



FEB 21, 2024

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Protocol Citation: Nimalka Weerasuriya 2024. Qubit 4 Fluorometer (Common Peanut Lab). [protocols.io](#)
<https://protocols.io/view/qubit-4-fluorometer-common-peanut-lab-c78ezrte>

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Protocol status: Working
We use this protocol and it's working

Created: Jan 25, 2024

Last Modified: Feb 21, 2024

PROTOCOL integer ID: 94182

Qubit 4 Fluorometer (Common Peanut Lab)

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ABSTRACT

This protocol is to guide the use of the Qubit 4 Fluorometer in the Stillwater, OK, ARS USDA facility. The complete user guide is also available for download at thermofisher.com/qubit. This guide contains some additional information for Common Lab use and may not be applicable for everyone.

The Qubit 4 Fluorometer is a benchtop fluorometer that can be used for the quantitation of DNA, RNA, microRNA, and protein, as well as for the measurement of RNA integrity and quality using the highly sensitive and accurate fluorescence based Qubit assays. You can also use the Qubit 4 Fluorometer to directly measure the fluorescence of samples or to create new assays using the MyQubit software preprogrammed into the instrument.

GUIDELINES

Please ensure that all reagents have been stored appropriately and are not expired before running this protocol.

MATERIALS

Assay Tubes for the Qubit 4:

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

To Perform Assays:

- A Qubit assay kit appropriate for quantifying your samples (see page 61 of the full manual for available Qubit kits and ordering information)
- DNA, RNA, or protein samples
- Qubit assay tubes or other appropriate 0.5 mL assay tubes
- Appropriate standards for the assay
- Optional: USE drive or USB cable for data transfer

BEFORE START INSTRUCTIONS

To power up the Quabit, plug the power cord into the electrical outlet. It will power on and first display the splash screen, and then the Home screen.

Introduction

- 1 General Guidelines:
 - **Wear gloves during all sample handling.**
 - **Bring all kit reagents to room temperature** and insert all assay tubes into instrument only for as much time as it takes for the instrument to measure the sample.
 - **Do not hold the assay tubes in your hand before performing the measurement.**
 - **Incubate the DNA and RNA assay tubes for 2 minutes after mixing the sample or standard with the working solution.**
 - Incubate the protein assay tubes for 15 minutes after mixing the sample or standard with the working solution.
 - Incubate the Broad Range Protein assay tubes for 10 minutes after mixing the sample or standard with the Protein BR Assay Reagent.
 - **If you are performing multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.**

Note: multiple readings of RNA samples is not recommended.

- 2 From the Home screen, you can:
 - Select one of the assays: dsDNA, RNA, oligo (ssDNA), protein, or Ion Sphere™.
 - Select the Fluorometer mode (the instrument behaves like a mini-fluorometer).

- Access and export saved data.
- Configure instrument settings.
- Use the Reagent Calculator to determine the exact volumes of Qubit™ buffer and reagent required to prepare the Qubit™ working solution.

Reagent Calculator (Optional)

3 Skip this section if the kit comes with a bottle of Working Solution, or if you are using the Protein Broad Range Assay.

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

1. On the **Home** screen, press **Reagent Calculator**.
2. Enter the total number of samples and standards that you will be running. Press Enter.

Optional: Select **Include Overage** if you want to include an additional tube in your calculations in case of pipetting error.

The screenshot shows a mobile application window titled "Reagent Calculator". At the top, there are two input fields: "How many samples?" with the value "0" and "How many standards?" with the value "0". Below these is a checked checkbox labeled "Include overage [1 extra tube]". At the bottom is a numeric keypad with digits 1 through 9, a decimal point, and a "Clear" button ("x") on the left and an "Enter" button on the right.

Enter number of samples and standards required to run assays.

4 Prepare the Qubit Working Solution with the shown inputs.

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

Press **Done** to return to the **Home** screen to run the assay.

Preparing Samples

2m

5 Multiple kits can be used for one sample to determine purity:

- Use the dsDNA BR Assay Kit together with the RNA BR Assay Kit. These measurements give you a better indication of sample purity than that produced by measuring the A260/A280 ratio. To measure protein

contamination in nucleic acid samples, simply run 1–20 µL of the sample in the Qubit Protein Assay.

