive commands you execute or select from a drop-down or shortcut menu. For example: Select Settings ▶ Instrument settings.

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.



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**. See "Product description" on page 10 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 "Set up the Qubit™ 4 Fluorometer" on page 13 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. You can also use the Qubit™ 4 Fluorometer to directly measure the fluorescence of samples or to create new assays using the MyQubit software and web tool.

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, Portuguese, German, simplified Chinese, and Japanese.

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Instrument exterior components

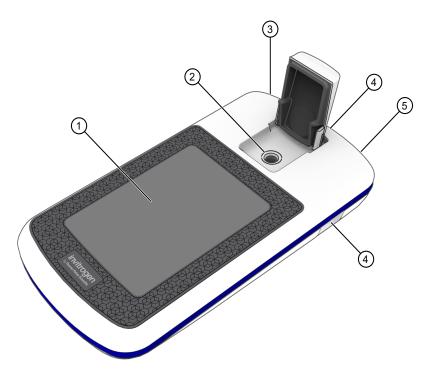


Figure 1 Top view



Figure 2 Rear view

- 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

Instrument type:	Benchtop fluorometer
Instrument dimensions:	5.4 in (w) × 10 in (l) × 2.2 in (h)
	(13.6 cm × 25 cm × 5.5 cm); rectangular shape
Weight:	26.2 oz (743 g)
Operating power:	100–240 VAC, 1.0 A/1.2 A
Frequency:	50/60 Hz
Electrical input:	12 VDC, 2.5 A
Installation site:	Indoor use only
Operating temperature:	10-40°C
Operating humidity:	20–80% (non-condensing)

Technical specifications

Dynamic range:	5 orders of magnitude
Processing time:	≤5 seconds/sample
Light sources: Blue LED (max ~470 nm) Red LED (max ~635 nm)	
Excitation filters:	Blue 430–495 nm Red 600–645 nm
Emission filters:	Green 510–580 nm Red 665–720 nm
Detectors: Photodiodes; measurement capability from 300–1000 nm	
Calibration type: 2- or 3- point standard	
Tube type: 0.5-mL Real Time PCR (polypropylene) tubes	
Warm-up time: <35 seconds	

USB drive

Capacity:	4 Gigabyte
Туре:	FAT32-formatted



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. Connect 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. Connect 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 off the Qubit[™] 4 Fluorometer, unplug it.

IMPORTANT! Use the power cord plug adapter supplied with the instrument that is appropriate for the electrical outlet configuration in the country. Powering the instrument with an unapproved power cord can 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), or Protein.
- 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.



Figure 3 Home screen

Create username and Sign In

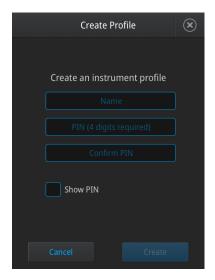
To follow new cybersecurity legal standards, the latest firmware for Qubit[™] 4 instruments require users to login to access specific menu options (such as **instrument settings**, **Update software**, and **system verification**).

Note: When not logged these menu options will appear to be inactive. Guest users attempting to make changes in **Settings** will be redirected to the **Sign-in** page.

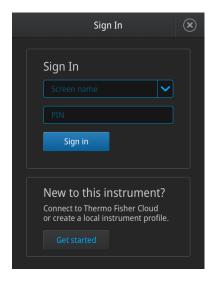
Instructions for login:

- 1. Power on the device.
- 2. From the **Home** screen, click on the profile (person) icon on the top left corner.
- 3. Tap the **Get Started** button.
- 4. Tap the **Create Profile** button.

5. Enter information for username and pin.



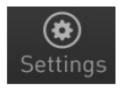
6. To Sign in, click on the profile (person) icon again and enter the **Username** and **PIN** in the provided fields.



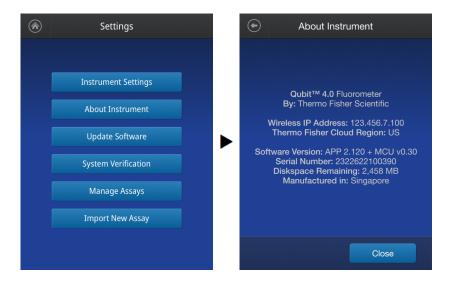
About instrument screen

The **About Instrument** screen displays information about the Qubit[™] 4 Fluorometer, including the currently installed software version. To go to the **About Instrument** screen:

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



2. On the Settings screen, press About Instrument.



3. Press Close or Back () to return to the Settings screen.

Optional: Configure instrument settings

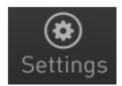
Introduction

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

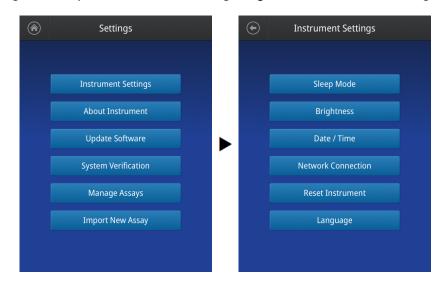
- Sleep mode ("Adjust the sleep mode" on page 18)
- Brightness ("Adjust screen brightness" on page 19)
- Date/Time ("Adjust the date and time" on page 19)
- Reset instrument ("Reset function" on page 20)
- Language ("Change the display language" on page 21)

Access the instrument settings screen

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



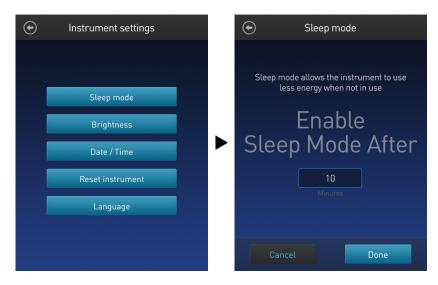
2. On the **Settings** screen, press **Instrument settings** to go to the **Instrument settings** screen.



