## Qubit<sup>™</sup> Flex Fluorometer USER GUIDE

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### Contents

Ab	out this guide	3
1.	Product information	5
	Product contents	5
	Product description	6
	Instrument exterior components	7
	Product specifications	
2.	Getting started	10
	Set up the Qubit <sup>™</sup> Flex Fluorometer	
	Connect to the network	
	After instrument setup	
	Sign in	16
	Guidelines for using the Qubit <sup>™</sup> Flex Fluorometer	23
3.	Perform assays	24
-	Before you begin	
	(Optional) Use the Assay Range Calculator to determine the assay range	
	Use the Reagent Calculator to prepare Qubit <sup>™</sup> Working Solution	
	Run standards for assay calibration	
	Read samples	
	Results	
	(Optional) Use the Molarity Calculator to determine sample molarity	45
	(Optional) Use the Normalization Calculator to determine how to dilute the samples to the	
	same molarity, concentration, or mass	48
4.	Manage data	58
	View detailed sample data	58
	Edit sample name	63
	Export data	66
	Delete data	
5.	Configure instrument settings	72
	Instrument settings	
	Sleep mode	
	Brightness	74
	Date and Time	
	Network connection	
	Reset instrument	
	Language	85
	Cloud region	86

6.	Instrument maintenance	87
	Maintenance and cleaning	
	Software updates	
	System verification	
	Replace battery	
Ap	pendix A: Troubleshooting	97
-	Troubleshooting	
	Critical Qubit <sup>™</sup> Assay considerations	
Ар	pendix B: Ordering information	101
	Qubit <sup>™</sup> Flex Fluorometer and accessories	
Ар	pendix C: Safety	102
	Symbols on instruments	
	Safety labels on instruments	
	General instrument safety	
	Chemical safety	
	Chemical waste safety	
	Electrical safety	
	Biological hazard safety	
	Safety, Electromagnetic compatibility (EMC), and Environmental standards.	
Do	cumentation and support	111
	Obtaining support	

### 1. Product information

### **Product contents**

The Qubit<sup>™</sup> Flex Fluorometer (Cat. No. Q33327) is shipped with the following components:

Component	Quantity
Qubit <sup>™</sup> Flex Fluorometer	1 each
Qubit <sup>™</sup> Flex power cord (shipped separately) <sup>[1]</sup>	1 each
USB drive	1 each
Qubit <sup>™</sup> Flex LAN cable	1 each
Qubit <sup>™</sup> Flex Fluorometer Quick Reference Card (QRC)	1 each
Certificate of Conformity (COC)	1 each
Qubit <sup>™</sup> screen cleaning cloth	1 each
Wi-Fi Dongle	1 each
<sup>1</sup> The power cords for the Qubit <sup>™</sup> Flex Fluorometer are not interchang he other Qubit <sup>™</sup> Fluorometer models. Powering the instrument with a ord 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<sup>™</sup> Flex Fluorometer.

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

See page 10 for instructions to set up the Qubit<sup>™</sup> Flex Fluorometer.

Register your Go to thermofisher.com/qubit to register your instrument. You will be asked to 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<sup>™</sup> Flex Fluorometer.

### **Product description**

Qubit™ FlexThe Qubit™ Flex Fluorometer is a benchtop fluorometer for the quantification of<br/>DNA, RNA, microRNA, and protein. With the Qubit™ Flex Fluorometer, you can<br/>directly measure the fluorescence of up to 8 samples simultaneously using the<br/>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<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> account using the Wi-Fi dongle (supplied with the instrument).
- ④ Power inlet connects the Qubit<sup>™</sup> 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**

Physical	Instrument type:	Benchtop fluorometer
characteristics	Instrument dimensions:	7.3 in (w) $\times$ 11.1 in (l) $\times$ 4.1 in (h)
	moti uniciti uniciiono.	$(18.6 \text{ cm} \times 28.2 \text{ cm} \times 10.3 \text{ cm})$ ; rectangular shape
	Weight:	60 oz. (1.7 kg)
	Operating power:	100–240 ±10% VAC, 1.3 A
	Frequency:	50/60 Hz
	Electrical input:	48 VDC, 1.87 A
	voltage, a power line reg	pplied power fluctuates ±10% beyond the rated gulator may be required. High or low voltages can tronic components of the instrument.
Operating	Installation site:	Indoor use only
conditions	Altitude:	Between sea level and 2000 m (6500 ft.) above sea level
	<b>Operating temperature:</b>	10–30°C
	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.
Technical	Dynamic range:	4 orders of magnitude
specifications	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
	Sample chamber:	Accommodates one Qubit <sup>™</sup> Flex Tube Strip
	Tube type:	Qubit <sup>™</sup> Flex Tube Strip (8× 0.2-mL thin-wall polypropylene tubes; Cat. No. Q33252)
	Warm-up time:	<35 seconds

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<sup>™</sup> Flex Fluorometer

**Install the** The Qubit<sup>m</sup> Flex Fluorometer is a stand-alone instrument that does not require connection to a computer.