Kit Options:

1. **dsDNA Broad Range Kit:** The assay is highly selective for double-stranded DNA (dsDNA) over RNA and is accurate for initial sample concentrations from **100 pg/µL to 1000 ng/µL**.
2. **dsDNA High Sensitivity Kit:** The assay is highly selective for double stranded DNA (dsDNA) over RNA. Depending on sample volume, the assay is accurate for initial sample concentrations from **5 pg/µL to 120 ng/µL** providing an assay range of 0.1–120 ng.
3. **RNA BR Kit:** This assay is highly selective for RNA over double-stranded DNA (dsDNA) and is accurate for initial sample concentrations from **0.5 ng/µL to 1200 ng/µL**, providing an assay range of 10–1,200 ng. The RNA BR assay is intended for total **RNA, rRNA, or large mRNA**. For small RNA (~20 nt or bp), we recommend the microRNA Assay Kit (Cat. Nos. Q32880, Q32881).
4. **Protein BR Kit:** provides a quick and accurate method to quantitate protein samples over a broad range of concentrations for sample concentrations from **100 µg/mL to 20 mg/mL**.

The screenshot shows a mobile application interface titled "Reagent Calculator". At the top, there are input fields for "How many samples?" (set to 10) and "How many standards?" (set to 3). Below these is a checked checkbox labeled "Include overage (1 extra tube)". Under the heading "Results:", a message box contains the instruction: "Add 14µL dye to 2786µL buffer for a total volume of 2800µL". At the bottom right is a blue "Done" button.

Prepare your Working Solution as shown above in a separate sterile tube.

The kits include concentrated assay Reagent, dilution Buffer [or pre-mixed working solution] and two prediluted DNA standards.

- **If there is no pre-mixed working solution**, simply dilute the reagent using the buffer provided, add your sample (any volume from 1–20 µL is acceptable).
- The assays are performed at room temperature, and the signal is stable for 3 hours.

- 5.1**
1. Set up the required number of 0.5-mL tubes for standards (optional) and samples. Each assay requires 2 standards.
 2. Label the tube lids.

Note

Do not label the side of the tube as this could interfere with the sample read. Label the lid of each standard tube correctly. Calibration of the Qubit requires the standards to be inserted into the instrument in the right order.

- 5.2** *Optional:* Prepare the Qubit Working Solution (if it is not already provided) by diluting the kit's Reagent 1:200 in the kit's Buffer solution. Use a clean plastic tube each time you prepare working solution. **Do not mix the working solution in a glass container.**

Note

The final volume in each tube must be 200 μL . Each standard tube requires 190 μL of working solution, and each sample tube requires anywhere from 180–199 μL . Prepare sufficient working solution to accommodate all standards and samples.

For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 μL per tube in 10 tubes yields 2 mL of working solution (10 μL of Qubit reagent plus 190 μL of Qubit buffer).

5.3 (Optional) Standards:

1. Add $\text{pipette icon } 190 \mu\text{L}$ of working solution to each of the tubes used for standards.
2. Add $\text{pipette icon } 10 \mu\text{L}$ of each standard to the appropriate tube, then mix by vortexing 2–3 seconds.

Be careful not to create bubbles.

Note

Careful pipetting is critical to ensure that exactly **10 μL** of each Qubit standard is added to **190 μL** of working solution.

5.4 Samples:

1. Add working solution to individual assay tubes so that the final volume in each tube after adding sample is 200 μL .

Note

Your sample can be anywhere from $\text{pipette icon } 1\text{--}20 \mu\text{L}$. Add a corresponding volume of working solution to each assay tube: anywhere from $\text{pipette icon } 180\text{--}199 \mu\text{L}$.

- 5.5** 1. Add each sample to the assay tubes containing the correct volume of working solution, then mix by vortexing 2–3 seconds. The final volume in each tube should be **200 µL**.

Note

If you are adding 1–2 µL of sample, use a 2-µL pipette for best results.

1. Allow all tubes to incubate at **Room temperature** for **00:02:00 minutes**.

Calibrating New Standards (Optional)

- 6** For each assay, you have the choice to run new standards for calibrating the assay, or to use values from the previous calibration (for more information see page 60 in the manual).

On the Home screen, select the assay type. Swipe left or right to view all pages.

