invitrogen

Qubit[™] Flex Fluorometer USER GUIDE

Catalog Number Q33327, Q45893, Q45894

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1. Product information

Product contents

The Qubit[™] Flex Fluorometer (Cat. No. Q33327) is shipped with the following components:

Component	Quantity
Qubit [™] Flex Fluorometer	1 each
Qubit [™] Flex power cord (shipped separately) ^[1]	1 each
USB drive	1 each
Qubit [™] Flex LAN cable	1 each
Qubit [™] Flex Fluorometer Quick Reference Card (QRC)	1 each
Certificate of Conformity (COC)	1 each
Qubit [™] screen cleaning cloth	1 each
Wi-Fi Dongle	1 each

^[1] The power cords for the Qubit™ Flex Fluorometer are not interchangeable with those for the other Qubit™ Fluorometer models. Powering the instrument with an unapproved power cord can irreversibly damage the instrument.

The complete user guide is available for download at thermofisher.com/qubit. See page 6 for the description and specifications of the Qubit[™] Flex Fluorometer.

instrument

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

See page 10 for instructions to set up the Qubit[™] Flex Fluorometer.

Register your Go to thermofisher.com/qubit to register your instrument. You will be asked to instrument supply the serial number, your name, and your contact details. Registering your instrument ensures that you will receive notifications of software upgrades and information on new assays for use with the Qubit[™] Flex Fluorometer.

Product description

Qubit[™] Flex The Qubit[™] Flex Fluorometer is a benchtop fluorometer for the quantification of Fluorometer DNA, RNA, microRNA, and protein. With the Qubit™ Flex Fluorometer, you can directly measure the fluorescence of up to 8 samples simultaneously using the highly sensitive and accurate fluorescence-based Qubit[™] assays.

Features •

- Fast and highly accurate quantification of DNA, RNA, and protein of up to 8 samples simultaneously in ~3 seconds.
- 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 10,000 samples.
- 8-inch, state-of-the-art color touchscreen for easy workflow navigation.
- Instrument indicates samples that are in the extended range or out of range.
- Saves sample data as a CSV (comma separated value) file.
- On-board Reagent and Range Calculators provide instructions to prepare Qubit[™] working solution using your sample and standard inputs and to select the most accurate assay for your expected concentration range.
- On-board Molarity and Normalization Calculators allow you to calculate molarity of your samples based on nucleic acid length and determine how to dilute the samples to the same concentration, respectively, using the results from your assays.
- Allows easy definition and saving of assay preferences.
- Exports data to a USB drive, to a network drive, or to the Connect™ cloud-based platform.
- Connects to the local area network via the LAN (RJ-45) port using an Ethernet cable or wirelessly using the supplied Wi-Fi adaptor.
- Instrument user interface can be personalized to display in the language of your choice including English, French, Spanish, Italian, German, simplified Chinese and Japanese.

Instrument exterior components

Top view



Rear view



- 1 **Touchscreen** is the user interface containing the controls for all the functions needed and displays data from the assays.
- 2 Sample chamber is used to load the Qubit[™] Flex Tube Strip containing your samples into the fluorometer for analysis.
- ③ **USB drive ports (Type A)** allow you to transfer and save data to your computer using a USB flash drive or wirelessly to a network drive or a Connect[™] account using the Wi-Fi dongle (supplied with the instrument).
- (4) **Power inlet** connects the Qubit[™] Flex Fluorometer to an electrical outlet using the supplied power cord and the appropriate plug.
- (5) LAN port (RJ-45) allows you to connect to the network using an Ethernet cable.

Product specifications

characteristics

Physical Instrument type: Benchtop fluorometer

> **Instrument dimensions:** 7.3 in (w) \times 11.1 in (l) \times 4.1 in (h)

> > $(18.6 \text{ cm} \times 28.2 \text{ cm} \times 10.3 \text{ cm})$; rectangular shape

Weight: 60 oz. (1.7 kg)

Operating power: 100-240 ±10% VAC, 1.3 A

Frequency: $50/60 \, \text{Hz}$ **Electrical input:** 48 VDC, 1.87 A

IMPORTANT! If the supplied power fluctuates ±10% beyond the rated voltage, a power line regulator may be required. High or low voltages can adversely affect the electronic components of the instrument.

Operating conditions

Installation site: Indoor use only

Altitude: Between sea level and 2000 m (6500 ft.) above

sea level

10-30°C Operating temperature:

Operating humidity: 15–80% (non-condensing)

Pollution degree: The instrument has a Pollution Degree rating of II.

> The instrument may only be installed in an environment that has nonconductive pollutants. Typical environment with a Pollution Degree II rating are laboratories and sales and commercial

areas.

specifications

Technical Dynamic range: 4 orders of magnitude **Processing time:** ≤3 seconds/sample

> **Light sources:** Blue LED (max 460-480 nm)

Red LED (max 620-640 nm)

Excitation filters: Blue 456-484 nm

Red 612-644 nm

Emission filters: Green 513-563 nm

Far-Red 671–693 nm

Detectors: Photodiodes; measurement capability from

320-1100 nm

Calibration type: 2- or 3-point standard

Accommodates one Qubit[™] Flex Tube Strip Sample chamber: Oubit[™] Flex Tube Strip (8× 0.2-mL thin-wall Tube type:

polypropylene tubes; Cat. No. Q33252)

<35 seconds Warm-up time:

Hardware Display: 8-inch capacitive touchscreen with high resolution

color display

Output ports: 3× USB ports

Networking capability: Connection via the LAN (RJ-45) port using an

Ethernet cable or wirelessly using the supplied

Wi-Fi adaptor

Power supply: AC adaptor with country-specific power cords

USB drive Capacity: 4 Gigabyte

2. Getting started

Set up the Qubit[™] Flex Fluorometer

Install the The Qubit[™] Flex Fluorometer is a stand-alone instrument that does not require instrument connection to a computer.

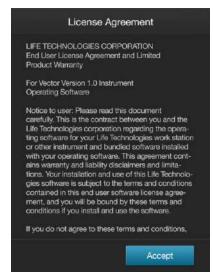
- 1. After unpacking the instrument, place the instrument on a flat, level, dry
- Plug one end of the supplied power cord into the Qubit[™] Flex Fluorometer.
- Attach the appropriate plug adaptor to the other end of the power cord.
- Plug the power cord into the electrical outlet. Ensure that the power adaptor plug remains accessible to allow disconnection.



IMPORTANT! Use the power cord plug adapter supplied with the instrument that is appropriate for the electrical outlet configuration in your country. Powering the instrument with an unapproved power cord can irreversibly damage the instrument. Note that the power cords for the Qubit[™] Flex Fluorometer are not interchangeable with those for the other Qubit[™] Fluorometer models.

The instrument automatically powers on, first displaying the splash screen, then the End User License Agreement (EULA) screen.







Note: The End User License Agreement (EULA) screen is displayed on the first use of the instrument. On subsequent uses, the **Home screen** (page 14) is displayed after the splash screen.

Click **Accept** to accept the terms of the agreement and proceed to "Set language and date/time options" (page 11).



Note: You can also view and export the EULA from the About **Instrument** screen (page 15).

To power down the Qubit[™] Flex Fluorometer, unplug it.

date/time options

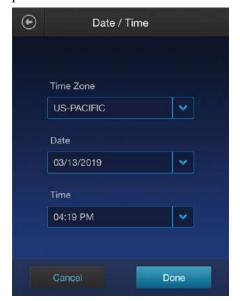
Set language and After you accept the EULA, the instrument shows the Language displayed and Date/Time screens, which allow you to set language and date/time options. If you wish, you can later change the language settings from the **Settings** ▶ **Instrument Settings** ► **Language** screen (page 85).

> 1. On the Language displayed screen, select the Language you want your instrument to display, then press Next.

Available options are English, French, German, Italian, Chinese, Japanese, and **Spanish**.



2. On the Date/Time screen, select the Time Zone, set the Date and Time in the desired format, then press Next.





Note: For detailed instructions on how to configure date/time options and to set the date and time, see "Set the date and time", page 75.

Connect to the network

(Optional) Connect After you set language and date/time options, the instrument displays the to the network Network Connection screen, which allows you to configure network options. If you wish, you can skip this step and connect to the network later from the **Settings** ► **Instrument settings** ► **Network connection** screen (page 78).

> 1. On the **Network Connection** screen, select **Wireless** or **Wired** connection. If you wish to use the instrument without joining a network, press **Skip**. You can always join a network and configure network settings later.



2. Depending on your choice, the instrument displays the **Choose Network** or the **IP Configuration** screen (for Wireless and Wired connection, respectively).



Choose Network screen (for Wireless connection)



IP Configuration screen (for Wired connection)

3. For wireless connection, select the network you want to join, then follow the on-screen instructions to configure the network options. When finished, press **Ioin**.

For wired connection, configure the network connection options, then press **Done**.

For detailed instructions on how to join a network (wireless or wired) and configure network options, see "Network connection", page 78.

4. On the **Network Connection** screen, click **Network Drive** to map the location on the network where you want to save your Qubit[™] Flex files.

For detailed instructions on how to map a network drive, see "Map a network drive", page 81.



1

Note: You must have an established network connection to map a network drive. If you wish, you can to map the network drive later.

After instrument setup

Home screen After you have set instrument preferences, the instrument automatically displays the **Home** screen each time it is powered on.



From the Home screen, you can:

- Sign in to your local instrument profile or your Connect[™] account.
- Select the assays to perform:
 - 1X dsDNA High Sensitivity (HS)
 - dsDNA High Sensitivity (HS)
 - dsDNA Broad Range (BR)
 - RNA High Sensitivity (HS)
 - RNA Broad Range (BR)
 - Protein
 - Oligo (ssDNA)
 - microRNA
- Access saved data.
- Filter, delete, or export 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.
- Use the Range Calculator to determine the best assay to use for your sample.

screen

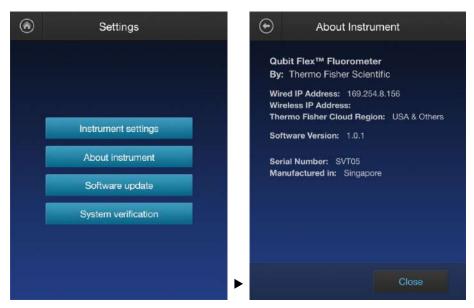
About Instrument The **About Instrument** screen displays information about your Qubit[™] Flex Fluorometer, including the currently installed software version.

To access the About Instrument screen:

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



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



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

Sign in

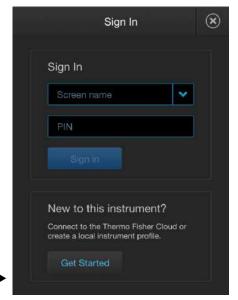
instrument profile

Create a local Qubit[™] Flex Fluorometer allows you to create a local instrument profile for each user. A local instrument profile allows you to save to a mapped network location and it is also required to connect to your Connect[™] account. If you wish, you can skip this step and create a profile later from the **Profile** screen.