- 1. After unpacking the instrument, place the instrument on a flat, level, dry surface.
- 2. Plug one end of the supplied power cord into the Qubit<sup>™</sup> Flex Fluorometer.
- 3. Attach the appropriate plug adaptor to the other end of the power cord.
- 4. 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<sup>™</sup> Flex Fluorometer are not interchangeable with those for the other Qubit<sup>™</sup> Fluorometer models.

5. 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.

6. 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).

7. To power down the Qubit<sup>™</sup> 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.

Date / Time	
Time Zone	
US-PACIFIC	<b>•</b>
Date	
03/13/2019	~
Time	
04:19 PM	~
Cancel	Done
	Time Zone US-PACIFIC Date 03/13/2019 Time 04:19 PM



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).



(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 **Join**.

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<sup>™</sup> Flex files.

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



**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<sup>™</sup> 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<sup>™</sup> buffer and reagent required to prepare the Qubit<sup>™</sup> working solution.
- Use the Range Calculator to determine the best assay to use for your sample. •

### About InstrumentThe About Instrument screen displays information about your Qubit™ FlexscreenFluorometer, 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.

۲	Settings	$\odot$	About Instrument
		003.52	<b>it Flex™ Fluorometer</b> Thermo Fisher Scientific
			l IP Address: 169.254.8.156 ess IP Address:
	Instrument settings		mo Fisher Cloud Region: USA & Others vare Version: 1.0.1
	About instrument		I Number: SVT05
	Software update	Manu	Ifactured in: Singapore
	System verification		
		▶	Close

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

### Sign in

### instrument profile

**Create a local** Qubit<sup>™</sup> 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<sup>™</sup> 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.

٢	Qubit Flex		Sign In	۲
×	A S H S	A A A A A A A A A A A A A A A A A A A	Sign In	
1X dsDNA High Senativity (849)	dsDNA High Screditivity (HS)	dsDNA Bread Hange (BH)	Screen name PIN	
RNA High Bandlivity (HG)	RNA Bread Farger (6P)	Protein		
	• •		New to this instrument	
		æ	Connect to the Thermo Fisher C create a local instrument profile.	
Data	Calculators	Settings	Get Started	
0		17/2019 02:46 PM		

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<sup>™</sup> account, Connect<sup>™</sup> 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<sup>™</sup> account or create one. To create your Connect<sup>™</sup> 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<sup>™</sup> 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.

٢	Qubit Flex		Sign In	∢
1×6	(ش	ب	Sign In	
1X dsDNA Hugn Genstitivity (145)	dsDNA Hgri Bensthing (HE)	dsDNA Bread Range (BR)	PIN	
RNA	RNA -	Protein	Sign in	
rilgin Samallivity (HS)	Bried Range (BR)		New to this instrument?	
(A	••	۲	Connect to the Thermo Fisher Cloud or create a local instrument profile.	
Data	Calculators	Settings	Get Started	
0		7/2019 02:46 PM		

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<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> Flex instrument. When finished, go to Step 8 (page 22).



- 6. To connect to your Thermo Fisher Connect<sup>™</sup> account with **Dashboard on PC**:
  - a. Go to **thermofisher.com/cloud** and sign in to your Thermo Fisher Connect<sup>™</sup> account.
  - b. On the Connect<sup>TM</sup> dashboard, press the **Instrument Connect** button.



Instrument Connect screen 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<sup>™</sup> Flex instrument; see page 19) to display the linking code.



f. Enter the linking code displayed on the Qubit<sup>™</sup> Flex instrument into the Add an instrument dialog, then press **Send**.

	Add an instrument	
Insert linking co	de:	
Get the code from y	our instrument	
Type your linking or	ode here	Send

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



b. Enter your **User name** and **Password** for your Thermo Fisher Connect<sup>™</sup> account, then press **Connect**.



8. When you have signed in to your Thermo Fisher Connect<sup>™</sup> Account, the Profile button on the Home screen becomes blue.

When signed in, you can export your data to your Connect<sup>™</sup> account.



### Guidelines for using the Qubit<sup>™</sup> Flex Fluorometer

**Recommendations** To obtain the best results, follow the recommendations below. For more information, see "Critical Qubit<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution.
- Incubate the tubes for the Qubit<sup>™</sup> 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<sup>™</sup> assays that have been tested using the Qubit<sup>™</sup> Flex Fluorometer.

Assay tubes for<br/>the Qubit<sup>™</sup> Flex<br/>FluorometerOnly thin-wall, clear 0.2-mL PCR tube strips are appropriate for use in the Qubit<sup>™</sup><br/>Flex Fluorometer. For best results, we recommend using Qubit<sup>™</sup> Flex Tube Strips<br/>(Cat. No. Q33252).

### 3. Perform assays

### Before you begin

Materials needed •

• A Qubit<sup>™</sup> assay kit appropriate for quantifying your samples (see page 101 for available Qubit<sup>™</sup> assay kits and ordering information)

- DNA, RNA, or protein samples in Qubit<sup>™</sup> Flex Tube Strips
- Appropriate standards for your assay in Qubit<sup>™</sup> Flex Tube Strips
- Single channel pipette (1–20 µL), multichannel pipette (200 µL)
- Qubit<sup>™</sup> 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*<sup>M</sup> *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<sup>™</sup> account wirelessly, if you have set up a wireless connection.