Note: The software displays the available assays for the assay type you selected.

Assay options:

- dsDNA (genomic or PCR products): High sensitivity or Broad range
- RNA Broad Range
- Protein Broad Range

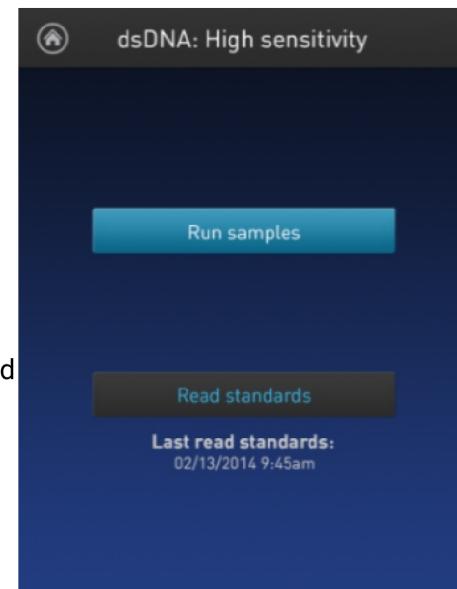
6.1

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

Note: To apply the previous calibration to your sample readings, press **Run samples**. The software prompts you to insert the assay tube containing the sample. See "Read Samples" section 8.

6.2 Prior to running samples

1. At the prompt, insert the prepared **Sample** into the sample chamber and press **Read standard**.



- 6.3** 1. At the prompt, insert the prepared **Sample** into the sample chamber and press **Read standard**.

2. For Qubit protein and RNA IQ assays only: At the prompt, insert Standard #3 into the sample chamber and press **Read standard**.
3. The calibration is complete and the software displays the results.

6.4

For Qubit quantitation assays

If the calibration is successful, the software displays the *Read standard* screen with the *Fluorescence vs. Concentration* graph.

The standard data points are connected by a line.

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



If the calibration is not successful, see Troubleshooting section.

Note

The Read standard screen displays raw fluorescence values for Standard #1 and Standard #2 (and Standard #3 if applicable). These values can assist in making judgements regarding the calibration results.

- For the dsDNA BR, dsDNA HS, ssDNA, microRNA, RNA HS, RNA BR, RNA XR and protein assays, the reading given by **Standard #2 should be at least 10x higher than Standard #1**.
- For the protein assay, the reading given by Standard #3 should be at least 40% higher than that of Standard #2.

If you receive a "Calibration error" message, see [go to step #15 Troubleshooting](#)

Read Samples

7 Before you begin:

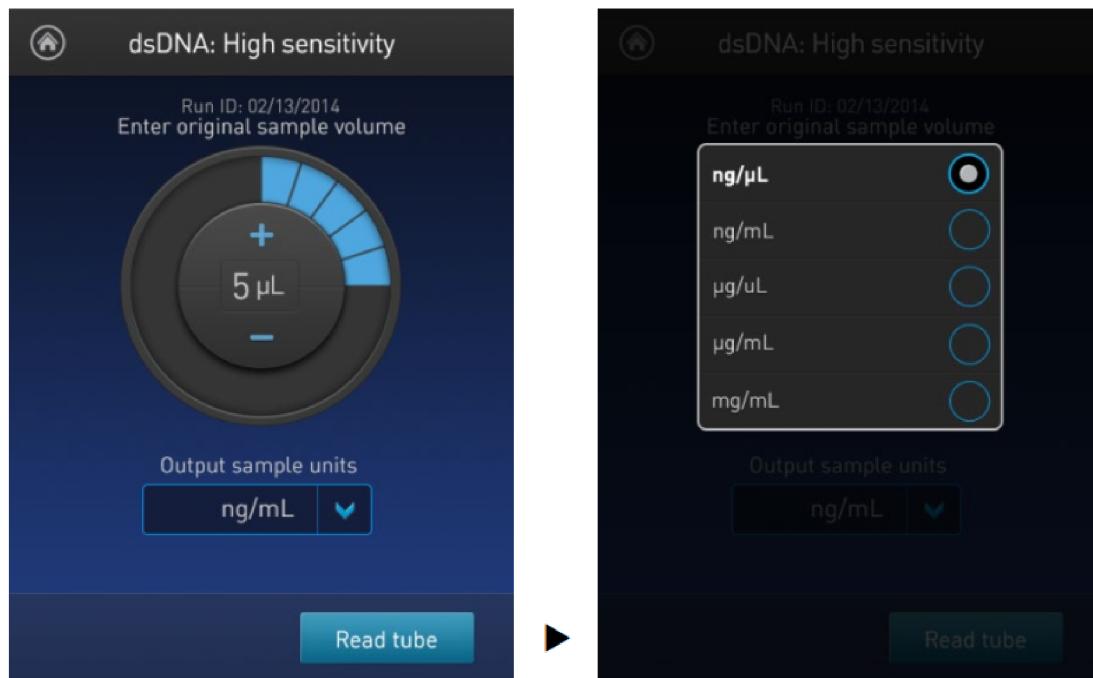
- Make sure you have either run new standards [Calibrating New Standards](#) or accepted values from the previous calibration.
- Prepare the samples. Refer to [go to step #5 Preparing Samples](#)