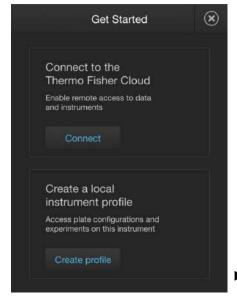
> 1. On the **Home** screen, press the **Profile** button on the top left corner of the screen to open the Sign In screen.

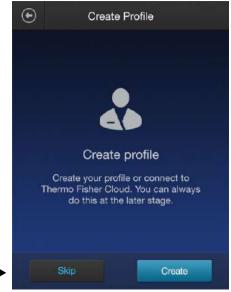




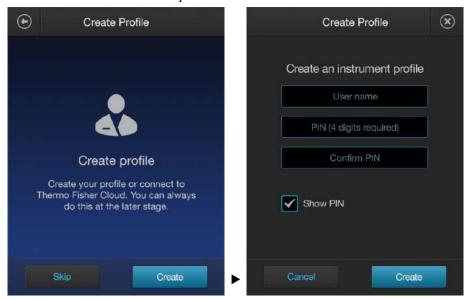


2. If you are new to the instrument and have not yet created a profile, press Get Started to open the Get Started screen, then press Create Profile.



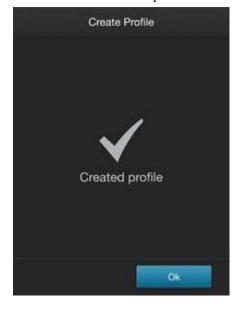


3. On the **Create Profile** screen, press **Create**.



If you wish to use the instrument without creating a local profile, press **Skip**. You can always create an instrument profile later.

- 4. Press the **User name** field, enter the desired user name for the profile (1–20 alphanumeric characters, no spaces), then press **Done**.
- 5. Press the **PIN** field, enter a 4-digit PIN, then press **Done**.
- 6. Enter the PIN in the **Confirm PIN** field, then press **Done**.
- 7. Press **Create** to create the local instrument profile.



Sign in to your After you have joined a network, you can also connect to your Connect[™] account, **Connect™ account** Thermo Fisher's cloud-based platform, to store and access your data files.

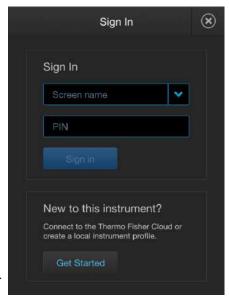


Note: To connect to the Thermo Fisher Cloud, you must have a Connect[™] account or create one. To create your Connect[™] account online or to sign in to your existing account, go to thermofisher.com/cloud.

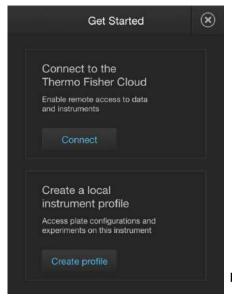
- 1. Ensure that you are connected to the network on your Qubit™ Flex instrument (page 12).
- 2. On the **Home** screen, press the **Profile** button on the top left corner of the screen to open the Sign In dialog.





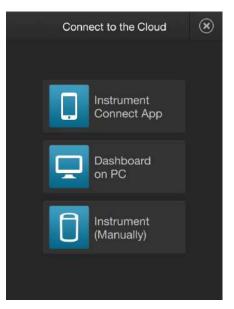


3. On the Sign In screen, press **Get Started** to open the Get Started screen, then press **Connect** to open the Connect to the Cloud screen.

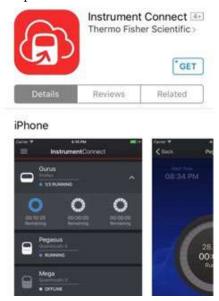




- 4. Connect to the Cloud screen offers three methods to sign in to your Thermo Fisher Connect[™] account:
 - **Instrument Connect App** on your mobile phone (Step 5, page 19)
 - Dashboard on PC (Step 6, page 20)
 - **Instrument (Manually)** (Step 7, page 21)



- 5. To connect to your Thermo Fisher Connect[™] account with the **Instrument Connect App** on your mobile phone:
 - a. Download the **Instrument Connect Mobile App** from the application store on your mobile phone.

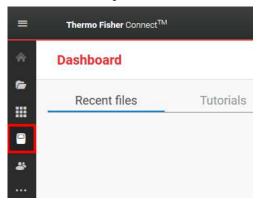


b. Press **Instrument Connect App** on the Connect to the Cloud screen, then follow the steps on the Qubit[™] Flex instrument. When finished, go to Step 8 (page 22).



- 6. To connect to your Thermo Fisher Connect[™] account with **Dashboard on PC**:
 - a. Go to **thermofisher.com/cloud** and sign in to your Thermo Fisher Connect $^{\text{\tiny TM}}$ account.
 - b. On the Connect $^{\text{\tiny IM}}$ dashboard, press the **Instrument Connect** button.





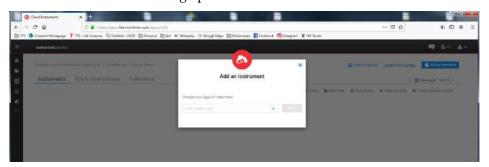
Instrument Connect screen opens.



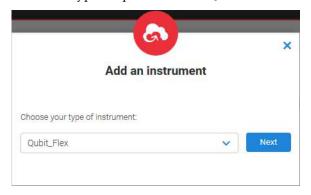
c. On the Instrument Connect screen, press **Add an instrument**.



Add an instrument dialog opens.



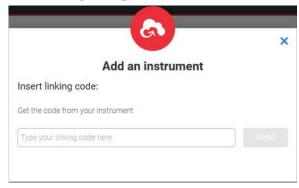
d. From the instrument type dropdown, select Qubit_Flex, then press Next.



e. Press **Dashboard on PC** on Connect to the Cloud screen (on the Qubit[™] Flex instrument; see page 19) to display the linking code.



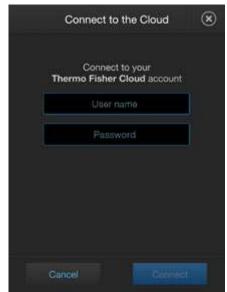
f. Enter the linking code displayed on the Qubit[™] Flex instrument into the Add an instrument dialog, then press **Send**.



- g. When finished, go to Step 8 (page 22).
- 7. To connect to your Thermo Fisher Connect[™] account with **manually with the Qubit**[™] **Flex instrument**:
 - a. Press **Instrument (Manually)** on the Connect to the Cloud screen (on the Qubit™ Flex instrument; see page 19).



b. Enter your **User name** and **Password** for your Thermo Fisher Connect[™] account, then press **Connect**.



8. When you have signed in to your Thermo Fisher Connect $^{^{\text{\tiny{TM}}}}$ Account, the Profile button on the Home screen becomes blue.



When signed in, you can export your data to your Connect $^{\text{\tiny TM}}$ account.



Guidelines for using the Qubit[™] Flex Fluorometer

Recommendations

To obtain the best results, follow the recommendations below. For more information, see "Critical Qubit™ Assay considerations", page 99.

- Do **not** operate the instrument in direct sunlight.
- Wear gloves during sample handling.
- Use the instrument at room temperature only (22–28°C).
- Bring all kit reagents to room temperature and insert all assay tubes into the
 instrument only for as much time as it takes for the instrument to measure the
 fluorescence.
- Do not hold the assay tubes in your hand before performing a measurement.
- Make sure that you have calibrated the Qubit[™] Flex Fluorometer using the appropriate standards.
- The assay volume must be 200 µL for an accurate read.
- Take care not to create air bubbles when mixing the sample or standard with the working solution.
- Incubate the tubes for the Qubit[™] DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution.
- Incubate the tubes for the Qubit[™] protein assays for 15 minutes after mixing the sample or standard with the working solution.
- 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 are not recommended.
- Visit 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[™] Flex Fluorometer.

Assay tubes for the Qubit™ Flex Fluorometer

Only thin-wall, clear 0.2-mL PCR tube strips are appropriate for use in the Qubit[™] Flex Fluorometer. For best results, we recommend using Qubit[™] Flex Tube Strips (Cat. No. Q33252).

3. Perform assays

Before you begin

Materials needed •

- A Qubit[™] assay kit appropriate for quantifying your samples (see page 101 for available Qubit[™] assay kits and ordering information)
- DNA, RNA, or protein samples in Qubit[™] Flex Tube Strips
- Appropriate standards for your assay in Qubit[™] Flex Tube Strips
- Single channel pipette (1–20 μL), multichannel pipette (200 μL)
- Qubit[™] Flex Reservoir (Cat. No. Q33253) or other suitable sample reservoir



Note: For instructions on the preparation of the assay standards, see the instructions that accompany the assay you are using or the *Qubit*TM *Flex Fluorometer Quick Reference Card (QRC)* (Pub. No. MAN0018187).

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



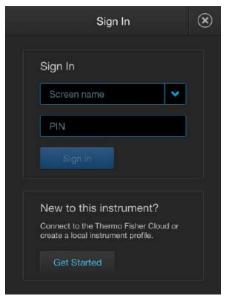
Note: You can also transfer your data to a network location or your Connect[™] account wirelessly, if you have set up a wireless connection.

Sign in to your 1. profile

. Press the **Profile** button on the top left corner of the screen to open the Sign In dialog.







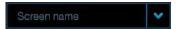
2. If you are new to the instrument and have not yet created a local instrument profile or signed in to your Thermo Fisher Connect™ account, press **Get Started**.

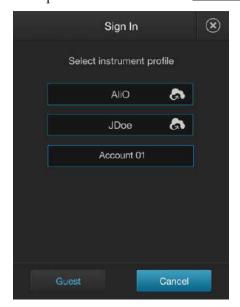


To create a local instrument profile, see page 16.

To sign in to your Thermo Fisher ConnectTM account, see page 18. Otherwise, go to Step 3 (page 25).

3. Press **Screen name**, then select your instrument profile from the available options.







Note: The Connect icon next to a screen name indicates that the profile has an associated ConnectTM account.



When the Connect icon is blue, the profile is signed in to the associated $Connect^{TM}$ account.

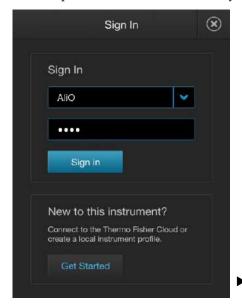


4. Press **PIN**, enter the PIN for your profile, then press **Enter**.



5. Press **Sign in** to sign in to your account and return to the Home screen. The blue profile button indicates that you have signed in to your account.