Sign in to your 1. profile

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



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

To create a local instrument profile, see page 16.

To sign in to your Thermo Fisher Connect<sup>™</sup> account, see page 18.

Otherwise, go to Step 3 (page 25).

Get Started

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



5.

# *(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<sup>™</sup> assay provides the most accurate quantification based on your sample volume and estimated sample concentration.

- Use the Assay 1.
- 1. On the **Home screen**, press **Calculators**.
- Range Calculator 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.



Output Units	۲
Select units:	
ng/µL	
ng/mL	
μg/μL	
µg/mL	
mg/mL	

4. Select the **Assay** for which you wish to view the assay accuracy 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.

Range	Sample Concentration
Extended low	0.007 - < 0.01
Core	0.01 - 6.7
Extended high	> 6.7 - 8
0.01	- 6.7

**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.

Assay Rar	nge Calculator	(j)	$\odot$	Assay Ra	ange Calculator	(i)
Enter samp	Enter sample volume used: 1X dsDNA HS			Enter sample volume used: 1X dsDNA HS		
	+ 20 µL -				+ 5 µL -	
Range	Sample Concentration	1		Range		
Extended low Core Extended high	0.005 - < 0.01 0.01 - 5 >5 - 6			Extended low Core Extended high	0.02 - < 0.04 0.04 - 20 > 20 - 24	
0.01 -	5			0.04	1-20	
	Done				Done	
Range	Sample Concent	ration	Ra	ange	Sample Concent	ration
Extended low	0.005 - < 0.0	)1		ended low	0.02 - < 0.0	4
Core	0.01 - 5		Con		0.04 - 20	
Extended high	>5-6		Exte	ended high	> 20 - 24	
0.01 - 5		0.04 - 20				
dsDNA HS Assay range for 20 μL sample volume		dsDNA HS Assay range for 5 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.

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.



	Range Details			×
	accuracy r	iys have core ai anges. Sample have relative e	s within these	
		<15%	mlative error	
		<25%	relative error	
		Out of	Range •	
•	The sample v accuracy rang		will change the assay	
•			may be required if th what the assay can	
•	Use a P-2 pip sample.	ette for best results w	/hen adding 1-2 μL of	
			Close	

- 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<sup>™</sup> Working Solution

Use the on-board Reagent Calculator to determine the amount of Qubit<sup>™</sup> dye and buffer required to prepare the Qubit<sup>™</sup> Working Solution for your samples and standards.

- Select Calculator

  Reagent

  Assay Range

  1

  0

  7

  8

  7

  8

  7

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  7

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  </t
- Use the Reagent 1. On the Select Calculator screen, press Reagent to open the Reagent Calculator. 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<sup>™</sup> dye and buffer required to prepare the Qubit<sup>™</sup> Working Solution with these inputs.

G	Reagent Calculat	or
H	ow many samples?	16
H0 (0, 2	ow many standards?	2
Resu	lts: Id <b>35µL</b> dye to <b>6965µL</b> buff volume of <b>7000µL</b>	
		Done



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

- 5. Press **Done** to return to the **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<sup>™</sup> Flex Fluorometer or use the values from the previous calibration. For more information, see "Qubit<sup>™</sup> 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.



2. 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<sup>™</sup> 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**.

left dsDNA: High Sensitivity	dsDNA: High Sensitivity
Insert Standard 2	
Load tubes as shown:	
	<ul> <li>A)2</li> </ul>
Hop (BELU)	Reading Standard 2
Concentration	
Concentration,	
Run standards	Cancel

5. *For Qubit<sup>™</sup> 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 Qubit<sup>™</sup> 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.



dsDNA: High Sensitivity There was one or more errors with the Qubit standards.

Done

• 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 1. In the Calibration error screen, press Done.
  - standards after 2. calibration error
    - 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<sup>™</sup> 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<sup>™</sup> DNA and RNA assays, 15 minutes for the Qubit<sup>™</sup> protein assay).

**Insert samples** 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.

۲	dsDNA: High Sensitivity	dsDNA: High Sensitivity
	Insert samples	Insert samples
	Load tubes as shown:	Load tubes as shown:
Pit	su tó deselect tube positions that do not contain a sample	S1         C2         C3         C4         C5         C5         C4         C4<
	Output sample units:	Output sample units:
N	fore options Next	More options Next

All 8 tubes contain samples

No sample in positions S7 and S8

Output sample units:

ng/µL

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

Select units: ng/µL ng/mL µg/µL µg/mL	
ng/mL μg/μL μg/mL	
μg/μL μg/mL	
µg/mL	
mg/mL	

- 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  $\mu$ 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.



Note: The sample volume used (1–20 μL) 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.
More options

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

- (Optional) Enter 1. Press More options to open More Options screen, where you can:
  - 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.

$\odot$	More Options	$\odot$	Add Tag
			Last Used Alphabetical
	Enter assay kit lot # 1503596		Create new run tag
	,,	×	Tag 🗸
	Add Tags		Tag 1
	Add Sample IDs		
	Cancel Done	•	Delete Cancel Apply

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. 5. 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.