Note

Incubate the samples for the appropriate amount of time after mixing them with the Working Solution (2 minutes for DNA and RNA assays, 15 minutes for protein, or 10 minutes for the Protein Broad Range Assay).

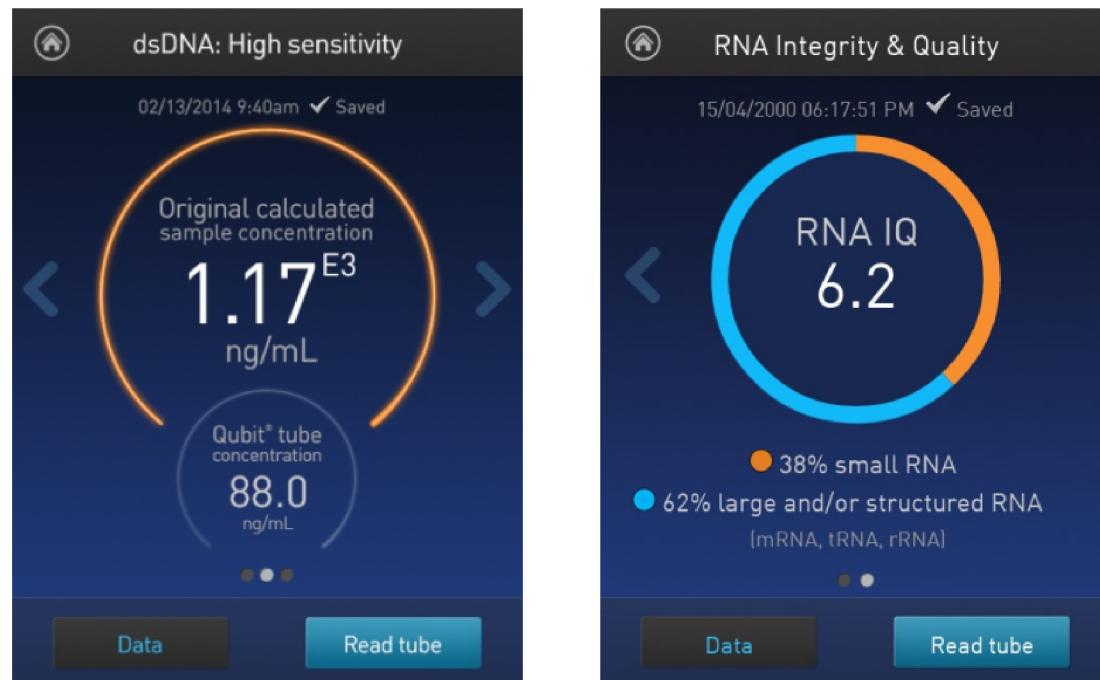
8

1. Press **Run samples**.
2. In the *Sample volume* screen, select the sample volume and units for the quantitation assays or the RNA IQ assay:
 - Press the + or - buttons to select the sample volume added to the tube (between 1 and 20 μL)
 - Select the units for the output sample concentration in the dropdown menu. Typical units are ng/uL for DNA assays.



Selecting sample volume and output units for quantitation assays.