(Optional) Use the Assay Range Calculator to determine the assay range

The on-board Assay Range Calculator displays the core sample concentration range for which the selected assay is most accurate, as well as the extended low and high ranges based on your sample volume. Knowing the assay range can help you determine which Qubit™ assay provides the most accurate quantification based on your sample volume and estimated sample concentration.

Use the Assay 1. Range Calculator 2.

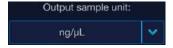
- 1. On the **Home screen**, press **Calculators**.
- 2. On the **Select Calculator** screen, press **Assay Range** to open the Assay Range Calculator.



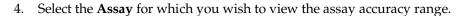




3. Press **Output sample unit**, then select the **units** in which you wish to view the assay range.









5. Enter the **sample volume** to be used directly in the sample volume text box. You can also use the + and – buttons or adjust the sample volume wheel.



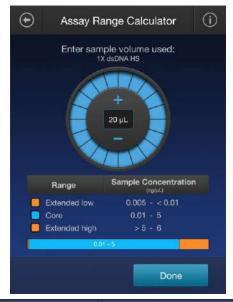
The Assay Range Calculator displays the Core sample concentration range for the selected assay and the Extended low and high ranges based on your input.

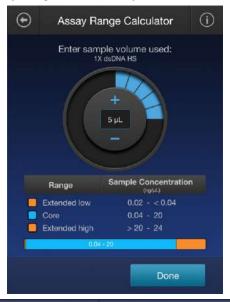


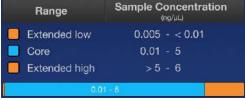


Note: Samples with concentrations within the Core range of the assay will have <15% relative error for the given sample volume. Samples with concentrations within the extended range will have <25% relative error for the given sample volume.

6. Increase or decrease the sample volume to observe how changes in the sample volume affect Core and Extended accuracy ranges for the assay.









Range

dsDNA HS Assay range for 20 µL sample volume

dsDNA HS Assay range for 5 µL sample volume

Sample Concentration

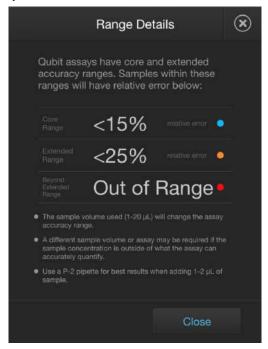


Note: The sample volume used (1–20 μ L) changes the assay accuracy range. For highest accuracy, use the maximum sample volume that would keep the concentration measurements within the core range.

Note that a different sample volume or assay may be required if the sample concentration is outside of what the assay can accurately quantify.

7. Press the **Information** icon on the header bar to view the Range Details (relative errors for the core and extended ranges) and guidelines for obtaining best assay results.





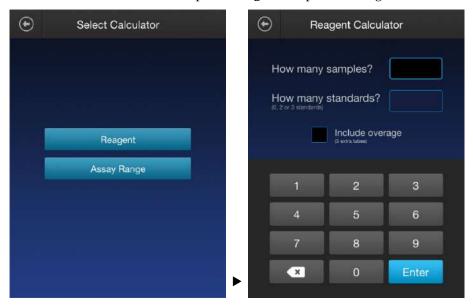
- 8. Press **Close** to return to the Assay Range Calculator.
- 9. (*Optional*) If desired, repeat the procedure for another assay to determine whether it would provide more accurate results in the expected concentration range.
- 10. Press **Done** to return to the Home screen.

Use the Reagent Calculator to prepare Qubit™ Working Solution

Use the on-board Reagent Calculator to determine the amount of Qubit^m dye and buffer required to prepare the Qubit^m Working Solution for your samples and standards.

Use the Reagent Calculator

Use the Reagent 1. On the **Select Calculator** screen, press **Reagent** to open the Reagent Calculator.

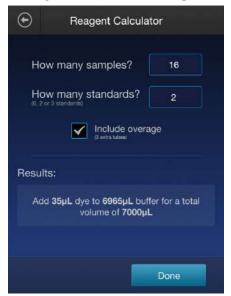


2. Enter the total **number of samples and standards** that you plan to run.



3. (Optional) Select **Include overage**, if you want to include reagents for three additional tubes ($600 \mu L$) in the total calculated volume.

4. Press **Enter** to calculate the amount of Qubit^m dye and buffer required to prepare the Qubit^m 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 **Select Calculator** screen.
- 6. Press the **Back** button to return to the **Home screen** or press **Assay Range** to open the Assay Range Calculator (page 26).

Run standards for assay calibration

For each assay, you can run new standards to calibrate the assay on the Qubit[™] Flex Fluorometer or use the values from the previous calibration. For more information, see "Qubit™ Flex Fluorometer calibration", page 100.



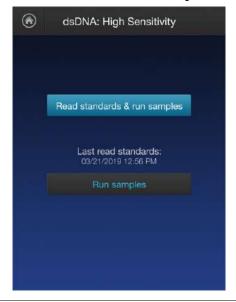
IMPORTANT! Be sure to use the appropriate standards for your assay. For best results, run new standards each time you perform an assay.

Run new standards 1. On the Home screen, press to select the Assay to perform. To view the next screen of available assays, swipe to the left. To return to the previous page, swipe to the right.





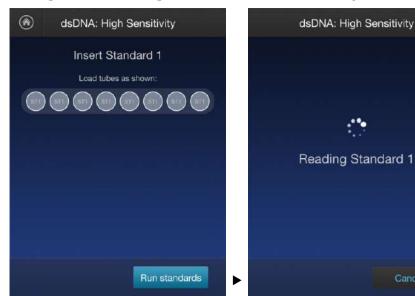
When prompted, press **Read standards & run samples** to read new standards.



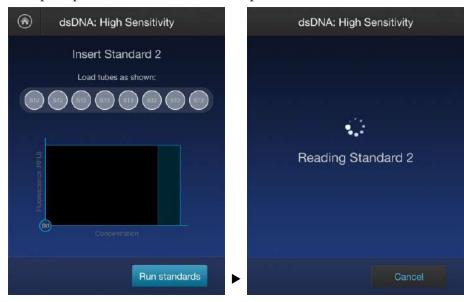


Note: To apply the previous calibration to your assay, press Run samples. See "Read samples", page 36.

3. When prompted, load the Qubit[™] Flex Tube Strip containing Standard #1 into the sample chamber, then press **Run standards**. The reading takes ~3 seconds.



4. When prompted, insert Standard #2, then press Run standards.



5. *For Qubit*[™] *protein assays only:* When prompted, insert Standard #3, then press **Run standards**.

The calibration is complete after Standard #2 is read (or after Standard #3 for the QubitTM protein assay) and the software displays the results (see "Calibration results", page 34).

6. If your calibration is successful, press **Next** to proceed to "Read samples", page 36.

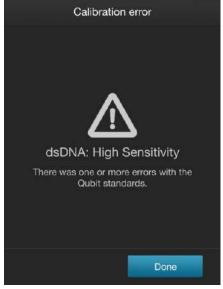
Calibration results •

If the calibration is successful, Standards complete screen with the Fluorescence vs. Concentration graph is displayed.
 In the Fluorescence vs. Concentration graph, the standard data points are connected by a line and open circles

represent correct standards.

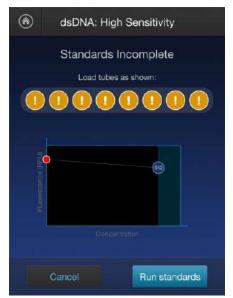


• If the calibration is not successful, Calibration error message is displayed. If you receive the Calibration error message, you can re-run the standards (see "Re-run standards after calibration error", page 35).



(Optional) Re-run standards after calibration error

- (Optional) Re-run 1. In the Calibration error screen, press Done.
 - 2. If you wish to re-run the standards, or run new standards, prepare a fresh set of standards, then load Standard #1 into the instrument.



3. Press **Run standards**, then repeat the calibration procedure (page 25).

Read samples

Before you begin •

- Calibrate the Qubit[™] Flex Fluorometer as described on page 25. (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^{TM} DNA and RNA assays, 15 minutes for the Qubit^{TM} protein assay).

Insert samples 1.

1. When prompted, load the tube strip containing the samples as shown in the **Insert samples** screen. If you have fewer than 8 samples, press to deselect the tube positions that do not contain a sample.



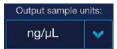


All 8 tubes contain samples

No sample in positions S7 and S8

2. Press **Output sample units** to open the **Output Units** screen, then select the desired units.

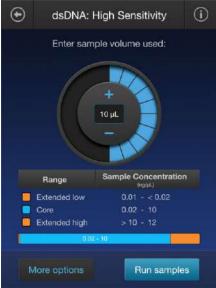




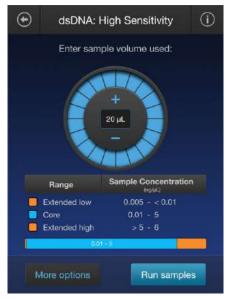
- 3. Press **Next** to go to the Sample volume screen.
- 4. In the **Sample volume** screen, enter the **sample volume** added to the assay tube (between 1 and 20 μ L).

You can enter the volume directly in the sample volume text box, use the + and – buttons, or adjust the sample volume wheel.

When you enter the sample volume, the assay range information on the screen automatically changes to reflect the new core and extended accuracy ranges based on the sample volume.



dsDNA HS Assay range for 10 µL sample volume



dsDNA HS Assay range for 20 µL sample volume



Note: The sample volume used (1–20 $\mu L)$ changes the assay accuracy range. For highest accuracy, use the maximum sample volume that would keep the concentration measurements within the core range. If the sample concentration is outside of what the assay can accurately quantify, a different sample volume or assay may be required.

(Optional) Enter Assay kit lot #, Add Tags, Add Sample IDs

(Optional) Enter 1. Press More options to open More Options screen, where Assay kit lot #, you can:

More options

- Enter assay kit lot # (Step 2, page 38)
- Add Tags to your sample run (Step 3, page 39)
- Add Sample IDs (Step 6, page 40)

The information you have entered will be available on the Data Details of your samples (page 62).



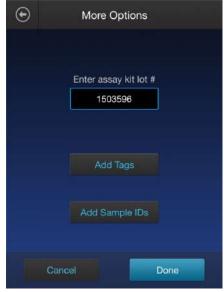


Note: You can open the More Options screen from the Insert Samples (page 36) or the Sample Volume (page 37) screens.

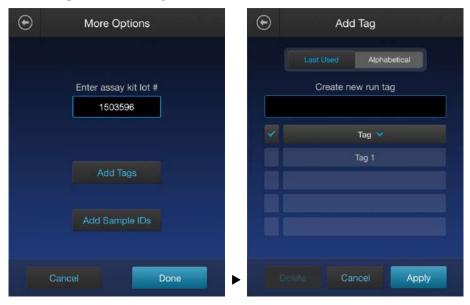
2. To enter an assay kit lot number, press the **Enter assay kit lot** # text box, enter the assay kit lot number, then press **Enter**.