$\odot$	Add Tag	$\odot$	More Options
	Last Used Alphabetical		
	Create new run tag		Enter assay kit lot # 1503596
	Tag 💙		
	Tag 1		
	Tag 2		Edit Tags
			Add Sample IDs
D	Delete Cancel Apply	•	Cancel Done

**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.

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



3

**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".

2	A	В	С
1	Plate Barcode	Well	Sample Id
2	96W207	A1	GS2072-2
3	96W208	A2	GS2072-3
4	96W209	A3	GS2072-4

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

Date	File name		Sample	Sample ID	Plate ID	We
23/09/2019	Sample ID.csv	8	S1	GS2072-2	96W207	A
			S2	GS2072-3	96W208	A
			S3	GS2072-4	96W209	A
			S4	GS2072-5	96W210	A
			S5	GS2072-6	96W211	
			<b>S</b> 6	GS2072-7	96W212	A
			S7	GS2072-8	96W213	A
			S8	GS2072-9	96W214	A

- 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.

۲	dsDN	IA: F	ligh Sensitivity	
	04/05/2	019 0	5:04 PM 🖌 Saved	
	Sample		Concentration	
	S1		2.84	
	S2		2.68	
	S3		2.47	
	<b>S</b> 4		2.43	
			2.47	
	S6		2.45	
	S7		2.45	
			1.43	
	xport	G	alculators Add samp	bies

**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. 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.



- (LL)
- 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.

 $\odot$ 

Desired units:

Molecular weight:

Sample Concentration

Desired units:

۲

Auto-populate DNA length

Use the Molarity 1. Calculator 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.

Units Selection

Choose the units you want to use

ng/µL

ng/mL



Calculators

nM

Molarity

Calculate

to

nM

g/mol

Molarity Calculator

ng/µL to

Length

ng/µL

660

μg/μL μg/mL mg/mL Input units

Output units



Note: The Qubit<sup>™</sup> Flex Fluorometer auto-populates the Molecular weight (MW) depending on the Qubit<sup>™</sup> 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).

Molecular weight:	660	g/mol
-------------------	-----	-------

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. S1 2 44

Desired	d units:	ng/µL	. to	nM
Molecu	ular weight:	660	g/m	iol
Auto	p-populate DN			
Sample	Concentratio	n Le	ength	Molarity
sample	(ng/µL)		(bp)	(nM)
Sample S1	(ne/uL) 2,44		(dap) 200	(Mn)
	(ng/µL)			(nM)
	(ngyul) 2.44			(nM)

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

Auto-populate DNA length

Desire	d units:	ng/µL to	nM
Molecu	ılar weight:	660 g/m	nol
	Concentration		Molarity
	2.44	200	
	2.44	200	
		200	
	2.57	and the second s	

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.

### Calculate





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

To export your results, press Export. The instrument exports the complete CSV 6. 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.

- On the Results screen, press Calculators, then press Select the 1. Calculators Normalization. Normalization Calculator  $\odot$ Calculator  $\odot$ Normalization Calculator Select a calculator: Select normalization calculator: Molarity Molarity Normalization Concentration Mass
  - 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.

1.	On the Normalization	Calculator screen,	select Molarity.

Normalize your 1 samples to the same molarity

$\odot$	Normalization Calculator	$\odot$	Norma	lization Calo	culator
			Fina	I sample mola	arity: M
	Select normalization calculator:				
	Molarity		Fina	I sample volu	ime:
	Concentration				il i
	Mass				
			1	2	3
			4	5	6
			7	8	9
			×	0	Enter

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

Normalization Calc	culator	$\odot$	Normalizatio	on Calculator
Final sample mola			Final samp	le molarity:
Final sample volu				ple volume:
1 2	3		Calc	ulate
4 5	6			
7 8 × 0	9 Enter			
	LING			

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

2

### Calculate

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.

(	1	Normalization Ca	lculator	
	Final	Molarity: 5 nM Final	Volume: 10 µL	
	Sample	Required Dilution	Diluted conc.	
		N/A	N/A	
		N/A		
	<b>S</b> 3	N/A		
<	<b>S</b> 4			
	S6			
	<b>S</b> 7			
		Page 2 of 3		
	Ex	port	Done	

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

	Final N	lolarity: 5 nM Final Volume: 10 µL
	Sample	Concentration
		18.5
		18.5
		19.5
<	<b>S</b> 4	18.6
		18.6
	S6	18.7
	S7	18.6
		18.4

- 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 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).

Final N	/lolarity: 100 pM  I	Final Volume: 20 μL
Sample	Add sample	Add buffer
S1	1.1 (†.9)	18.9
S2	1.1 (1:9)	18.9
	/lolarity: 100 pM  I	Final Volume: 20 µL
		Final Volume: 20 µL
Final N	Aolarity: 100 pM I	Final Volume: 20 μL n Diluted conc.
Final N Sample	Aolarity: 100 pM I Required Dilutio <sub>(sample:buffer)</sub>	Final Volume: 20 µL n Diluted conc. (pM)

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

- Normalize your 1. On the Normalization Calculator screen, select Concentration. samples to the  $\odot$  $\odot$ Normalization Calculator Normalization Calculator same concentration Final sample concentration: Select normalization calculator: Final sample volume: Molarity Concentration Mass 2 3 6
  - 2. Enter the Final sample concentration and select units.
  - 3. Enter the **Final sample volume** and select **units**, then press **Enter**.