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 ("Access the instrument settings screen" on page 17), press **Sleep mode** to go to the **Sleep mode** screen.

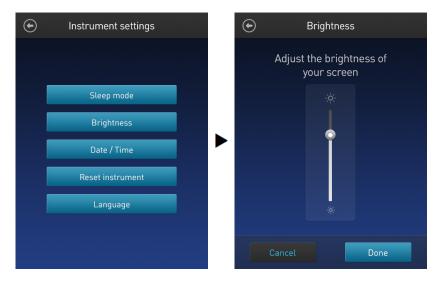


- 2. Enter the time in minutes allowed before the instrument goes into sleep mode by pressing the **minutes** field, then using the number pad to select a value. The software requires a minimum of 1 minute and a maximum of 60 minutes.
- Press Done to save the changes and return to the Instrument settings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

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Adjust screen brightness

1. On the **Instrument settings** screen ("Access the instrument settings screen" on page 17), press **Brightness** to go to the **Brightness** screen.

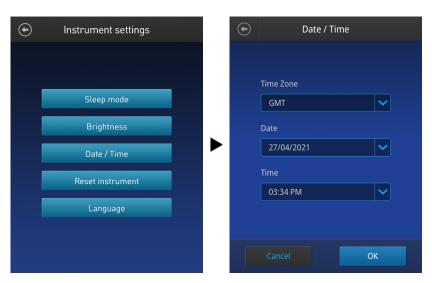


- 2. Move the **brightness slider** button up or down to adjust the brightness of the display.
- Press Done to save the changes and return to the Instrument settings screen.
 Press Cancel or Back () to return to the Instrument settings screen without saving the changes.

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 ("Access the instrument settings screen" on page 17), 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.

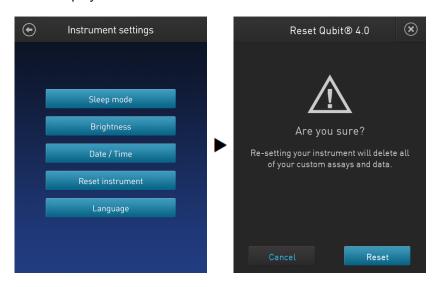
Reset function

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 ("Access the instrument settings screen" on page 17), 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 (((x)) to return to the Instrument settings screen without saving the changes.

Change the display language

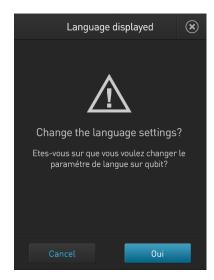
You can change the language that the Qubit[™] 4 Fluorometer displays to English (default), French, German, Italian, Portuguese, Spanish, simplified Chinese, and Japanese.

1. On the **Instrument settings** screen ("Access the instrument settings screen" on page 17), press **Language** to go to 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" on page 80.

- 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.
- Ensure 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 Assay 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.

Go to 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.



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 Appendix B, "Ordering information" 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) https://assets.thermofisher.com/TFS-Assets/BID/manuals/MAN0017210_Qubit_4_Assays_QR.pdf.

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

Qubit[™] 4 Fluorometer User Guide

Optional: Use reagent calculator to prepare Qubit™ working solution

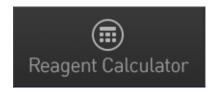
Introduction

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.

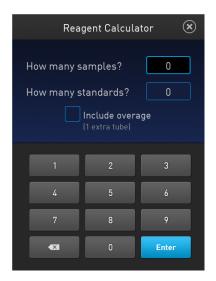
Use the Reagent Calculator

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.

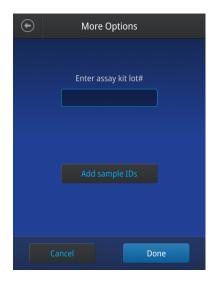


Note: You can change the total number of tubes that you plan to run or the overage selection on this screen.

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

Optional: Enter Assay Kit Lot numbers and Sample IDs

 Press More options to access the More options screen, where you can Enter assay kit lot # and Add sample IDs.

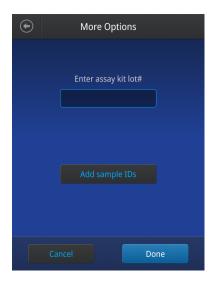


Note: The information you have entered will be available on the Data Details of your samples.

2. Click the Enter assay kit lot # text box, type the assay kit lot number, then press Enter.



3. To add sample IDs to your samples, insert a **USB drive** with the sample names into the instrument, then press **Add Samples IDs**.



Note: The file containing the sample IDs must be in .CSV format and filled out like the example below: first Plate Barcode, then Well and Sample ID.



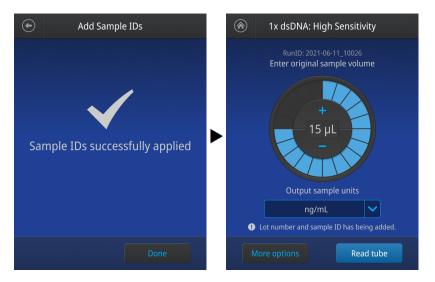
4. Select the file containing the sample IDs from the list of available files, then press **Apply**.