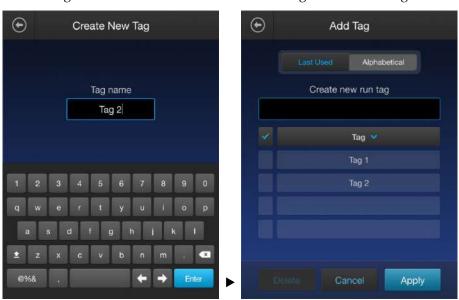


3. To add a tag to your samples in the run, press **Add Tags** on the More Options screen to open the Add Tag screen.



4. To create a new tag, press the **Create new run tag** text box to open the Create New Tag screen, enter the new tag, then press **Enter**.

The new tag will be added to the list of available tags on the Add Tag screen.

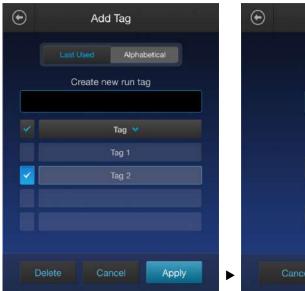




Note: To filter the list of available tags for the last used tag, press **Last Used**.

To display all existing tags alphabetically, press **Alphabetical**. To sort the list of available tags alphabetically in ascending or descending order, press the Tag column header.

 Select the desired tag from the list of available tags, then press Apply to add the selected tag to your samples and return to the More Options screen.
 The tag you have applied to your sample run is displayed on the More Options screen and the Add Tags button changes to Edit Tags.







Note: To return to the More Options screen without applying a tag to your samples, press **Cancel**.

To delete an existing tag, select the tag from the list of available tags, then press **Delete**.

To change the tag applied to your sample run, press **Edit Tags** on the More Options screen.

6. To add sample IDs to your samples, press Add Samples IDs, then select Cloud (your Connect[™] account; see page 18 for sign in instructions)) or USB for the location of the sample IDs you want to import.



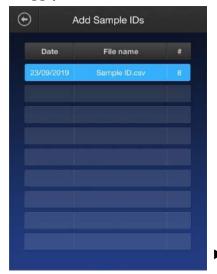




Note: The file containing the sample IDs must be in CSV (comma separated value) format and filled out like the example below: first "Plate Barcode" then "Well" and "Sample Id".



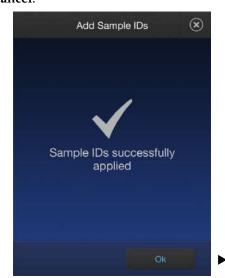
7. Select the file containing the sample IDs from the list of available files, then press **Apply**.





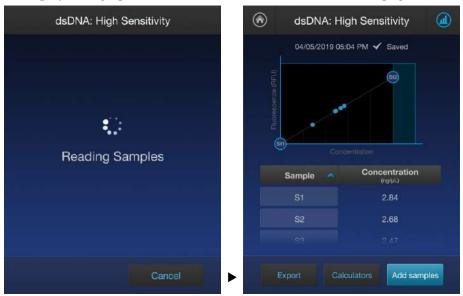
- 8. Press **OK** at the confirmation page.
- 9. When finished entering assay kit lot number and applying tags and sample IDs, press **Done** at the More Options screen. The assay screen displays the new information added to your samples at the bottom of the screen.

To go back to the assay screen without applying the new information, press **Cancel**.





Run Samples 1. Press **Run samples**. The reading takes approximately 3 seconds and the results are displayed in graph view in the Results screen (see "Results", page 43).



2. To display the results in list view, press the **Graph** button to unselect it. The Results screen lists the concentration of each original sample using the output units selected at the beginning of the assay.





7:

Note: By default, the Results screen displays the measurements in graph view. However, the graph settings are "sticky", so that if you close the graph, the next time anyone runs an assay, the graph view is hidden and the results are shown in list form.

3. To run more samples, press **Add samples**, and repeat the procedure.

Results

View results 1.

1. The instrument automatically displays the Results screen after the completion of each sample run.

By default, the results are displayed in graph view, which shows the Fluorescence vs. Concentration graph and lists the concentration of each original sample below the graph.

In the graph:

- Open circles represent correct standards.
- Blue circles represent samples that fall within the assay's core range.
- Orange circles represent samples that fall within the assay's extended range.
- Red circles represent samples that fall outside the assay's range.



2. To view a sample on the Fluorescence vs. Concentration graph, press the desired sample on the sample list. The selected sample is displayed as a gray circle on the graph.



3. To display the results in list view, press the **Graph** button to hide the graph.



The Results screen shows the concentration of each original sample in a list form, using the output units selected at the beginning of the assay.





- If the concentration of a sample is within the assay's extended range, the concentration value is displayed in orange, and an "extended range" message and an orange circle are displayed next to the concentration value.
- If the concentration of a sample is outside of the assay's range, an "out of range" message and a red circle are displayed next to the sample.
- 4. To display the results in graph view again, press the **Graph** button.



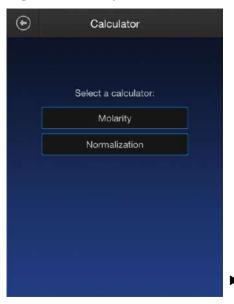
(Optional) Use the Molarity Calculator to determine sample molarity

The on-board Molarity Calculator allows you to calculate the molarity of your samples based on nucleic acid length and their measured concentration.

Use the Molarity Calculator

1. On the **Results** screen, press **Calculators**, then select **Molarity** to open the Molarity Calculator.







2. On the **Molarity Calculator** screen, press the **Desired units** fields to select the **input** and **output** units.





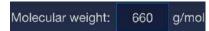
Input units



Output units



Note: The QubitTM Flex Fluorometer auto-populates the Molecular weight (MW) depending on the QubitTM assay performed (for example, for the dsDNA HS assay, it uses a default value of 660 g/mol for the average molecular weight of one DNA base pair).



To change the auto-populated MW value, press the **Molecular weight** field and enter the desired average molecular weight of your sample.

3. Press **Length (bp)** field for Sample 1 (S1), enter the length (bp) of Sample 1, then press **Enter**.





4. If all your samples have the same length, select **Auto-populate DNA length**.

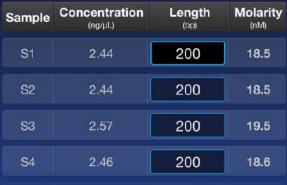




5. Press **Calculate** to calculate the molarity of your samples based on the assay results and DNA length in the output units that you have selected.









Note: When you press Calculate, the instrument saves the data from molarity calculations with the sample data in the CSV file.

6. To export your results, press **Export**. The instrument exports the complete CSV file with all sample data, including the molarity calculation results.

To go back to the Calculator screen, press the **Back** button.

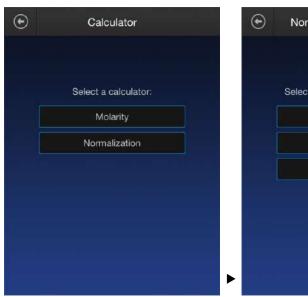
(Optional) Use the Normalization Calculator to determine how to dilute the samples to the same molarity, concentration, or mass

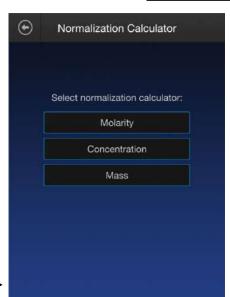
The on-board Normalization Calculator helps you to normalize your samples of variable concentration to the same molarity, concentration, or mass using the results from your assay.

Select the 1. Normalization Calculator

 On the Results screen, press Calculators, then press Normalization.







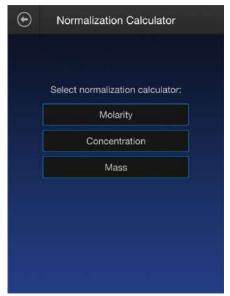
- 2. On the Normalization Calculator screen, select:
 - **Molarity** to determine how to dilute your samples to the same final mass and volume (page 49).
 - **Concentration** to determine how to dilute your samples to the same final concentration (page 52).
 - Mass to determine how to dilute your samples to the same final mass and volume (page 55).



Note: The option to normalize your samples based on molarity is available only if you have run the Molarity calculator (page 45) on your samples.

Normalize your samples to the same molarity

Normalize your 1. On the Normalization Calculator screen, select Molarity.





- 2. Enter the **Final sample mass** and select **units**.
- 3. Enter the **Final sample volume** and select **units**, then press **Enter**.



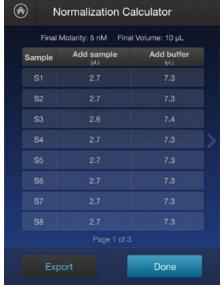


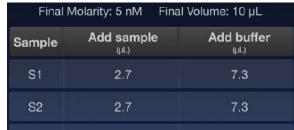


Note: The minimum allowed sample volume on the Normalization Calculator is $5\,\mu L.$

4. Press **Calculate** to see how much sample and buffer to mix to achieve the desired final sample concentration and volume.









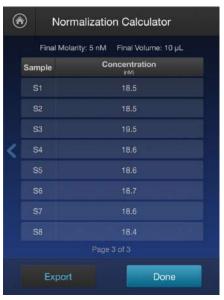
Note: When you press Calculate, the instrument saves the data from normalization calculations with the sample data in the CSV file.

5. Press the **right arrow** to view page 2 of results, which displays the required sample:buffer dilution before mixing ("Required Dilution", if applicable) and the sample concentration after the dilution ("Diluted conc.").

If dilution is not required before mixing, then "N/A" is displayed in the Required Dilution and Diluted conc. columns for the sample.



6. Press the **right arrow** again to view page 3, which displays the actual sample concentration ("Concentration").



7. Press the **left arrow** to go back to the previous page.

S2

- 8. To export your calculations, press **Export**. The instrument exports the complete CSV file with all sample data, including the normalization calculation results.
- 9. Otherwise, press **Done** to close the Normalization calculator and go back to the Calculator screen.



Note: If your sample needs further dilution before mixing to achieve the desired final molarity, the required sample:buffer dilution is indicated in the Add sample column (in red) and in the Required Dilution column (on page 1 and 2 of calculation results, respectively).



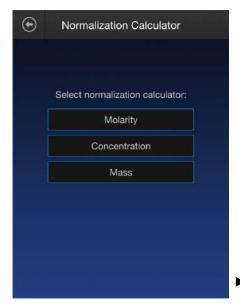
If dilution is not required before mixing, then the Required Dilution and Diluted conc. columns display "N/A" for the sample.

1850

1:9

Normalize your samples to the same concentration

Normalize your 1. On the Normalization Calculator screen, select Concentration.





- 2. Enter the Final sample concentration and select units.
- 3. Enter the **Final sample volume** and select **units**, then press **Enter**.



