CALIBRATION, USE, AND MAINTENANCE OF THE QUBIT 2.0 FLUOROMETER

1. SCOPE AND APPLICATION

1.1. This method is based on the Qubit 2.0 Fluorometer User Manual and uses the Qubit® dsDNA HS (High Sensitivity) Assay Kit. The assay is highly selective for double stranded (dsDNA). It can be used to determine the concentration of gBlocks® and the salmon DNA standard used for qPCR analyses.

2. SUMMARY OF METHOD

2.1. Interferences: Temperature fluctuations can affect measurement accuracy. Assay tubes should be read at room temperature. Do not hold assay tubes in your hand before reading a measurement or leave samples in the chamber longer than is necessary as this can raise the temperature of the assay tube.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation –

Instrument Name	Serial Number	Manufacturer	Catalog Number
Qubit 2.0 Fluorometer	1301008411	Invitrogen	Q32866

3.2. Equipment –

Equipment	Notes
Various pipettes	N/A
Fisher Mini Vortex	N/A
Fisher Mini Vortex	N/A
PCR#9, Thermo Refrigerator	S/N 7R1562211012; Maintained at 4 ± 2°C, Room B246A
Or Equivalent	

3.3. Supplies/Consumables –

Supply/Consumable	Notes
Gloves, Latex or nitrile	N/A
Qubit assay tubes (0.5 mL)	Invitrogen, Catalog Number Q32856
Tube rack	N/A
Filter tips, Sterile PCR grade	N/A
Or Equivalent	

4. REAGENTS AND STANDARDS

4.1. Qubit Reagents and Standards –

Reagents/Standards	Components	Notes
Qubit dsDNA HS kit, Catalog Number Q32851	Assay Reagent	Store at ≤4°C. When using on the bench, store in mini cooler
	Assay Buffer	Store at room temperature.

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	Assay Standard #1	Store at ≤4°C. When using on the bench, store in mini cooler
	Assay Standard #2	Store at ≤4°C. When using on the bench, store in mini cooler

5. PREPARATION OF THE QUBIT ASSAY AND CALIBRATION OF THE QUBIT FLUOROMETER

NOTE: This protocol provides instructions on calibration of the Qubit Fluorometer and determination of the concentration of the resuspended gBlock®. The standards used to calibrate the instrument are called Standard 1 (STD1) and Standard 2 (STD2) and are provided in the kit. The Qubit Fluorometer only reads concentrations ≥ 0.5 ng/mL. This equates to a concentration of approx. 7E7 TSC/5 µL for both gBlocks.

5.1. Set up of assay tubes –

Prior to starting, make sure all reagents are at room temperature. Place 11 Qubit tubes (included in the kit) in the standing rack in Lab A. Label one STD 1, one STD 2, and three tubes for each gBlock® dilution (1/10, 1/100 and E7*).

***NOTE:** The E7 solution will be prepared and tested after the determination of the gBlock concentration from the 1/10 and 1/100 solutions. To prepare the E7, use TE and the 1:100 dilution.

5.2. Preparing the working solution –

HS Buffer	199 x n µL
HS Reagent	1 x n µL

Mix the HS Buffer well by inverting the bottle 2–3 times and the HS reagent by tapping the bottom of the tube followed by a pulse spin. The ratio for the working solution is 1 µL of HS Reagent for every 199 µL of HS Buffer. To calculate the amount of working solution needed use the following formula: 1 x n µL HS Reagent and 199 x n µL HS Buffer, where n= number of standards and samples plus one.

For example, for 11 Qubit tubes n=12; thus, 12 µL of HS Reagent and 2,388 µL of HS Buffer is needed. Transfer the calculated amount of HS Buffer into a 15 mL falcon tube then add the HS reagent. This step will dilute the HS Reagent in the HS Buffer. Mix and vortex prior to use.

5.3. Prepare gBlock® dilutions –

Dilute the resuspended HFG2GD gBlock® in TE buffer (See table below) to measure in the Qubit fluorometer. Gently mix each dilution and pulse centrifuge to coalesce droplets.

Concentration	**1:10**	**1:100**
Stock Name	HFG2GD gBlock	1:10
Amount of Stock	10 µL	50 µL
Amount of TE Buffer	90 µL	450 µL

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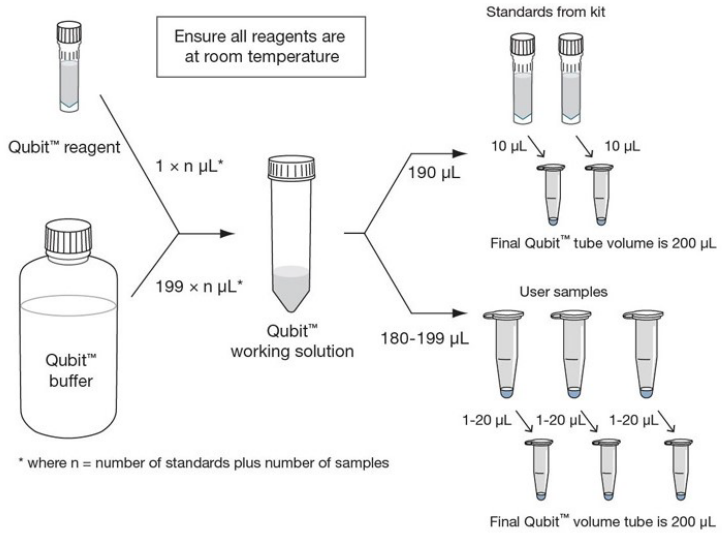
5.4. Add working solution, standards and samples to assay tubes –

The final volume of each Qubit assay must be 200 µL. Sample volume may vary between 1-20 µL, with the volume of the working solution varying between 180-199 µL. See the table below for typical volumes used.