5. Press **OK** at the confirmation page.

Chapter 3 Perform assays Timer

6. When finished entering assay kit lot number and sample IDs, press **Done** at the **More Options** screen. The assay screen displays the new information added to your samples at the bottom of the screen. To go back to the assay screen without applying the new information, press **Cancel**.



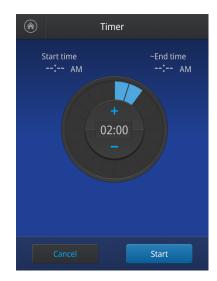
Timer

Optional: Use Assay Incubation Timer to keep track of sample incubation.

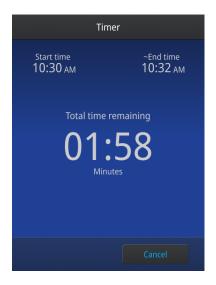
1. Select Timer.



2. Set the timer for the recommended incubation assay time (2 minutes for nucleic acid assay samples, 10 minutes for protein BR assay, 15 minutes for the protein assay samples).



3. Tap the **Start** button.



Chapter 3 Perform assays Timer

4. Screen will indicate when time is complete.



Calibrate assays on the Qubit[™] 4 Fluorometer

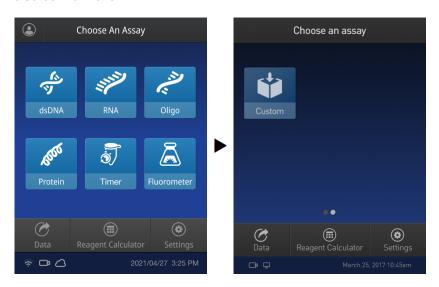
Introduction

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 calibration" on page 81").

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

Read new standards for calibration

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.



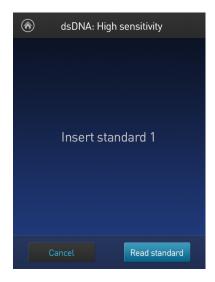
Note: The software displays the available assays for the assay type you selected in step 1 on page 31. The example in step 2 on page 31 shows the available assays for the dsDNA assay type.

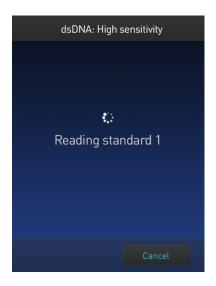
3. If you have already performed a calibration for the selected assay, the software prompts you to choose between reading new standards and running samples using the previous calibration. A notification will notify users if the last standard reading was more than six months ago. For best results, perform a new standard calibration each time. Press Read standards.



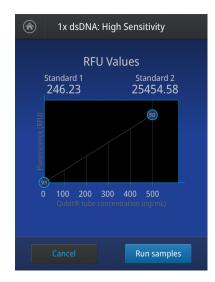
Note: To apply the previous calibration to your sample readings, press **Run samples**. The software prompts you to insert the assay tube containing sample. See ""Read samples" on page 37".

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.



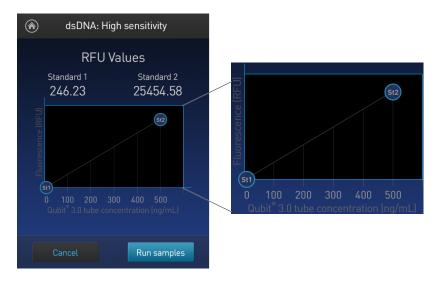


- 5. At the prompt, insert Standard #2 into the sample chamber and press **Read standard**. Be sure to use the Standard #2 appropriate for the assay you are performing.
- **6.** For Qubit[™] protein and RNA IQ assays only: At the prompt, insert Standard #3 into the sample chamber and press **Read standard**.
- 7. 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.



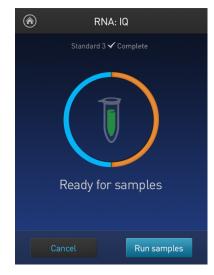
Calibration results

• 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 ("Calibration error" on page 35).

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.

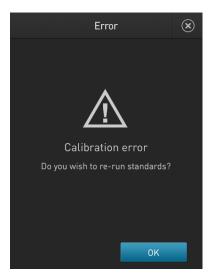
For the Qubit™ protein assay, the reading given by Standard #3 should be at least 40% higher than that of Standard #2.

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

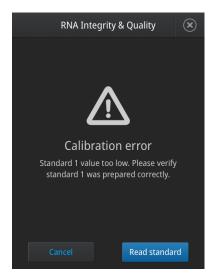
If you receive the "Calibration error" message, you can re-run the standards:

- 1. In the Error screen, press OK.
- 2. Review the **Read standard** screen (see "Calibration results" on page 34).
- 3. If you wish to re-run the standards, or run new standards, press **Read standards**, then repeat the calibration procedure (beginning in "Optional: Use reagent calculator to prepare Qubit™ working solution" on page 24).



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**.

Qubit[™] 4 Fluorometer User Guide



Read samples

Before you begin

- Calibrate the Qubit[™] 4 Fluorometer as described in "Optional: Use reagent calculator to prepare
 Qubit[™] working solution" on page 24. (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:
 - a. Press the + or buttons on the wheel to select the sample volume added to the assay tube (between 1 and 20 µL).

Chapter 3 Perform assays Read samples

b. For the Qubit™ quantitation assays, select the units for the output sample concentration from the dropdown list.