Normalization Calculator	Normalization Calculator
Final sample concentration:	Final sample concentration:
Final sample volume:	Final sample volume:
1 2 3 4 5 6	Calculate
7 8 9	
X 0 Enter	

►



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

8

×

9

Enter

#### Calculate

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.

¢		Normalization C	alculator	
	Final Con	centration: 1 ng/µL	Final Volume: 200 µL	
	Sample	Required Dilution	Diluted conc.	
	<b>S</b> 1	N/A	N/A	
	<b>S</b> 3	N/A		
<	S4	N/A		>
	S6			
	<b>S</b> 7			
		Page 2 of 3		
	Ex	port	Done	

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

Final C	ncentration: 1 ng/µL Final Volume: 200 µL
Sampl	e Concentration
S1	2,44
	2,44
<b>S</b> 3	2.57
S4	2.46
	2.45
S6	2.47
S7	2,45
	2.43
	Page 3 of 3

- 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).

0			
Final Cond	centration: 2 ng/mL	Final Volume: 200 µL	
Sample	Add sample (µL)	Add buffer (µL)	
S1	1.1 <mark>(1:0</mark> )	199	
S2	1.1 (1:0)	199	
Final Cond	centration: 2 ng/mL	Final Volume: 200 µL	
Final Cond Sample	centration: 2 ng/mL Required Dilution (sample:buffer)	Final Volume: 200 µL Diluted conc. (ng/mL)	
	Required Dilution	Diluted conc.	
Sample	Required Dilution (sample:buffer)	Diluted conc. (ng/mL)	

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

. On the Normalization Calculator screen, select	ect Mass.
--	-----------

 $\odot$  $\odot$ Normalization Calculator Normalization Calculator Final sample mass: Select normalization calculator: Final sample volume: Molarity Concentration Mass 2 3 6 8 9 × Enter ►

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

Norma	lization Calo	culator		Normalizatio	n Calculator	
Fina	al sample ma	ss:		Final sam	ple mass:	
1	n	g		1	ng	
Fina	l sample volu	me:		Final samp	le volume:	
10	р При р	L		10	μL	
1	2	3		Calc	ulate	
4	5	6				
7	8	9				
×	0	Enter	▶			



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

Normalize your 1 samples to the same mass and volume

### Calculate

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.

(	1	Normalization Ca	lculator	
	Fin	al Mass: 1 ng Final V	olume: 10 µL	
	Sample	Required Dilution	Diluted conc.	
		1:2	0.813	
			0.813	
			0.857	
<	S4		0.82	
		1:2	0.817	
	S6		0.823	
	S7		0.817	
			0.81	
		Page 2 of 3		
	Ex	port	Done	

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).

Fina	al Mass: 1 ng Final Vo	olume: 10 µL	
Sample	Add sample	Add buffer (µL)	
S1	1.2 (1:2)	8.8	
S2	1.2 (1:2)	8.8	
Fina	al Mass: 1 ng Final Vo	olume: 10 µL	
Fin: Sample	al Mass: 1 ng Final Vo Required Dilution (sample:buffer)	Dlume: 10 µL Diluted conc. (ng/µL)	
	Required Dilution	Diluted conc.	

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<sup>™</sup> Flex Fluorometer can save data for up to 10,000 samples.

For the saved data, the Qubit<sup>™</sup> 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<sup>™</sup> account (page 64).
- Delete data files (page 70).

### View detailed sample data

# 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.



07/25/2019 1X dsDNA HS 16 07/17/2019 dsDNA HS 8	Date	Assay name	
07/25/2019 1X dsDNA HS 12 07/25/2019 1X dsDNA HS 16 07/17/2019 dsDNA HS 8	07/30/2019	dsDNA BR	
07/25/2019 1X dsDNA HS 16 07/17/2019 dsDNA HS 8	07/29/2019	1X dsDNA HS	
07/17/2019 dsDNA HS 8	07/25/2019	1X dsDNA HS	12
	07/25/2019	1X dsDNA HS	
06/10/2010 deDNA HS 9	07/17/2019	dsDNA HS	
00/19/2019 USDIAA HS 8	06/19/2019	dsDNA HS	

- 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.