Mix using the vortex for 2-3 seconds and centrifuge briefly.

	STD1 and STD2	1:10	1:100	E7
Working solution	190 µL	190 µL	190 µL	180 µL
Sample volume	10 µL	10 µL	10 µL	20 µL

5.4.1. Qubit Assay Summary –



5.5. Incubate the tubes at room temperature for 2 minutes

5.6. Plug in the Qubit Fluorometer –

On the Home screen, press **DNA**, and then select the **dsDNA High Sensitivity** assay.

5.7. Perform a new calibration in the Qubit Fluorometer –

Select **Yes** on the touch screen to run the calibration. When prompted, insert Standard 1 and select **Read**. Remove Standard 1, insert Standard 2 and select **Read**. When complete, the graph will display the standard points as open circles.

6. DETERMINATION OF THE GBLOCK CONCENTRATION

6.1. Insert the first sample assay tube and select **Read**. When reading is completed, select **Calculate Stock Concentration** and select the volume of sample added to the assay tube. The fluorometer will then calculate the concentration of the original sample. Select **Save**.

6.2. Repeat Step 6.1. until all 1:10 and 1:100 dilution samples are read. The graph will display each sample point as a black circle.

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6.3. After the final sample is read, read Standard 2 again. This serves as a bracketing QC check (See Section 9.1.). Select the units to change to ng/µL.

6.4. Once all samples and standards are run, select **Data**. Insert USB key to export the data in a spreadsheet format. Each sample name is listed as a timestamp. Keep track of the order of samples analyzed so the names can be matched with the timestamp once the data are exported. Determine the concentrations of the 1:10 and 1:100 dilutions in Excel.

6.5. Prepare and determine the concentration of the E7 dilution.

6.5.1. Use the formula $C_1V_1=C_2V_2$ to prepare an E7 dilution using TE buffer and the 1:100 dilution prepared in Section 5.3.

6.5.2. Add 20 µL of E7 and 180 µL of the working solution to the three assay tubes set up in Section 5.1. See Section 5.4. for details on the Qubit assay.

6.5.3. Mix using the vortex for 2-3 seconds and centrifuge briefly.

6.5.4. Incubate the tubes at room temperature for 2 minutes.

6.5.5. Using the previous calibration, read the three samples with the Qubit fluorometer, as in Section 6.1.

6.5.6. Export the data following Section 6.4.

6.5.7. Determine the concentration of the E7 in Excel. This is the concentration of the standard and will be used for quantification of samples in qPCR analysis.

7. DATA ARCHIVAL

7.1. To save the data, insert the Qubit 2.0 USB drive into the fluorometer USB port, select Data, then select the USB icon. The data are saved in a .CSV file and named with a timestamp. The USB icon should display a green dot if it recognizes the USB drive. A red dot is displayed when there is no USB drive inserted or if it is not recognized by the fluorometer.

7.2. Transfer the file to \\FLDEP1\DEAR\Labs\Lab3\Biology\qPCR\Qubit Data. Open the Excel file and rename the file and samples as appropriate then save as an .**xlsx** file. Once the file is transferred, clear the data from the Qubit and power off by using the power button.

8. MAINTENANCE

8.1. After use, disconnect the power cable from the fluorometer and wipe it down with a paper towel dampened with 10% bleach. Then wipe the instrument down with a paper towel dampened with sterile water. Immediately dry with a clean paper towel. Make sure to wipe the touch screen softly. Excessive force can damage the screen.

9. QUALITY CONTROL

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9.1. Bracketing QC Check: Standard 2 must be read after all samples have been measured as a bracketing QC check. The Relative Fluorescence Units (RFU) should not be more than 15% different from the original RFU amount.

9.1.2. If the initial and final check of Standard 2 are more than 15% different, recalibrate the fluorometer and reread all the samples. Once prepared, the standards are stable for 3 hours.

9.2. Monitoring of Standard Quality: The Qubit calibration is composed of two standards: STD1, isa blank, and STD2, which has a concentration of 10 ng/µL. The concentration of STD2 may deteriorate over time, resulting in inaccurate calculations of the concentration of the prepared gBlock. STD2 value should be 8.5-11.5 ng/µl (+/- 15% of the true value). If the difference is greater than +/- 15%, discard the standard and use a new kit.

10. SAFETY/HAZARDOUS WASTE MANAGEMENT

10.1. Discard all tubes in the hazardous waste bin and autoclave for 45 minutes before disposal.

10.2. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management.

10.3. Read MSDS information for reagent handling and disposal.

11. REFERENCES

11.1. Qubit 2.0 Fluorometer User Manual: Qubit 2.0 Fluorometer (Invitrogen, Catalog Number: Q32866

11.2. Qubit dsDNA HS Assay Kit (Invitrogen, Fisher Catalog number: Q32851)

11.3. FDEP Quality Manual 2023

11.4. Laboratory Health and Safety Plan

Appendix of Significant Changes

08/06/2015	Updated hyperlinks to manuals and data entry. Formatted for 508 compliance.
10/13/2017	Updated hyperlink to manual.
07/12/2021	Updated hyperlink to manual.
07/12/2022	Updated hyperlink to manual.
07/06/2023	Formatted SOP, added tables and detailed information in Sections 5 and 6. Added check of E7 dilutions. Added instructions for monitoring standard quality in Section 9.

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