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 – sample quantitation" on page 40".



Figure 4 Results screen for quantitation assays

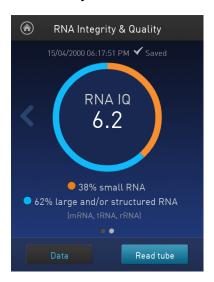


Figure 5 Results screen for quality assays

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 on page 37 ("Read samples" on page 37) and step 3 on page 38 ("Read samples" on page 37).

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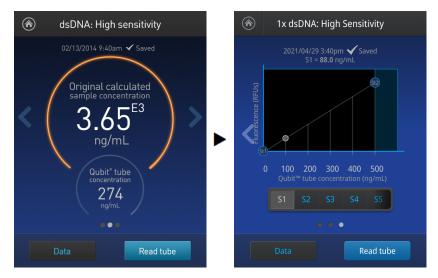
Chapter 3 Perform assays Results

Results

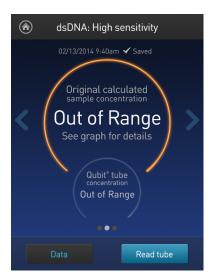
Results screen – sample quantitation

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.



Figure 6 Single sample



Figure 7 Multiple samples

Qubit[™] 4 Fluorometer User Guide



Figure 8 Out of range high



Figure 9 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, an error message is displayed.

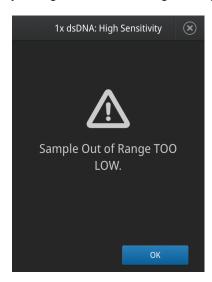


Figure 10 Measurement error - Sample concentration too low

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 select 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).

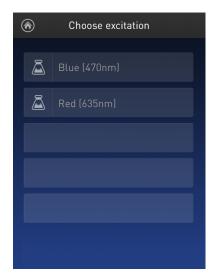
The reading is in raw fluorescence units (RFU).

Read sample in fluorometer mode

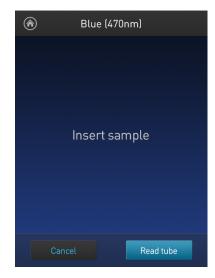
1. On the **Home** screen, press **Fluorometer**.



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.

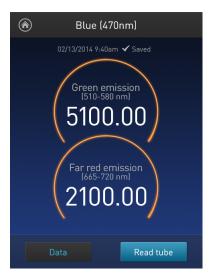


Figure 11 Blue (470 nm) excitation

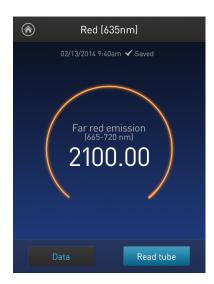


Figure 12 Red (635 nm) excitation

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.



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 ("View detailed sample data" on page 47).
- Rename data files ("Rename samples" on page 52).
- Save data as a CSV (comma separated value) or PDF file, and export the file to a USB drive or directly to your computer ("Export data" on page 53).
- Delete data files ("Delete data" on page 58)

View detailed sample data

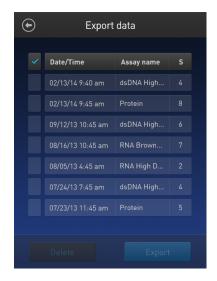
View detailed sample data

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



Chapter 4 Manage data View detailed sample data

2. When selecting data from the **Home** screen, a list of data files will appear on the **Export data** screen. Select the **dataset of interest**.



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



Figure 13 Data screen for quantitation assays, when accessing data from the sample acquisition screen



Figure 14 Data screen for quantitation assays when accessing data from the Home screen



Figure 15 Data screen for quality assays

Chapter 4 Manage data View detailed sample data

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



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



Figure 16 Data details for quantitation assays





Figure 17 Data details for quality assays

Rename samples

You can rename individual samples from the default name to your custom name by pressing the individual sample entry. You may rename samples during or after sample acquisition.

Rename samples

- 1. On the Concentration, Graph, RNA IQ results, or Home screen, press Data.
- 2. When selecting data from the **Home** screen, a list of data files will appear on the **Export data** screen. Select the **dataset of interest**.



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



Figure 18 Data screen for quantitation assays, when accessing from sample acquisition mode (left) or from the Home screen (right)

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



Export data

Introduction

The Qubit™ 4 Fluorometer is designed for stand-alone 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.

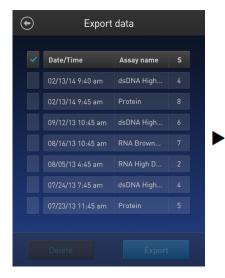
53

Export data using the USB drive

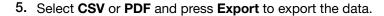
- 1. Insert the USB drive into the Qubit™ 4 Fluorometer.
- 2. On the Concentration, Graph, RNA IQ results, or Home screen, press Data.
- 3. On the Export data screen, check the selection box to the left of each dataset you wish to export.

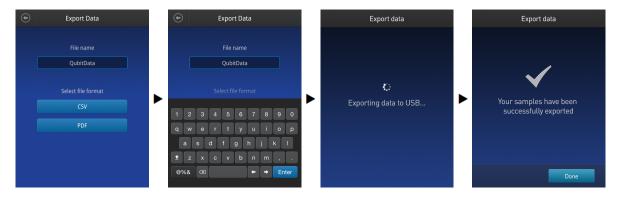


4. To save only individual data entries from a dataset, press the **dataset of interest**, and then check the **selection box** to the left of the samples you wish to export.