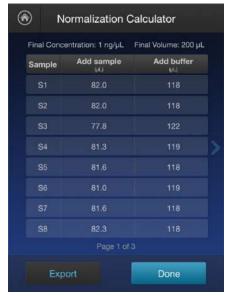


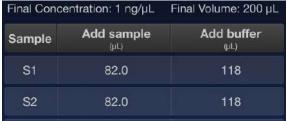


Note: The minimum allowed sample volume on the Normalization Calculator is 5 $\mu L.\,$

4. Press **Calculate** to see how much sample and buffer to mix to achieve the desired final sample concentration and volume.









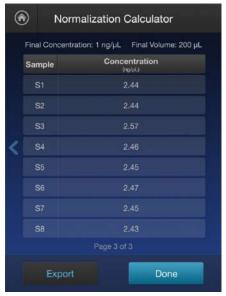
Note: When you press Calculate, the instrument saves the data from normalization calculations with the sample data in the CSV file.

5. Press the **right arrow** to view page 2 of results, which displays the required sample:buffer dilution before mixing ("Required Dilution", if applicable) and the sample concentration after the dilution ("Diluted conc.").

If dilution is not required before mixing, then "N/A" is displayed in the Required Dilution and Diluted conc. columns for the sample.



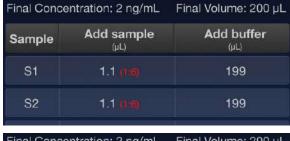
6. Press the **right arrow** again to view page 3 of results, which displays the actual sample concentration ("Concentration").

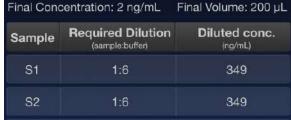


- 7. Press the **left arrow** to go back to the previous page.
- 8. To export your calculations, press **Export**. The instrument exports the complete CSV file with all sample data, including the normalization calculation results.
- 9. Otherwise, press **Done** to close the Normalization calculator and go back to the Calculator screen.



Note: If your sample needs further dilution before mixing to achieve the desired final concentration, the required sample:buffer dilution is indicated in the "Add sample" column (in red) and in the "Required Dilution" column (on page 1 and 2 of calculation results, respectively).

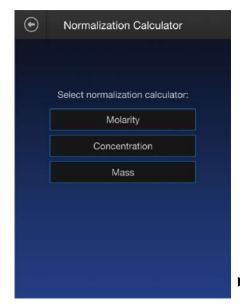




If dilution is not required before mixing, then the Required Dilution and Diluted conc. columns display "N/A" for the sample.

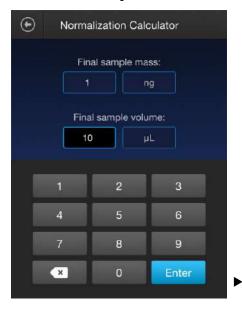
Normalize your samples to the same mass and volume

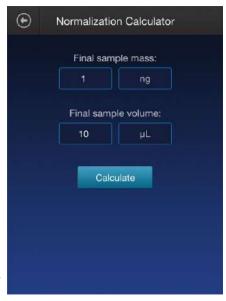
Normalize your 1. On the Normalization Calculator screen, select Mass.





- 2. Enter the **Final sample mass** and the desired **units**.
- 3. Enter the **Final sample volume** and the desired **units**, then press **Enter**.





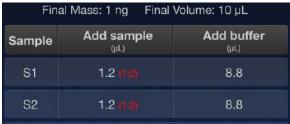


Note: The minimum allowed sample volume on the Normalization Calculator is 5 $\mu L.\,$

4. Press **Calculate** to see how much sample and buffer to mix to achieve the desired final sample concentration and volume.





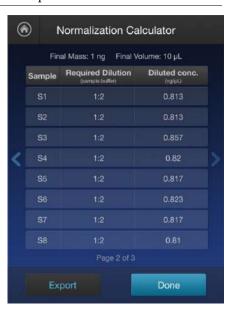




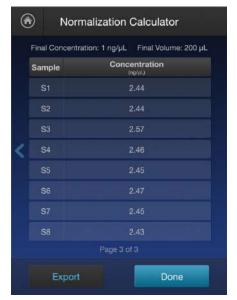
Note: When you press Calculate, the instrument saves the data from normalization calculations with the sample data in the CSV file.

5. Press the **right arrow** to view page 2 of results, which displays the required sample:buffer dilution before mixing ("Required Dilution", if applicable) and the sample concentration after the dilution ("Diluted conc.").

If dilution is not required before mixing, then "N/A" is displayed in the Required Dilution and Diluted conc. columns for the sample.



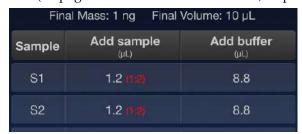
6. Press the **right arrow** again to view page 3 of calculation results, which displays the actual sample concentration ("Concentration").

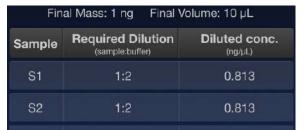


- 7. Press the **left arrow** to go back to the previous page.
- 8. To export your calculations, press **Export**. The instrument exports the complete CSV file with all sample data, including the normalization calculation results. Otherwise, press **Done** to close the Normalization calculator and go back to the Calculator screen.



Note: If your sample needs further dilution before mixing to achieve the desired final mass and volume, the required sample:buffer dilution is indicated in the "Add sample" column (in red) and in the "Required Dilution" column (on page 1 and 2 of calculation results, respectively).





If dilution is not required before mixing, then the Required Dilution and Diluted conc. columns display "N/A" for the sample.

4. Manage data

Overview The Qubit[™] Flex Fluorometer can save data for up to 10,000 samples.

For the saved data, the Qubit[™] Flex Fluorometer allows you to:

- View detailed data for each sample (page 58).
- Rename data files (page 63).
- Export data as a CSV (comma separated value) file to a USB drive, directly to your computer, or to your Connect[™] account (page 64).
- Delete data files (page 70).

View detailed sample data

sets

View list of data 1. On the **Home screen**, press **Data**. The Data screen opens and displays the list of data sets that are saved in the instrument.





- 2. By default, the data sets are arranged by date in descending order. To sort the data sets, press the appropriate category in the header row:
 - To sort the data sets by date in ascending order, press **Date**. To sort the data sets by date in descending order, press **Date** again.
 - To sort the data sets by Assay name in descending order, press Assay name.

To sort the data sets by Assay name in ascending order, press **Assay name** again.

To sort the data sets by the number of samples in descending order, press #.

To sort the data sets by the number of samples in ascending order, press # again.

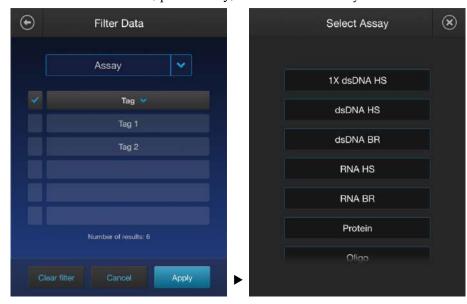
data sets

(Optional) Filter 1. To filter data sets by Assay or Tag, press Actions to open the Actions screen, then select Filter data.

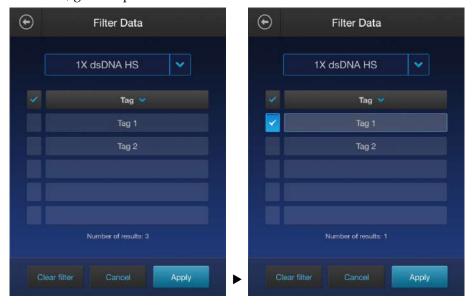




2. On the Filter Data screen, press Assay, then select the Assay of interest.



3. If you had applied a tag to the assay (page 38), select the **Tag** from the list. Otherwise, go to step 4.

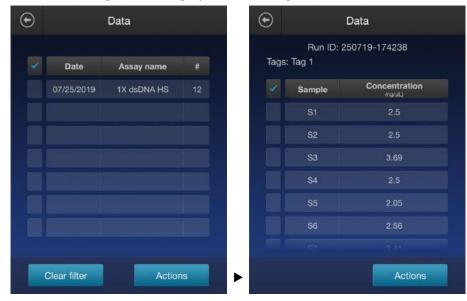


4. Press **Apply** to filter the data list by the assay and tag you have selected. Only the data sets that satisfy the filter criteria are displayed in the Data screen.



view detailed sample data

Select data set and 1. On the Data screen (filtered or not filtered), press the **data set of interest**. The Data set screen opens and displays a list of samples in that run.

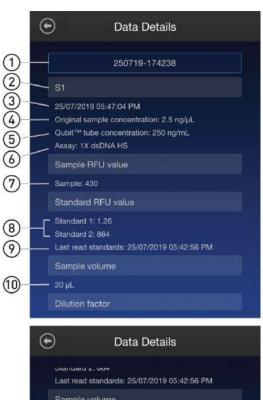


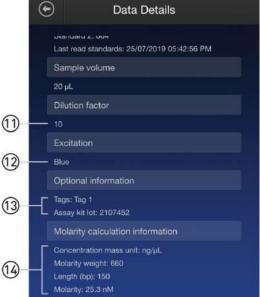
2. To view the sample details, press the **sample of interest**. A Data details screen opens. To view sample details that do not fit in the screen, scroll down.





Information in the detailed sample data





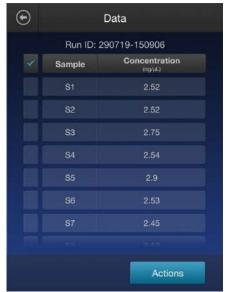
- 1 Run ID
- (2) Sample name
- (3) Assay date
- (4) Original sample concentration
- (5) Qubit™ tube sample concentration
- (6) Assay name
- (7) Sample RFU* value *RFU: Relative Fluorescence Units

- (8) RFU values for the standards
- (9) Date of last read standards
- (10) Sample volume
- (11) Dilution factor
- (12) Excitation channel
- (13) Optional information (Tags, Reagent lot etc.)
- Molarity calculation information (units, nucleic acid length, MW, molarity)

Edit sample name

Edit 1. On the Data screen, select the **data set of interest**, then select the **sample** you want to rename.



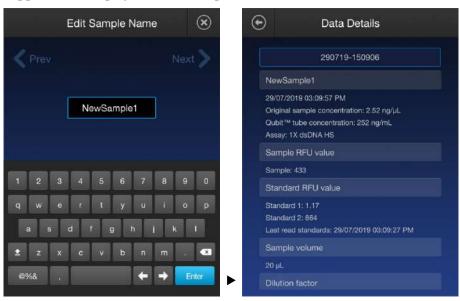


2. On the Data details screen, press the **Sample set** # field (indicated by red arrow). Edit Sample Name screen opens.





3. Enter the desired sample name, then press **Enter**. Data Details screen reappears and displays the new sample name.

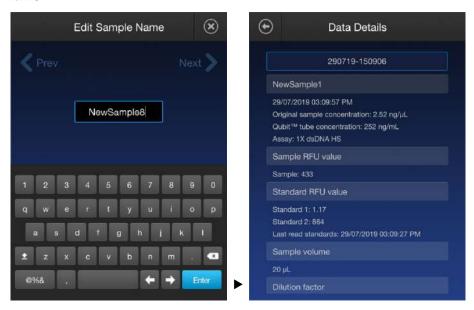


4. If you wish to rename all of the samples in the data set, press the **Next** button to go the next sample (instead of pressing **Enter** at step 3), then enter the new name for that sample.