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



8.2 To read multiple samples for the same assay:

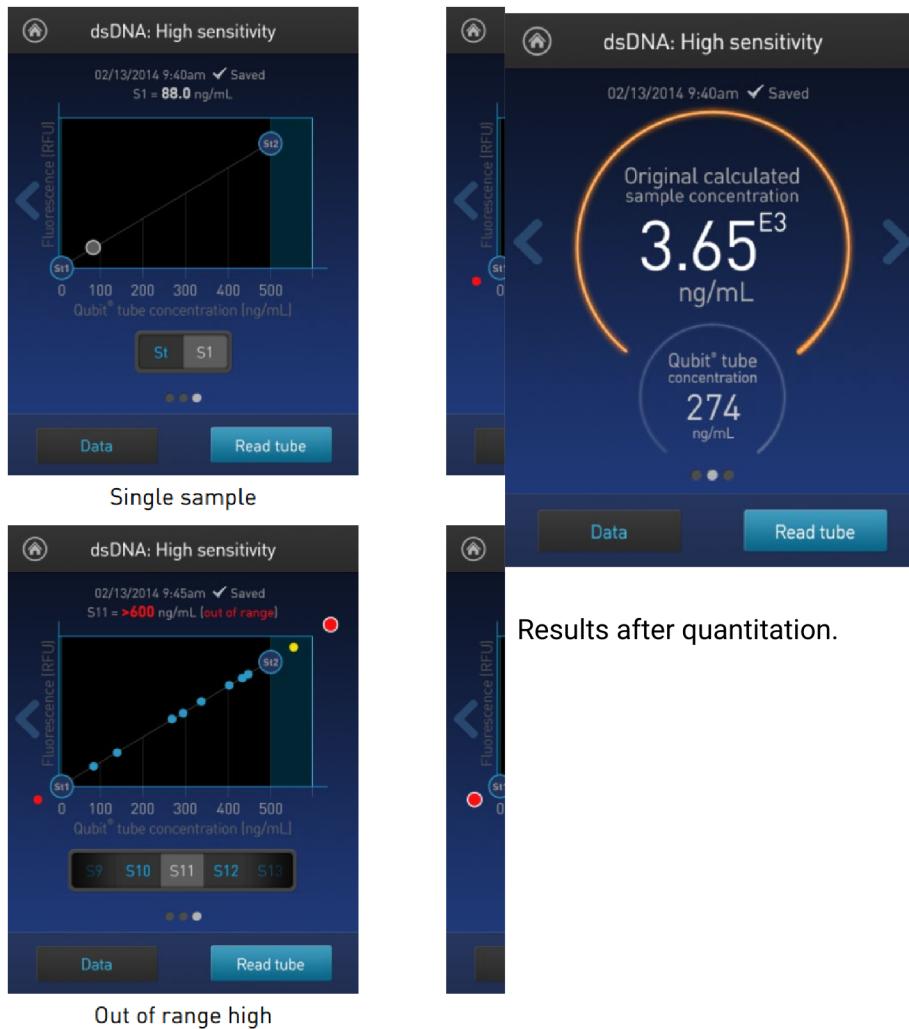
1. Remove the current sample, insert a new sample.
2. To change the sample volume, swipe **right** or press the **left arrow**.
3. Press **Read tube**.
4. Repeat step 2 and 3.

Results

9 The Results screen displays the results of the sample run.

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

10 To view the Fluorescence vs. Concentration graph, **swipe left** or press the **right arrow**. In the graph:



Fluorescence vs Concentration graph.

- Open circles represent the correct standards.
- The large gray circle represents the most recent sample.
- Blue circles represent samples that fall within the assay's core range.
- Yellow circles represent samples that fall within the assay's extended range.
- Red circles represent samples or standards that fall outside the assay's range.

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If the results are outside of the assay's range, an "Out of Range" message is displayed. See [go to step #15 Troubleshooting](#) for adjustments.

Managing Data

- 12 The Qubit can save data for up to 1000 samples. For the saved data, the instrument allows you to:
- View detailed data for each sample (manual page 32).
 - Rename data files (manual page 35).
 - Save data as a CSV or PDF file, and export to a USB drive (page 36).