Actions

$\odot$		Data			Actions	۲
	Date	Assay name				
	07/30/2019	dsDNA BR				
	07/29/2019	1X dsDNA HS			Export	
	07/25/2019	1X dsDNA HS				
	07/25/2019	1X dsDNA HS			Filter data	
	07/17/2019	dsDNA HS			Delete	
	06/19/2019	dsDNA HS				
		Actio	ie i			
		Action				

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

	· ·	. 5	5	
$\odot$	Filter Data		Select Assay	∕ ⊗
	Assay	~		
			1X dsDNA HS	
<u> </u>	Tag 💌		dsDNA HS	
	Tag 1 Tag 2		dsDNA BR	
Ē			RNA HS	
			RNA BR	
	Number of results: 6		Protein	
			Oligo	
c	lear filter Cancel	Apply	•	

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

€	Filter Data	Filter Data
	1X dsDNA HS	1X dsDNA HS 💌
×	Tag 👻	V Tag V
	Tag 1	Tag 1
	Tag 2	Tag 2
	Number of results: 3	Number of results: 1
	Clear filter Cancel Apply	Clear filter Cancel Apply

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.

$\odot$	Data				
*	Date	Assay name			
	07/25/2019	1X dsDNA HS	12		
	Clear filter	Action	ıs		

- 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.

)	Data Details	<u>ن</u>	Data Details
	250719-174238	Last read standard	ls: 25/07/2019 05:42:56 PM
S1		Sample volume	
25/07/201	9 05:47:04 PM	20 µL	
Original sa	ample concentration: 2.5 ng/µL	Dilution factor	
	be concentration: 250 ng/mL dsDNA HS	10	
Sample F	RFU value	Excitation	
Sample: 4	30	Blue	
Standard	I RFU value	Optional informa	ation
Standard	1:1.26	Tags: Tag 1	
Standard		Assay kit lot: 2107	452
Last read	standards: 25/07/2019 05:42:56 PM	Molarity calculation	tion information
Sample \	volume	Concentration mas	ss unit: ng/µL
20 µL.		Molarity weight: 66	50
		Length (bp): 150	
Dilution f	actor	Molarity: 25.3 nM	

Information in the detailed sample data



- 1 Run ID
- 2 Sample name
- 3 Assay date
- (4) Original sample concentration
- (5) Qubit<sup>™</sup> 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
- (1) Sample volume
- (11) Dilution factor
- 12 Excitation channel
- (13) Optional information (Tags, Reagent lot etc.)
- (1) Molarity calculation information (units, nucleic acid length, MW, molarity)

### Edit sample name

sample name

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

			Run ID: 2	90719-150906
Date	Assay name	×	Sample	Concentration (ng/µL)
07/30/2019	dsDNA BR		S1	2.52
07/29/2019	1X dsDNA HS		S2	2.52
07/25/2019	1X dsDNA HS		S3	2.75
07/25/2019	1X dsDNA HS		S4	2.54
07/17/2019	dsDNA HS		S5	2.9
06/19/2019	dsDNA HS			2.53
			S7	2.45

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<sup>™</sup> 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<sup>™</sup> 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.
  - 2. 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.

 $\odot$ Data  $\odot$ Data Date Assay name Date Assay name 07/30/2019 dsDNA BR 07/30/2019 dsDNA BR 07/29/2019 07/29/2019 1X dsDNA HS 1X dsDNA HS 07/25/2019 1X dsDNA HS 1X dsDNA HS 07/25/2019 1X dsDNA HS 07/17/2019 dsDNA HS 06/19/2019 dsDNA HS

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

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.



Three samples in the data sets selected

Run ID: 250719-174238 Tags: Tag 1						
	Sample	Concentration				
~	S1	2.5				
~	S2	2.5				
Image: A start of the start	S3	3.69				
~	S4	2.5				
~	S5	2.05				
~	S6	2.56				

All samples in the data set selected

#### Actions

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<sup>™</sup> account), **USB**, and **Network Drive**.
  - To export data to a USB drive, insert the USB drive into the Qubit<sup>™</sup> Flex Fluorometer.
  - To export data to your Connect<sup>™</sup> 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.
  - 2. On the Data screen, press the check box to the left of each data set you wish to 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.

€		Data		$\odot$		Data
~	Date			Tag	Run ID: 2 s: Tag 1	50719-174238
	07/30/2019	Assay name dsDNA BR	#		Sample	Concentration
	07/29/2019	1X dsDNA HS			S1	2.5
	07/25/2019	1X dsDNA HS			S2	2.5
	07/25/2019	1X dsDNA HS			S3	3.69
	07/17/2019	dsDNA HS			S4	2.5
	06/19/2019	dsDNA HS				2.05
					<b>S</b> 6	2.56
		Action	ns			Actions
	Salaat dat		1000		Coloct Com	Actions

Select data sets to delete

Select Sample files to delete

Actions

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<sup>™</sup> 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) •

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<sup>™</sup> 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**.

$\odot$	Instrument Settings	Sleep Mode	
	Sleep mode	Sleep mode allows the instr less energy when not	
	Brightness	Enable	)
	Date / Time	Sleep Mo	de
	Network connection	Off 0	n
	Reset instrument	Edit Time	
	Language	15 Minutes	
	Cloud region		
		Cancel	Done

- 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**

- brightness  $\odot$  $\odot$ Instrument Settings Brightness Adjust the brightness of your screen Sleep mode Brightness Date / Time Network connection **Reset instrument** Language Cloud region Done
- 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**

time  $\odot$  $\odot$ Instrument Settings Date / Time Sleep mode Time Zone US-PACIFIC Brightness Date / Time Date Network connection Reset instrument Language Cloud region

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

►



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

Done

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.