Note: To rename the file, select the box and change the default **QubitData** prefix to the name of your choice.

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.

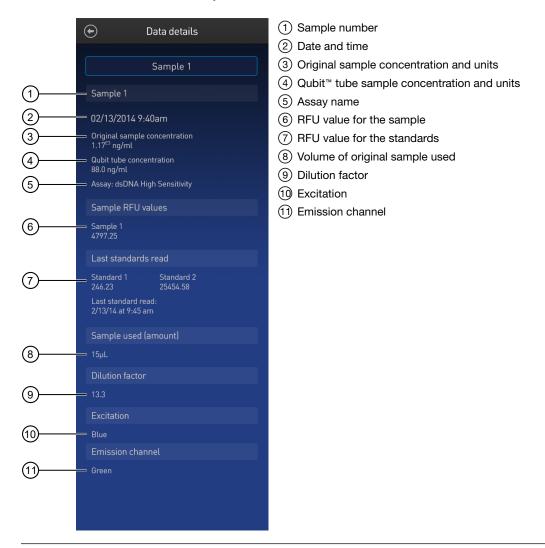
Note: The Windows[™] XP operating system requires the installation of a driver. The driver and installation instructions are available for download from Qubit[™] Technical Resources at thermofisher.com/qubit.

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.
- 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



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



Connect to Wi-Fi to enable cloud export

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 lastest 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.

Export data to the cloud using your connect account

- 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."

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

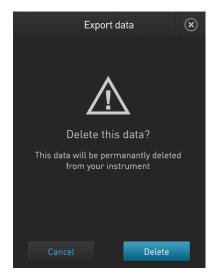
Delete data

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 dataset you wish to delete. To delete only individual data entries from a dataset, press the **dataset 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.



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



Manage, import, and create assays

Overview

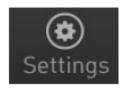
The Qubit™ 4 Fluorometer allows you to personalize the instrument's user interface to hide and show (unhide) assays or folders and to move them to different locations. In addition, you can add custom MyQubit assays by importing them from our website at thermofisher.com/qubit or create new assays using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

Manage assays

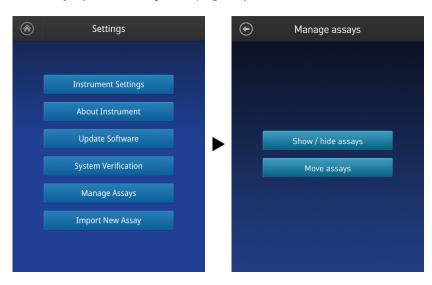
Manage assays function

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** ("Show/Hide assays" on page 61) or **Move assays** ("Move assays" on page 63).

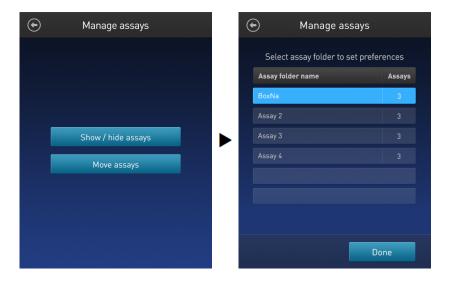


Show/Hide assays

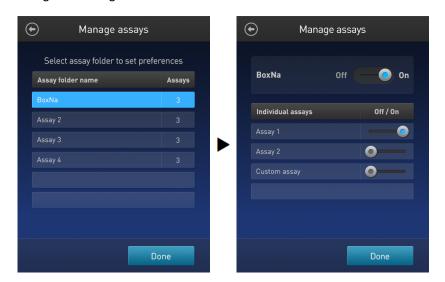
Show/Hide all assays within the assay folder

- 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 in the selected assay folder and the controls for showing and hiding them.



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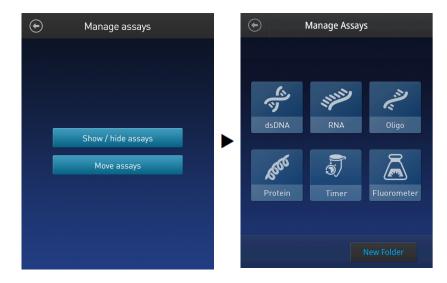
- 4. To **hide** all of the assays in the assay folder, swipe the **Assay folder On/Off** switch located to the right of the assay folder name to the **Off** position (to the **left**).
 - To **show** all of the assays in the assay folder, swipe the **Assay folder On/Off** switch to the **On** position (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.

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.

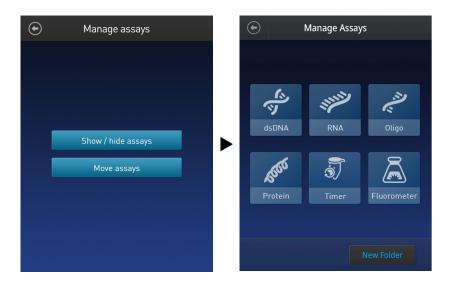


- 3. Press and hold on the desired assay folder or individual assay to detach it from its current position, then drag it to its new location.
 - To move the selected object to a different **Home** screen page, drag it up or down to the desired screen.
- 4. Release the drag to drop the selected assay folder or individual assay in its new location.
- 5. Press the **Back** button (((a)) 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 (((a))).
- 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", "Move assays" on page 63.
- 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