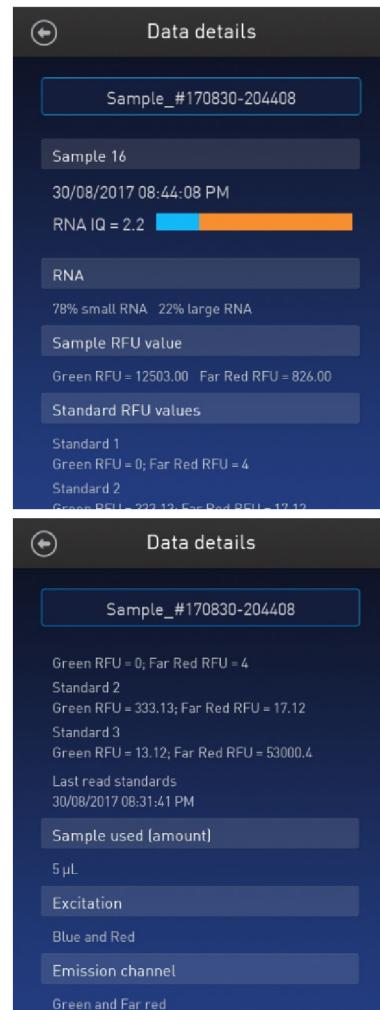
- Delete data files (page 40).

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1. Sample details can be seen by going to the **Home** screen, pressing **Data**.
2. On the Export data screen, press the **data set of interest**.
3. A *Data* screen displaying a list of data entries for that set appears. Scroll to view additional entries.
4. To view sample details, press the **sample of interest**. A *Data details* screen opens.



Data details for quantitation assays



Data details for quality assays



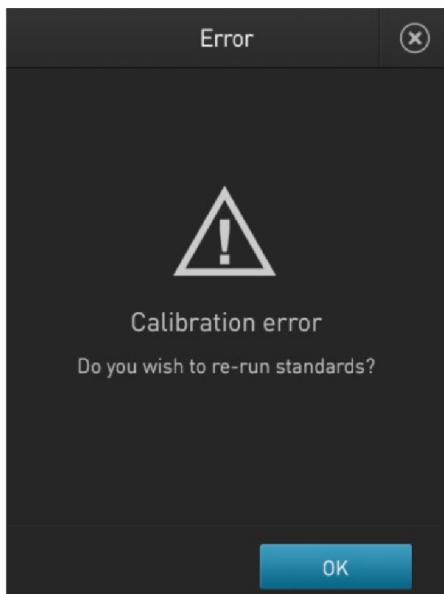
14

The Qubit can also be used as:

- a mini-fluorometer using the **Fluorometer** mode (see pages 29-30 in manual).
- **Ion Sphere Assay** for Ion Sphere Particles prior to a sequencing run on an Ion Personal Genome Machine Sequencer (see page 31)

Troubleshooting

15 Calibration error message:



1. In the Error screen, press **OK**.
2. Review the *Read standard* screen.
3. If you wish to re-run the standards

Calibration error screen.

[go to step #6.2 Running Standards](#) , or run new standards [Preparing Standards](#) , press **Read standards**, then repeat the calibration procedure.

16 Handling samples:

- For RNA tests, use appropriate RNAase-free handling techniques, including RNAase-free gloves, pipette tips, and tubes. Keep the tube lids closed whenever possible; do not press the pipette to the inside wall of the tube when withdrawing a sample. Return the RNA standards to -80°C as soon as possible.
- Ensure the assay tubes are at room temperature at time of reading. Do not hold them in your hand and do not leave them in the Fluorometer for longer than it needs to take a measurement.
- Use clean 0.5 mL PCR tube for each reading. Tubes must be dry on the outside. Moisture and condensation will lead to reading errors.
- Minute bubbles in samples will cause reading errors. Be sure not to introduce bubbles into samples. Slight tapping on the tube wall or brief centrifugation will often help dissipate bubbles.

17 Reading Out of Range (High):

- The sample is out of range. Use a sample that is less concentrated (for example, 10 µL in 190 µL instead of 20 µL in 180 µL)
- For quantitation assays, view the Fluorescence vs. Concentration graph in the Results screen to confirm that the values for the samples fall between the standards.
- Ensure that the lid is closed when reading standards and samples.
- Prepare samples and standards according to the assay kit you are using.

- Ensure that the assay is performed entirely at room temperature.