$\odot$	Date / Time	Э	Date /	Time
	Choose your time format 12 Hour 24 Hour		Choose your 12 Hour	time format D 24 Hour
	Choose a time		Choose	a time
l	4 35 O		16 Hours	35 Minutes
			nours	
	Cancel Done	с	ancel	Done
	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**.



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<sup>™</sup> 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.

۲	Settings	Instrument Settings
		Sleep mode
y	Instrument settings	Brightness
	About instrument	Date / Time
	Software update	Network connection
	System verification	Reset instrument
	Cystem venication	Language
		Cloud region

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<sup>™</sup> Flex files, go to page 81. ٠

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.

Network Connection	€	Choose	Network	
	Wirele	ess Networks	Security	Signal
Wireless	Lifetec			
Wireless	Redmi		WPA2 Per.	•
	sec2.t	ts.wireless	WPA2 Ent.	ŝ
器 Wired	sec2.t	fs.wireless	WPA2 Ent.	<u> </u>
Network Drive				
	Oth	ers Ca	ncel	Refresh

- 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.

area network (LAN)

- **Connect to a local** 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.

$\odot$	Instrument Settings	Network Connection
	Sleep mode	Wireless
	Brightness	
	Date / Time	器 Wired
	Network connection	
	Reset instrument	Network Drive
	Language	Network Drive
	Cloud region	

- 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.

۲	IP Configuration	IP Configuration
	DHCP Static	DHCP O Static
	IP address	Default Gateway
	123.456.789.10	123.456.789.10
	MAC address	Primary DNS Server
	123.456.789.10	123.456.789.10
	Subnet mask	Secondary DNS Server
	123.456.789.10	123.456.789.10
		Cancel Done

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<sup>™</sup> Flex files, then press **Enter**.



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

⊙	Network Drive					Do	mair	ו Na	me			۲
	Drive location											
	192.168.1.100/MyFolder					Do	mair	ו Na	me			
	Domain name						Arci	h01				
	User name		1	2	3	4	5	6	7	8	9	0
	Password		q	w	е	r	t	y	u	i	0	p
			1	1 5	i	1	F I		n	j k		
			٤	z	×				n	m		×
	Cancel	►	Ø9	68					+	+	En	ter

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



6. Press **Password**, enter your password for the network drive, then press **Enter**.

⊙	Network Drive					F	ass	word	ł			۲
	Drive location											
	192.168.1.100/MyFolder					ł	ass	word	ł			
	Domain name						••••	•••		1		
	Arch01											
	User name											
1	AliO			2		4				8		0
	Password		q	w	е	r	t	y	u	i	0	p
			2		6 (	1	f (	g   1	n	j k		1
			£	z	×	c	v	b	n	m		×
	Cancel	►	@9	68					+	•	Er	ter

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

۲	Network Drive
	Drive location
	192.168.1.100/MyFolder
	Domain name
	Arch01
	User name
	AliO
	Password
	•••••
	Cancel

## **Reset instrument**

**Reset instrument** Reset instrument function returns the Qubit<sup>™</sup> 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<sup>™</sup> Flex screen.

⊙	Instrument Settings		Reset Qub	it™ Flex	$\otimes$
	Sleep mode		^		
1	Brightness		<u>_!</u>	7	
	Date / Time		Are you	sure?	
	Network connection		Re-setting your instr all of you		Э
	Reset instrument				
	Language				
	Cloud region				14
		►	Cancel	Reset	

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

the You can change the language that the Qubit<sup>™</sup> 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.

$\odot$	Instrument Settings	Language Displayed	۲
		Choose the language you want your instrument to display	
	Sleep mode	English	
	Brightness	français	
	Date / Time	Deutsch	
	Network connection	italiano	
	Reset instrument	日本語	
	Language	简体中文	
	Cloud region	español de España	
		Cancel	

- 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** (**I**) to return to the Instrument settings screen without saving the changes.



## **Cloud region**

Change the cloud 1. On the Instrument Settings screen (page 72), press Cloud region. 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<sup>™</sup> 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<sup>™</sup> Flex Fluorometer to avoid damaging the instrument.
- Do not expose the Qubit<sup>™</sup> 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**<sup>™</sup> We recommend that you clean the Qubit<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 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.

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

software



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<sup>™</sup> 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.

۲	Software Upda	te	e	) Softw	are Update	
				Select the up	date file from the	ə list
				Firmware Package	9	
	A			Qubit8 1.0.0		
				Qubit8 1.0.1		
	C		1	Qubit8 1.2.0		
So	ftware update is a	available		Oubit8 2.2.0		
An	ew version of software [1.1.1].	is available				
		14				
	Cancel	Update		Cancel	Up	date

- 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<sup>™</sup> Flex Fluorometer and requires the use of the Qubit<sup>™</sup> 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 1. On the Home screen, press Settings, then select System Verification. verification test



2. On the System Verification screen, press Next.



3. When prompted, set up three Qubit<sup>™</sup> Flex Tube Strips and label the tube strip lids 1–3.



- 4. Add 200 μL of Blank Reagent to each tube of tube strip #1, 200 μL of Green Fluorescence Reagent to each tube of tube strip #2, and 200 μL 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<sup>™</sup> Flex hardware test (Step 1 of System Verification). This test takes approximately 15 seconds.