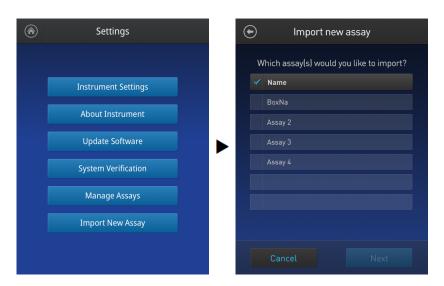
MyQubit assay files

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

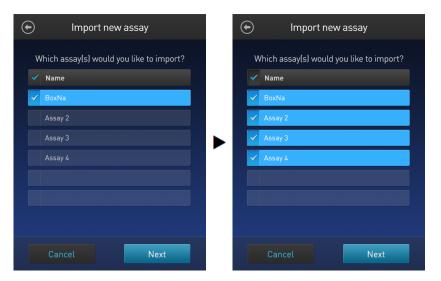
Note: MyQubit assay files have the file extension .qbt.

Import assays from a USB drive

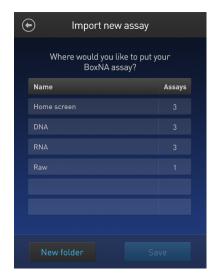
- To import one or more new assays, go to thermofisher.com/qubit and download the MyQubit assay files (.qbt file) you wish to install to the root directory of a USB drive.
 Do not download the .qbt files to a folder in the USB drive.
- 2. Insert the USB drive containing the new assay files into the USB drive port of the Qubit™ 4 Fluorometer.
- 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.

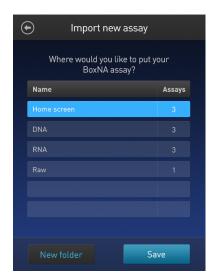


Press the **Back** button (or **Cancel** to return to the **Settings** screen without importing the assays.

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 on page 68).

Alternatively, press New folder to create a new destination folder (see Step 8 on page 68).



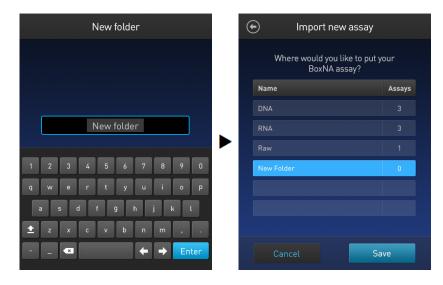
Press the **Back** button (or **Cancel** to abort the import process and return to the **Settings** screen.

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.

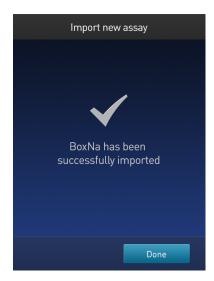
Press **Enter**, then press **Done** to return to the **Import new assay – Where** screen. The newly created folder will appear on the list of folders.

Select the new destination folder, then press **Save**. The **Verification** screen opens (see Step 9 on page 68).



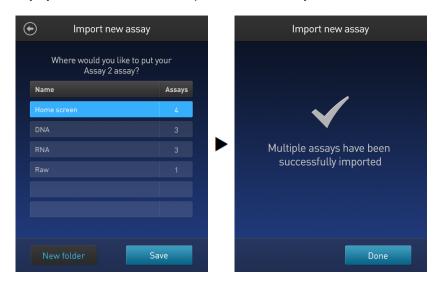
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8. In the **Verification** screen, press **Done** to complete the import process. If importing only one assay, you will be returned to the **Home** screen.



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 on page 68).

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 on page 66.
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.

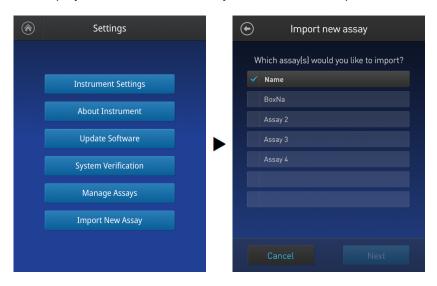
Import assays from a computer

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 on page 66 of the import procedure described in "Import assays from a USB drive", "Import assays from a USB drive" on page 65.

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 Fluorometer 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.



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



Instrument maintenance

Maintenance and cleaning

Maintenance

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

- 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 can damage the instrument or alter its functionality, which can 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 decontamination method other than that specified by the manufacturer can 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

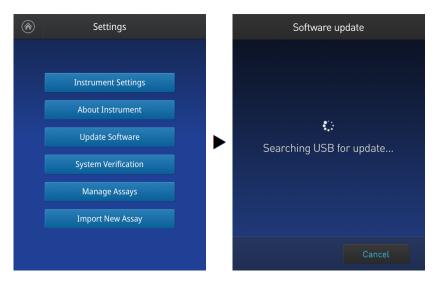
Update the software

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

- Download the latest software to a USB drive or to your computer from https://www.thermofisher.com/us/en/home/ industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/fluorometers/ qubit/qubit-technical-resources.html#firmware-updates.
- 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.

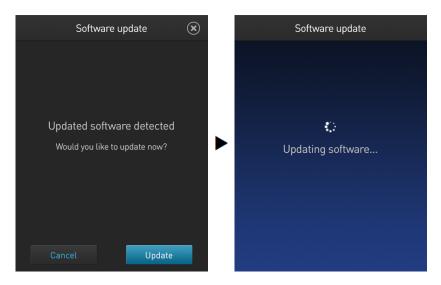
- a. On your computer, find the Qubit™ software update file that you wish to upload.
- b. Copy and paste or click-drag the software file to the Qubit™ instrument drive. This saves the software file to the root directory of your Qubit™ instrument; however, the software file transfer will not be visible 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.