18 Reading Out of Range (Low):

- 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 quantitation assays, view the Fluorescence vs. Concentration graph in the Results screen to confirm that the values for the samples fall between the standards.
- Ensure that you have prepared the Working Solution correctly (1:200 dilution using kit buffer).
- Ensure that the standard sample tubes are filled to 200 µL.
- Protect the reagent and working solutions from light.
- Calibrate the standards again. Standards must be used in the correct order.
- Ensure that the assay is performed entirely at room temperature.



Sample High Reading

19 Critical Considerations:

- Allow for the appropriate incubation time.
- Prevent photobleaching of reagents.
- The temperature inside the Qubit may be as much as 3°C above room temperature after 1 hour. If you want to perform multiple readings of a single tube, remove the tube and wait 30 seconds before re-inserting.
- Assays were designed to be performed at 22-28°C and temperature fluctuations can influence the accuracy of the assay. Store all kit reagents at room temperature and do not hold tubes in your hand before a measurement.
- For each assay you can run a new calibration or use the values from previous calibrations. Calibration data may be affected by pipetting accuracy and temperature fluctuations.

20 System verification test:

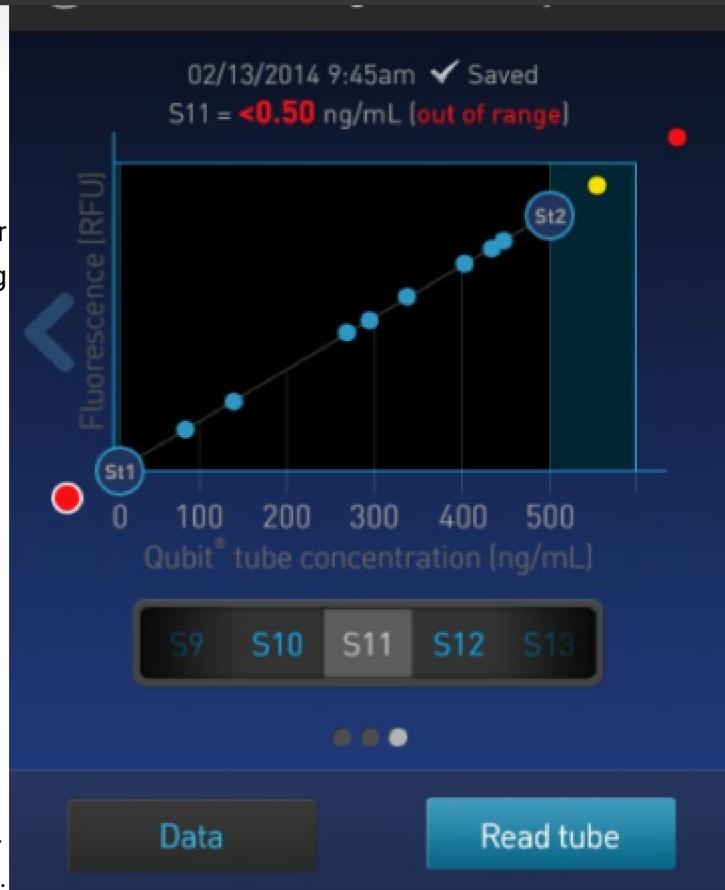
The system verification test checks the internal components and requires the use of the System Verification Assay Kit (Cat. no. Q33237). Perform the system verification when a problem with the instrument is suspected. It is not necessary to perform this regularly. See pages 55-56 in the manual.

Cleaning & Updates

21 The Qubit does not require regular maintenance.

To clean the fluorometer periodically:

- the touchscreen can be cleaned after disconnecting the power cable, using a soft cloth lightly moistened with LCD cleansing detergent.
- To disinfect, disconnect the power, and clean using a soft cloth lightly moistened with 70% ethanol, 70% isopropanol or 10% bleach.
- The cloth included with the instrument is only recommended for use with LCD detergent.



22 Software updates can be done by downloading the latest software to a USB drive from thermofisher.com/qubit.

- If using a USB, insert into instrument.
- On the Home screen, press **Settings**.
- Press **Update software**. The instrument will search the USB drive.
- When update files are detected, press **Update** to update the software.
- When prompted, **Restart** to complete the update.

Sample Low Reading

Read tube