6. When prompted, ensure that the Qubit<sup>™</sup> Flex Verification Assay is prepared, then press **Next**.



- 7. Insert tube strip #1 into the sample chamber, close the lid, then press **Read**.
- 8. 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).



System Verification Pass



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).

 System Verification
 System Verification

 20/09/2019 02:22:00 PM ✓ Saved
 20/09/2019 02:28:33 PM ✓ Saved

 System Verification
 Result

 Hardware Test
 PASS

 Red LED check
 PASS

 Photodiode check
 PASS

 Green Fluorescence Channel
 PASS

 Far Red Fluorescence Channel
 FAIL

 Data Log
 Close

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<sup>™</sup> 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<sup>™</sup> 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. Disconnect the Qubit<sup>M</sup> Flex Fluorometer from the power source.
  - 2. Remove the four screws (as indicated by the red arrows) on the bottom chassis of the Qubit<sup>™</sup> 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<sup>™</sup> microRNA, Qubit<sup>™</sup> RNA HS, and Qubit<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> assays are very sensitive and even small amounts of material from a previous sample may result in errors. Use a clean Qubit<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> assay kit you are using.
  - Ensure that the assay is performed entirely at room temperature.



- - For Qubit<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> working solution).



- Ensure that the standard and sample tubes are filled to 200 µL.
- Protect the Qubit<sup>™</sup> reagent and working solutions from light.
- Select the correct Qubit<sup>™</sup> Flex Fluorometer assay for the Qubit<sup>™</sup> 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<sup>™</sup> Assay considerations

How the Qubit<sup>™</sup> Flex Fluorometer calculates concentration The Qubit<sup>™</sup> Flex Fluorometer generates concentration data based on the relationship between the two standards used in calibration (three for the Qubit<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> RNA HS assay. Data for other Qubit<sup>™</sup> quantification assays are generated by similar algorithms.

**Incubation time** To allow the Qubit<sup>™</sup> 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<sup>™</sup> ssDNA assay, which is stable for up to 30 minutes.

The Qubit<sup>™</sup> 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<sup>™</sup> reagents The Qubit<sup>™</sup> DNA and protein exhibit high photostability in the Qubit<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> Flex Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, because the Qubit<sup>™</sup> 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<sup>™</sup> dsDNA BR assay. Qubit<sup>™</sup> dsDNA HS, Qubit<sup>™</sup> ssDNA, Qubit<sup>™</sup> RNA HS, and Qubit<sup>™</sup> protein assays show similar sensitivities over the same range.

#### Qubit<sup>™</sup> 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<sup>™</sup> Flex Fluorometer calculates concentration" (page 99) for an example of the calibration curve used to generate the quantification results.

# Appendix B: Ordering information

## Qubit<sup>™</sup> Flex Fluorometer and accessories

The following products can be used with the Qubit<sup>™</sup> 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 <sup>™</sup> Flex Fluorometer	1 each	Q33327
Qubit <sup>™</sup> Flex Quantitation Starter Kit	1 kit	Q45894
Qubit <sup>™</sup> Flex NGS Starter Kit	1 kit	Q45893
Qubit <sup>™</sup> Flex USB Flash Drive	1 each	Q46009
Qubit <sup>™</sup> Flex Wi-Fi dongle	1 each	A26774
Qubit <sup>™</sup> Flex Fluorometer International Power Supply (replacement)	1 each	A36204
Qubit <sup>™</sup> Flex Tube Strips	125 strips	Q33252
Qubit <sup>™</sup> Flex Reservoir (10 mL)	100 each	Q33253
Qubit <sup>™</sup> Flex System Verification Assay Kit	1 kit	Q33254
Qubit <sup>™</sup> RNA BR Assay Kit *20–1,000 ng*	100 assays 500 assays	Q10210 Q10211
Qubit <sup>™</sup> RNA HS Assay Kit *5–100 ng*	100 assays 500 assays	Q32852 Q32855
Qubit <sup>™</sup> ssDNA Assay Kit *1–200 ng*	100 assays	Q10212
Qubit <sup>™</sup> dsDNA BR Assay Kit *2–1,000 ng*	100 assays 500 assays	Q32850 Q32853
Qubit <sup>™</sup> dsDNA HS Assay Kit *0.2–100 ng*	100 assays 500 assays	Q32851 Q32854
Qubit <sup>™</sup> 1X dsDNA HS Assay Kit	100 assays 500 assays	Q33230 Q33231
Qubit <sup>™</sup> Protein Assay Kit *0.25–5 µg*	100 assays 500 assays	Q33211 Q33212
Qubit <sup>™</sup> microRNA Assay Kit *0.5–100 ng*	100 assays 500 assays	Q32880 Q32881
Qubit <sup>™</sup> dsDNA HS Assay - Lambda DNA Standard	5 mL	Q33233