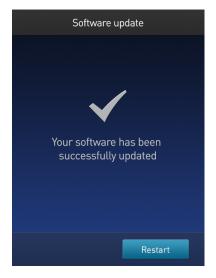
Note: If the USB drive is not inserted into the USB drive port or the instrument does not recognize the USB drive, a warning message is displayed.

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.



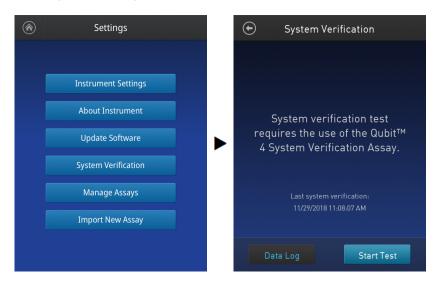
System verification test

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.

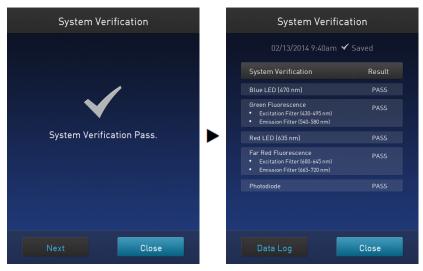
Note: Do not label the side of the tube as this could interfere with the sample read.

- 4. Add 200 μL of the Blank Reagent (Component A) to Tube 1.
- 5. Add 200 µL of the Green Fluorescence Reagent (Component B) solution to Tube 2.
- 6. Add 200 µL 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.

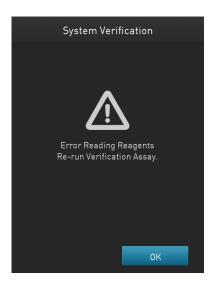
9. Insert Tube 3 containing the Far Red Fluorescence Reagent (Component C) into the sample chamber, close the lid, then press **Read tube**. When the reading is complete (~3 seconds), remove Tube 3.

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.

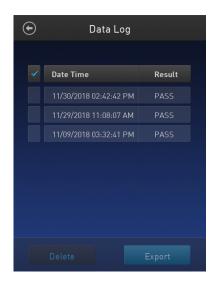


• If the **System Verification Failed** message persists after re-running the tube strips with the lid closed, contact Technical Support for help.

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Chapter 6 Instrument maintenance System verification test

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





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°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" on page 81".
- 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.

Appendix A Troubleshooting Troubleshooting

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 ("Results screen sample quantitation" on page 40).
- 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 μL in 180 μL instead of 10 μL in 190 μL).
- 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 ("Results screen sample quantitation" on page 40).
- 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 μL of each standard in 190 μL of Qubit™ working solution).
- Ensure that the standard and sample tubes are filled to 200 µL.
- 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.

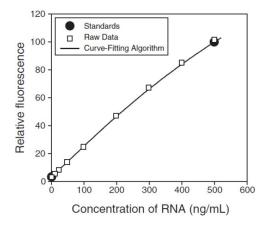


Figure 19 The curve-fitting algorithm used to determine concentration in the Qubit™ RNA HS assay. Data for other Qubit™ quantitation assays are generated by similar algorithms.

Incubation time

To allow the Qubit™ assay to reach maximum fluorescence, incubate the tubes for the DNA and RNA assays 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 20 in ""Assay temperature" on page 81"). The RNA assays including the RNA IQ assay should only be read once.

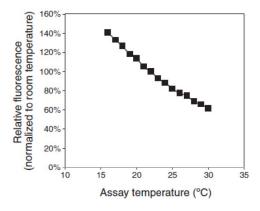


Figure 20 Effect of temperature on the Qubit™ dsDNA BR assay. Qubit™ dsDNA HS, Qubit™ ssDNA, Qubit™ RNA HS, Qubit™ RNA IQ, and Qubit™ protein assays show similar sensitivities over the same range.

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.

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. For most accurate results, it is recommended to perform a new calibration each time. However, 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. It is recommended to perform a new calibration when switching users, working with a new assay lot, or after 6 months of use. A notification will notify users if the last standard reading was more than six months ago. 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 19 in ""How the Qubit™ 4 Fluorometer calculates concentration" on page 80" ("How the Qubit™ 4 Fluorometer calculates concentration" on page 80) for an example of the calibration curve used to generate the quantitation results.



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** or contact Technical Support.

Product	Quantity	Cat. No.
Qubit [™] 4 Fluorometer, with WiFi	1 kit	Q33238
Qubit [™] 4 Quantitation Starter Kit, with WiFi	1 kit	Q33239
Qubit™ 4 NGS Starter Kit, with Wi-Fi	1 kit	Q33240
Qubit™ 4 RNA IQ Starter Kit, with WiFi	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

(continued)

Product	Quantity	Cat. No.
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

Safety





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, and so on). To obtain SDSs, visit thermofisher.com/support.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **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.

Standard safety symbols

	Symbol and description		
<u> </u>	CAUTION!	Risk of danger. Consult the manual for further safety information.	
A	CAUTION!	Risk of electrical shock.	
	CAUTION!	Hot surface.	
A	CAUTION!	Pinch point.	
	CAUTION!	Potential biohazard.	