5. Repeat for all remaining samples. When finished renaming all the samples, press **Enter**. Data Details screen reappears and displays the new sample name.



6. Press the **Back** button to return to the Data screen for the assay. All of the samples display the new sample names.



Export data

Introduction

The Qubit[™] Flex Fluorometer is designed for standalone use; it does not require an external computer. However, to archive data and generate reports, you can export the numeric data stored in the CSV file to a computer using a USB flash drive, or save to your Connect[™] account or a network drive wirelessly or via the Ethernet cable. You can then view the file in any spreadsheet program.

- **Export data** 1. On the **Home screen**, press **Data** to open the Data screen.
 - To export entire data sets, press the **check box** to the left of each data set that you wish to export. You can select multiple data sets.

To select all data sets to export, press the blue **check** icon on the header row.

 \odot



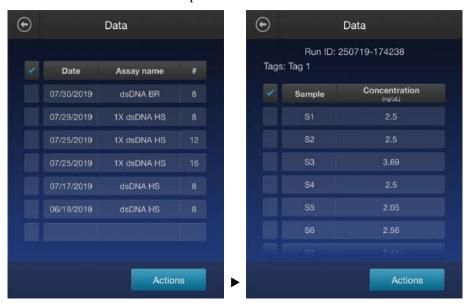


Data

Two data sets selected

All data sets selected

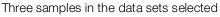
3. To export only individual data entries from a data set, press the **data set of interest** to view individual samples in the data set.



4. Press the **check box** to the left of the samples that you wish to export. You can select multiple samples to export.

To select all samples in the data set to export, press the blue **check** icon on the header row.



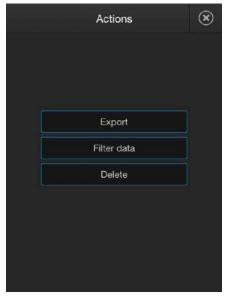




All samples in the data set selected

5. After you have selected the data sets or the samples, press **Actions**, then select **Export**.

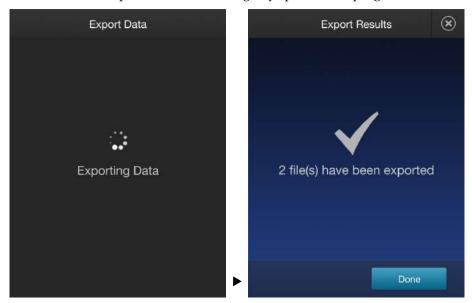




- 6. In the **Export data** screen, select the **Export method**. Available options are **Cloud** (i.e., your Connect[™] account), **USB**, and **Network Drive**.
 - To export data to a USB drive, insert the USB drive into the Qubit[™] Flex Fluorometer.
 - To export data to your Connect[™] account or a network drive, ensure that
 the instrument is connected to the network wirelessly or via an Ethernet
 cable.



7. Press **Export** to export the data. The numeric data is automatically saved as a CSV file. You can open the CSV file using any spreadsheet program.



Delete data

- **Delete data files** 1. On the **Home screen**, press **Data**.
 - On the Data screen, press the **check box** to the left of each data set you wish to 2. delete. To select all data sets, press the blue **check** icon on the header row.

To delete only individual sample files from a data set, press the data set of interest to view individual samples in the data set, then press the check box to the left of the samples you wish to delete.



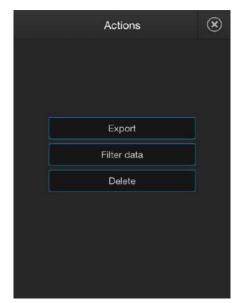


Select data sets to delete

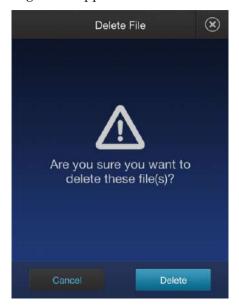
Select Sample files to delete

3. After you have selected the data sets or the samples, press Actions, then select Delete.





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



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

5. Configure instrument settings

Instrument settings

You can configure the following instrument settings for the Qubit[™] Flex Fluorometer from the **Settings ▶ Instrument Settings** screen:

- Sleep mode (page 73)
- Brightness (page 74)
- Date/Time (page 75)
- Network Connection (page 78)
- Reset instrument (page 84)
- Language (page 85)
- Cloud region (page 86)

Access the 1.
Instrument 2.
Settings screen

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





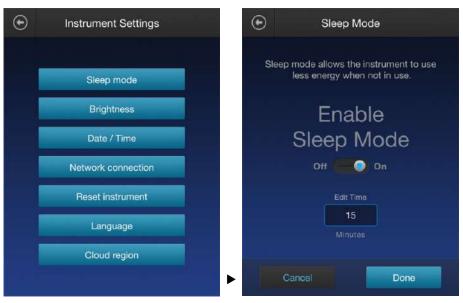


Sleep mode

Adjust the sleep mode

The Qubit[™] Flex 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.

1. On the **Instrument Settings** screen (page 72), press **Sleep Mode**.



- 2. To change the time of inactivity before the instrument goes into sleep mode, press **Edit Time** field, then enter the time between 1 minute and 60 minutes.
- 3. To disable the sleep mode, toggle the **Enable Sleep Mode** switch to the **Off** position.

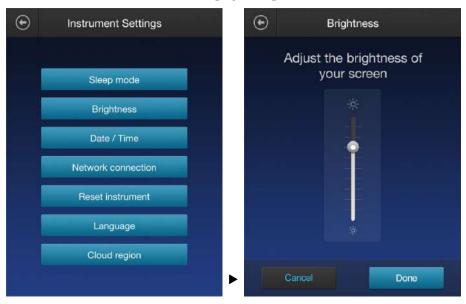


4. Press **Done** to save the changes and return to the Instrument Settings screen. Press **Cancel** or **Back** (to return to the Instrument Settings screen without saving the changes.

Brightness

Adjust screen brightness

Adjust screen 1. On the Instrument Settings screen (page 72), press Brightness.

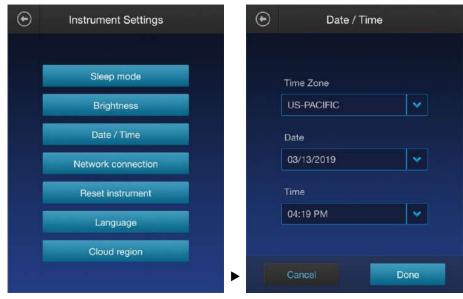


- 2. Move the **Brightness slider** up or down to adjust the brightness of the display.
- 3. Press **Done** to save the changes and return to the Instrument settings screen. Press **Cancel** or **Back** (to return to the Instrument settings screen without saving the changes.

Date and Time

Set the date and time

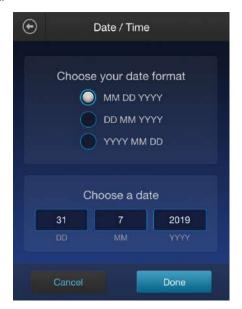
Set the date and 1. On the **Instrument Settings** screen (page 72), press **Date/Time**.



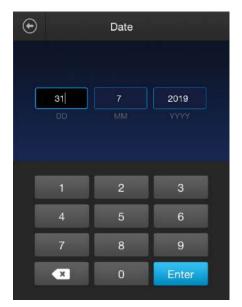
2. Press **Time Zone**, then select the time zone for your location from the list.



3. Press **Date**, then choose **MM DD YYYY**, **DD MM YYYY**, or **YYYY MM DD** for the date format.

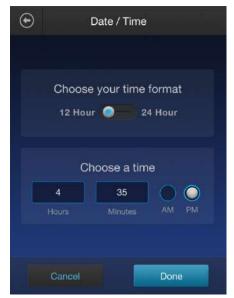


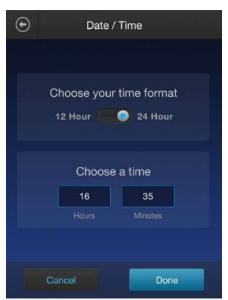
4. To set the date, press the **DD**, **MM**, and **YYYY** fields to enter the Day, Month, and Year.



5. Press **Enter** when finished entering the date, then press **Done**.

6. Press **Time**, then choose **12 Hour** or **24 Hour** for the time format.

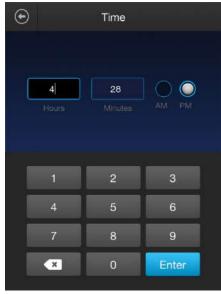


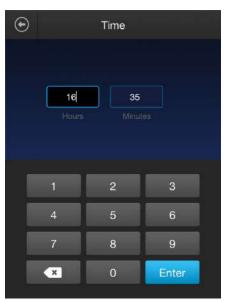


12-Hour format

24-Hour format

7. To set the time, press the **Hours** and **Minutes** fields to enter the Hours and Minutes. If you have chosen the 12 Hour format, select **AM** or **PM**.





12-Hour format

24-Hour format

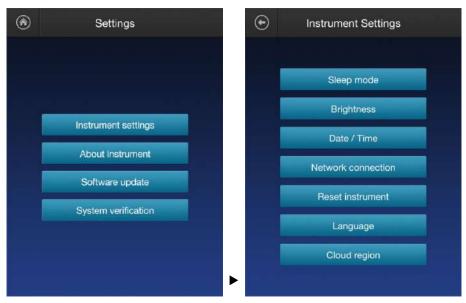
8. Press **Enter** when finished entering the time, then press **Done**.

Network connection

Network Connection screen

Access the Network Connection screen allows you to connect to an available wireless network using the supplied Wi-Fi adaptor, or to configure and join a local area network via the LAN (RJ-45) port using an Ethernet cable. After you have joined a network, you can also connect to Thermo Fisher's Connect[™] cloud-based platform to store and access your data files.

> 1. To access the Network Connection screen, press **Settings** ▶ **Instrument Settings**, then select **Network connection**.



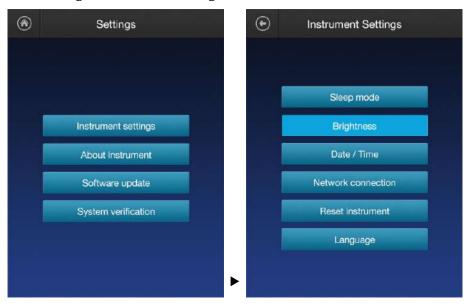
2. The Network Connection screen opens.



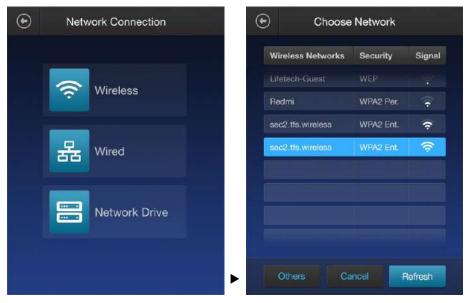
- To connect to a Wi-Fi network, go to page 79.
- To establish a wired connection to a local area network (LAN), go to page 80.
- To map a network drive to save your Qubit[™] Flex files, go to page 81.

Connect to a Wi-Fi 1. network

- 1. Ensure that your USB Wi-Fi dongle is inserted into one of the available USB ports on the instrument (see page 7).
 - If it is not, insert the Wi-Fi dongle, then restart the instrument by disconnecting and reconnecting the power supply.
- 2. Press **Settings** ▶ **Instrument Settings**, then select **Network connection**.



3. On the **Network Connection** screen, press **Wireless**. The instrument searches for available wireless networks within range.



- 4. On the **Choose Network** screen, press the network you want to join.
- 5. If required, enter the appropriate security credentials, then press **Join**. After the connection is established, the network is highlighted in blue.

Connect to a local area network (LAN)

- 1. Ensure that the instrument is connected to an active network jack via the LAN (RJ45) port (page 7) using a standard Category 6 Ethernet cable.
- 2. On the **Instrument Settings** screen, press **Network connection**, then select **Wired**.



- 3. On the **IP Configuration** screen, select **DHCP** or **Static**.
- 4. If you have selected **Static**, enter the static **IP address**, **MAC address**, **Subnet mask**, **Default Gateway address**, and primary and secondary **DNS server addresses** for the LAN port.





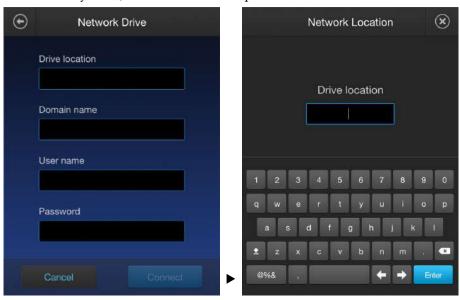
5. Press **Done** to join the local area network.

Drive

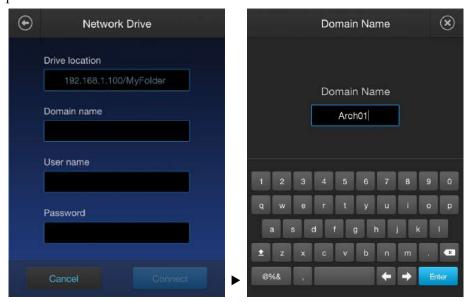
- Map a Network 1. Ensure that the instrument is connected to an active network and that you have signed in to your profile (page 24).
 - 2. On the **Instrument Settings** screen, press **Network connection**, then select Network Drive.



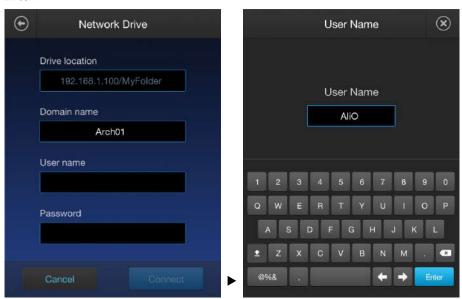
3. On the Network Drive screen, press **Drive location**, enter the location of the drive to save your Qubit[™] Flex files, then press **Enter**.

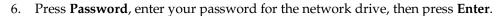


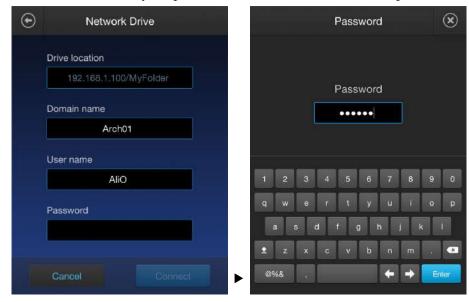
4. Press **Domain name**, enter the domain name where the drive is located, then press **Enter**.



5. Press **User name**, enter your user name for the network drive, then press **Enter**.







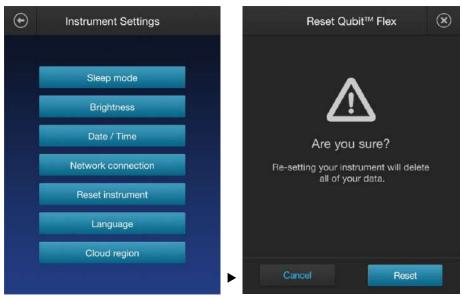
7. When finished entering all the required fields for the Network Drive, press **Connect**.



Reset instrument

Reset instrument Reset instrument function returns the Qubit[™] Flex Fluorometer to its default factory settings, and erases all saved data and user-defined instrument settings.

> 1. On the **Instrument Settings** screen (page 72), press **Reset instrument** to display the Reset Qubit[™] Flex screen.



2. To return the instrument to its default factory settings, press **Reset**. After the reset is complete, all data, user-defined instrument settings, and custom assays are removed, and the instrument displays the Home screen. Press Cancel or Exit () to return to the Instrument settings screen without saving the changes.



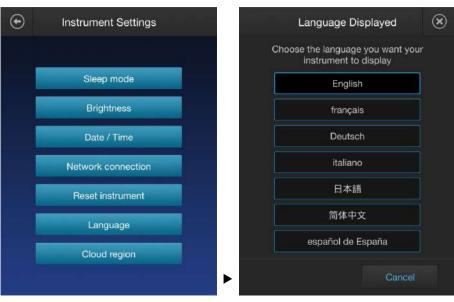
IMPORTANT! The reset function is **not** reversible.

Language

Change the displayed language

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

1. On the **Instrument Settings** screen (page 72), press **Language** to display the Language screen.



- 2. Press to select the desired language. Available options are **English**, **French**, **German**, **Italian**, **Chinese**, **Japanese**, and **Spanish**.
- 3. When prompted, press **Yes** to confirm the change and return to the Instrument settings screen.

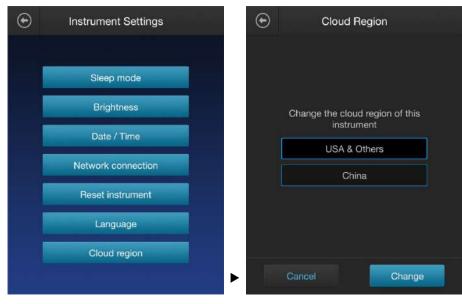
If you do not want to change the language settings, press **Cancel** or **Exit** (to return to the Instrument settings screen without saving the changes.



Cloud region

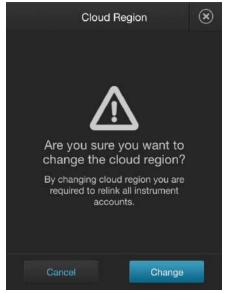
Change the cloud region

Change the cloud 1. On the **Instrument Settings** screen (page 72), press **Cloud region**.



- 2. Select the cloud region from the available choices, then press **Change**.
- 3. When prompted, press **Change** to close the warning screen, then press **Change** again to change the cloud region of the instrument. The instrument will restart after changing the cloud region.

If you do not want to change the cloud region, press **Cancel** to return to the previous screen.





Note: If you change the cloud region of the instrument, you must relink all instrument accounts.

6. Instrument maintenance

Maintenance and cleaning

Maintenance The Qubit[™] Flex Fluorometer does not need regular maintenance. To troubleshoot problems with the instrument, contact Technical Support (page 111).

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



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

Clean the Qubit™ Flex Fluorometer

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



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



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

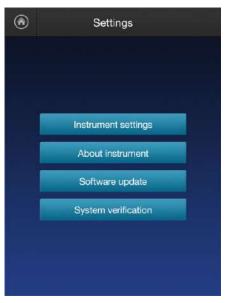
- Clean the surface of the Qubit[™] Flex Fluorometer with a damp cloth.
- To clean the touchscreen, disconnect the power cable, and clean the touchscreen with a soft cloth lightly moistened with LCD (liquid crystal display) cleansing detergent.
- Cleaning the screen with excessive force can damage the 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[™] Flex 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

- Before you begin 1. Download the latest software to a USB drive or to your network from thermofisher.com/qubit.
 - 2. If using a USB drive, insert the USB drive into the instrument. If using a network drive, ensure that the instrument is connected to the network wirelessly or via an Ethernet cable.

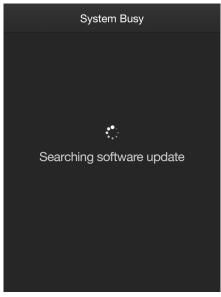
software

Update the 1. On the Home screen, press **Settings**, then select **Software update**.



2. On the Software Update screen, select Cloud, USB, or Network Drive. If you have selected Cloud or Network drive, enter your credentials to sign in. The instrument searches your Connect[™] account, the USB drive, or the network drive for the update.



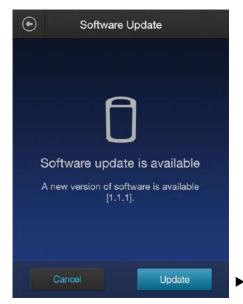




Note: If the USB drive is not inserted into the USB drive port or the instrument does not recognize the USB drive, a warning message is displayed.

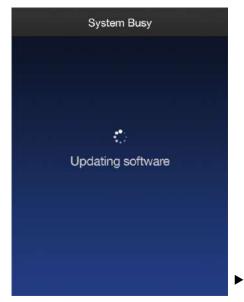
To proceed with the software update, insert the correct USB drive into the instrument, then press **Retry**.

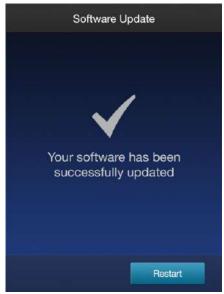
3. If a new update is available and the appropriate files are detected, the instrument displays "Software update is available". Press **Update** to view the available versions of the software.





- 4. Select the software version you want install on the instrument for the update, then press **Update**.
- 5. When prompted, press **Restart** to complete the software update.





System verification

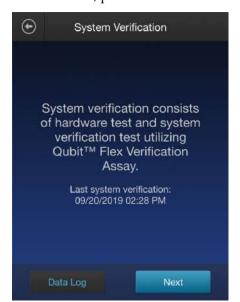
The system verification checks the internal components of the Qubit[™] Flex Fluorometer and requires the use of the Qubit[™] Flex System Verification Assay Kit (Cat. No. Q33254). Perform the system verification when a problem with the instrument is suspected. It is not necessary to perform the verification regularly.