Control and connection symbols

Symbols and descriptions	
	On (Power)
	Off (Power)
<u>_</u>	Earth (ground) terminal
	Protective conductor terminal (main ground)
	Direct current
\sim	Alternating current
\sim	Both direct and alternating current

Conformity symbols

Conformity mark	Description
c UL us	Indicates conformity with safety requirements for Canada and U.S.A.
25	China ROHS EFUP 25 (Environmental Friendly Use Period of 25 years)
C€	Indicates conformity with European Union requirements.
	Indicates conformity with Australian standards for electromagnetic compatibility.
	INDICATES CONFORMITY WITH THE WEEE DIRECTIVE 2012/19/EU. CAUTION! To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Appendix C Safety General instrument safety

General instrument safety



WARNING! PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified may result in personal injury or damage to the instrument.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all relevant Safety Data Sheets (SDSs). See "Safety Data Sheets (SDS)".

Safety precautions

Do not install the instrument in areas with high humidity, such as greenhouses or incubators, to prevent the risk of electric shock. If water or other materials enter the instrument, the adaptor, or the power inlet, disconnect the power cord immediately and contact a service technician:

- Make sure to only use the 12V power supply that comes with the Qubit™. Use of higher than 12V power supply will damage the Qubit™ 4 Fluorometer device.
- Do not press the main plug or power cord with wet hands.
- Ensure the power supply input voltage matches the voltage available at your location.
- Do not install the instrument on an inclined surface or in areas subject to vibrations, as this may cause malfunction or damage.
- Securely plug the power cord into both the wall outlet and the instrument.
- To avoid potential shock hazard, make sure that the power cord is properly grounded.
- Be sure to position the equipment such that it is easy to disconnect the instrument.
- If the instrument is broken or dropped, disconnect the power cord and contact technical services.

 Do not disassemble the instrument.
- Use only authorized accessories (adaptor, power cord, and USB drive).
- For operating environment, see "Product specifications" on page 12.
- If the instrument emits smoke, disconnect the power cord from the wall outlet and contact technical services.

Cleaning and decontamination



CAUTION! Cleaning and Decontamination. Using cleaning or decontamination methods other than those recommended by the manufacturer may compromise the safety or quality of the instrument.

Removing covers or parts of the instrument



WARNING! PHYSICAL INJURY HAZARD. The instrument is to be serviced only by trained personnel or vendor specified in the user guide. Do not remove any covers or parts that require the use of a tool to obtain access to moving parts.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety and EMC standards

Reference	Description
UL 61010-1/CAN/CSA- C22.2 No. 61010-1	Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements.
IEC/EN 61010-1	
UL 61010-2- 081/CAN/CSA-C22.2 No. 61010-2-081	Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 2–081: Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes.
IEC/EN 61010-2-081	
FCC Part 15 Subpart B (47 CFR)	This instrument has been tested to and complies with standard 47 CFR FCC Part 15 "Radio Frequency Devices"; Subpart B "Unintentional Radiators".
	Note: This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures: Reorient or relocate the receiving antenna.
	Increase the separation between the equipment and receiver.
	 Connect the equipment into an outlet on a circuit different from that to which the receiver is connected. Consult the dealer or an experienced radio/TV technician for help.
ICES-001	This instrument has been tested to and complies with standard ICES-001, "Industrial, Scientific and Medical (ISM) Radio Frequency Generators."
EU Directive 2014/35/EU	European Union "Low Voltage Directive"

(continued)

Reference	Description
EU Directive 2014/30/EU	European Union "EMC Directive"
EN 61326-1	EN 61326–1:2013 Class B, "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."
Restriction of Hazardous Substance (RoHS)	This instrument meets European requirements RoHS Directive 2011/65/EU. This instrument has been tested to and complies with standard EN 50581:2012, "Technical documentation for the evaluation of electrical and electronic products with respect to restriction of hazardous substances."
Waste of Electrical and Electronic Equipment (WEEE)	This instrument meets European requirement WEEE Directive 2012/19/EU.
AS/NZS CISPR 11	This instrument has been tested to and complies with standard AS/NZS CISPR 11, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."
China RoHS GB/T 26572–2011 SJ/T 11364–2014	 This instrument does not contain any hazardous substances above the limit and complies with China RoHS standards: GB/T 26572-2011, "Requirements on Concentration Limits for Certain Restricted Substances in Electrical and Electronic Products." SJ/T 11364-2014, "Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products."

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union "WEEE Directive" - Waste electrical and electronic equipment
EU Directive 2011/65/EU Commission Delegated Directive (EU) 2015/863	European Union "RoHS Directive"—Restriction of hazardous substances in electrical and electronic equipment
SJ/T 11364-2014	"China RoHS" Standard—Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products For instrument specific certificates, visit our customer resource page at www.thermofisher.com/us/en/home/technical-resources/rohs-certificates.html .

Chemical safety



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

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Chemical waste safety

Chemical waste hazard



CAUTION! HAZARDOUS WASTE!

Refer to Safety Data Sheets (SDSs) and local regulations for handling and disposal.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide 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.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis, if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument 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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Electrical safety



WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.



WARNING! Voltage Selector Switch. Before installing the instrument, verify that the voltage selector switch is set for the supply voltage. This will prevent damage to the instrument, reduce risk of fire, and enable proper operation.



WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.



WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility. If the line cord is damaged, contact Technical Support.



WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Overvoltage rating

The Qubit™ 4 Fluorometer, has an installation (overvoltage) category of II, and is classified as portable equipments.

Appendix C Safety Biological hazard safety

Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



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), 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020 cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
 who.int/publications/i/item/9789240011311

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

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