Perform System verification test

Perform System 1. On the **Home screen**, press **Settings**, then select **System Verification**.



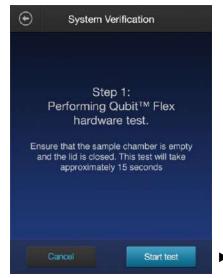
2. On the System Verification screen, press Next.



3. When prompted, set up three Qubit[™] Flex Tube Strips and label the tube strip lids 1–3.

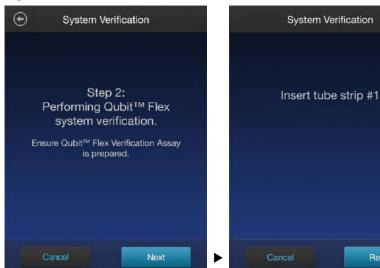


- 4. Add 200 μ L of Blank Reagent to each tube of tube strip #1, 200 μ L of Green Fluorescence Reagent to each tube of tube strip #2, and 200 μ L of Far Red Fluorescence Reagent to each tube of tube strip #3, then press **Next**.
- 5. When prompted, ensure that the sample chamber is empty and the lid is closed, then press **Start test** to run the Qubit[™] Flex hardware test (Step 1 of System Verification). This test takes approximately 15 seconds.

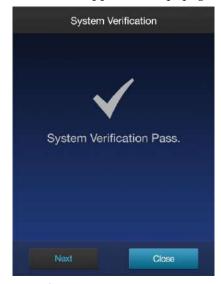




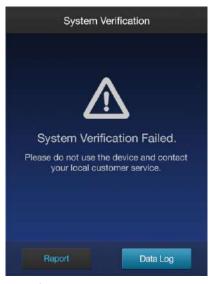
6. When prompted, ensure that the Qubit[™] Flex Verification Assay is prepared, then press Next.



- 7. Insert tube strip #1 into the sample chamber, close the lid, then press **Read**.
- When prompted, read tube strip #2 and tube strip #3 as described for tube strip #1.
- 9. When the test is complete, the software displays the error status.
 - If no errors are found, **System Verification Pass** message appears. Press Close to return to the Settings screen or press Next to view the System Verification Report (page 93).
 - If errors are found, 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, do not use the instrument and contact Technical Support for help (page 111).







Read

System Verification Failed

10. Press **Report** to view the *System Verification Report* or press **Data Log** to view and export the available data logs (page 93).

The *System Verification Report* shows the pass/fail status of the instrument components.

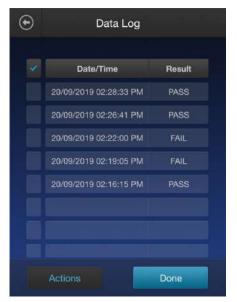




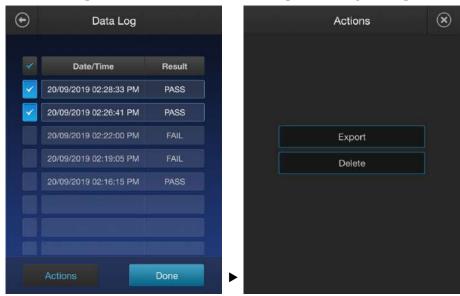
Far Red Fluorescence Channel Fail

All Pass

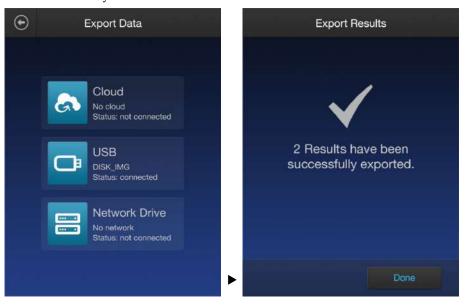
11. Press **Close** to return to the Settings screen or press **Data Log** to view the available data logs.



12. To export a Data Log as a PDF report, select the desired **Data Log**, press **Actions**, then press **Export**. You can select multiple Data Logs for export.



13. Select **Cloud** (Thermo Fisher Connect[™] cloud-based platform), **USB**, or **Network Drive** for the location where you want to save the PDF report of the Verification Assay Test Results.



14. To delete a Data Log, select the desired **Data Log**, press **Actions**, then press **Delete**. You can select multiple Data Logs for deletion.

Replace battery

The Qubit[™] Flex Fluorometer contains a 3 V CR2450 battery, which is required to record the export CSV file date and time. When the battery runs out, the system cannot keep the time setting, which indicates the need to replace the battery.

Replace battery 1.

- 1. Disconnect the Qubit[™] Flex Fluorometer from the power source.
- 2. Remove the four screws (as indicated by the red arrows) on the bottom chassis of the Qubit[™] Flex instrument using a Phillips-head screwdriver.



- 3. Flip the instrument so that the top chassis is facing up.
- 4. Open the instrument slightly (~ 3 cm) from the bottom right side.





5. Pry the old battery from its housing using a flat-head screwdriver and remove it.



- 6. Insert the new 3 V CR2450 battery to the battery housing.
- 7. Arrange two cable assemblies into the groove on the bottom chassis, place the top chassis on the bottom chassis so that the slots for the screws align properly, then tighten the four screws on the bottom chassis using a Phillipshead screwdriver.



Appendix A: Troubleshooting

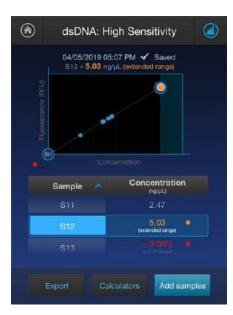
Troubleshooting

Handling samples •

- The calibration standards included in the Qubit™ microRNA, Qubit™ RNA HS, 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™ Flex Fluorometer for longer than it takes to read the fluorescence. See "Assay temperature", page 100.
- 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 Qubit[™] Flex Tube Strip for each reading.
- The tube **must be clean and dry** on the outside when taking readings. Moisture and condensation on the tube surface can lead to reading errors.
- Minute bubbles in samples will cause errors in readings. Be sure not to introduce bubbles into samples. Slight tapping on the tube wall or brief centrifugation will often help dissipate bubbles.

High reading •

- The sample is out of range. Use a sample that is less concentrated or add a smaller volume of sample into the assay to further dilute the sample.
- For Qubit[™] quantification assays, view the Fluorescence vs. Concentration graph in the Results screen to confirm that the values for the samples fall between the values of the standards (page 43).
- 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[™] quantification assays, view the Fluorescence vs. Concentration graph in the Results screen to confirm that the values for the samples fall between the values of the standards (page 43).
- 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).
- dsDNA: High Sensitivity

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 Saved
 S13 = <0.005 ng/µL (out of range)

 Concentration

 (ng/µL)

 S11

 2.47

 S12

 5.03
 (putanded range)

 S13

 Export

 Calculators

 Add samples
- 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[™] Flex 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™ Flex Fluorometer calculates concentration The Qubit™ Flex 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 quantification.

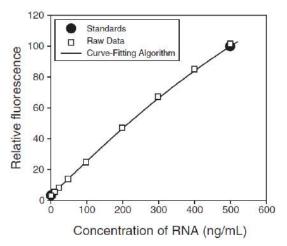


Figure 1. The curve-fitting algorithm used to determine concentration in the Qubit™ RNA HS assay. Data for other Qubit™ quantification assays are generated by similar algorithms.

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.

Photobleaching of Qubit™ reagents

The Qubit™ DNA and protein exhibit high photostability in the Qubit™ Flex 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™ Flex Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (see Figure 2 in "Assay temperature", page 100). The RNA assays should only be read once.

Note that the temperature inside the Qubit[™] Flex 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^{$^{\text{TM}}$} Flex Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, because the Qubit^{$^{\text{TM}}$} Flex Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before a measurement, because holding the tubes warms the solution and results in a low reading.

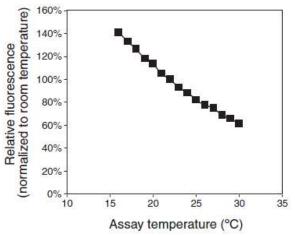


Figure 2. Effect of temperature on the Qubit[™] dsDNA BR assay. Qubit[™] dsDNA HS, Qubit[™] ssDNA, Qubit[™] RNA HS, and Qubit[™] protein assays show similar sensitivities over the same range.

Qubit[™] Flex Fluorometer calibration

For each assay, you have the choice to run standards for a new calibration or to use the values from the previous calibration.

As you first use the instrument, perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you can determine the level of comfort you have using the calibration data stored from the last time the instrument was calibrated.

Remember also that the fluorescence signal in the tubes containing the standards and the samples is stable for not longer than 3 hours. See Figure 1 in "How the Qubit^m Flex Fluorometer calculates concentration" (page 99) for an example of the calibration curve used to generate the quantification results.

Appendix B: Ordering information

Qubit[™] Flex Fluorometer and accessories

The following products can be used with the QubitTM Flex Fluorometer and are available separately from Thermo Fisher Scientific. For more information, visit **thermofisher.com** or contact Technical Support (page 111).

Product	Quantity	Cat. No.
Qubit [™] Flex Fluorometer	1 each	Q33327
Qubit [™] Flex Quantitation Starter Kit	1 kit	Q45894
Qubit [™] Flex NGS Starter Kit	1 kit	Q45893
Qubit [™] Flex USB Flash Drive	1 each	Q46009
Qubit [™] Flex Wi-Fi dongle	1 each	A26774
Qubit [™] Flex Fluorometer International Power Supply (replacement)	1 each	A36204
Qubit [™] Flex Tube Strips	125 strips	Q33252
Qubit [™] Flex Reservoir (10 mL)	100 each	Q33253
Qubit [™] Flex System Verification Assay Kit	1 kit	Q33254
Qubit [™] RNA BR Assay Kit *20–1,000 ng*	100 assays 500 assays	Q10210 Q10211
Qubit [™] RNA HS Assay Kit *5–100 ng*	100 assays 500 assays	Q32852 Q32855
Qubit [™] ssDNA Assay Kit *1–200 ng*	100 assays	Q10212
Qubit [™] dsDNA BR Assay Kit *2–1,000 ng*	100 assays 500 assays	Q32850 Q32853
Qubit [™] dsDNA HS Assay Kit *0.2–100 ng*	100 assays 500 assays	Q32851 Q32854
Qubit [™] 1X dsDNA HS Assay Kit	100 assays 500 assays	Q33230 Q33231
Qubit [™] Protein Assay Kit *0.25–5 μg*	100 assays 500 assays	Q33211 Q33212
Qubit [™] microRNA Assay Kit *0.5–100 ng*	100 assays 500 assays	Q32880 Q32881
$Qubit^{^{\mathrm{TM}}}dsDNAHSAssay-LambdaDNAStandard$	5 mL	Q33233

