Calibration, Use and Maintenance of 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. It can be used to determine the concentration of DNA in standards used for qPCR.

2. SUMMARY OF METHOD

   2.1 Interferences

      2.1.1 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 Qubit 2.0 Fluorometer (S/N 1301008411)

   3.2 Assay tubes, 0.5 mL PCR

4. REAGENTS AND STANDARDS

   4.1 dsDNA HS Assay Reagent

      4.1.1 Store at room temperature, away from light and humidity. Minimize light exposure as much as possible.

   4.2 dsDNA HS Assay Buffer

      4.2.1 Store at room temperature.

   4.3 dsDNA HS Assay Standard #1

      4.3.1 Store at ≤4ºC. When using on the bench, keep standard in mini cooler.

   4.4 dsDNA HS Assay Standard #2

      4.4.1 Store at ≤4ºC. When using on the bench, keep standard in mini cooler.

   4.5 Qubit working solution

      4.5.1 Prepare the working solution by diluting the HS reagent in the HS buffer (1 µL of HS reagent for every 199 µL of HS Buffer). Mix HS buffer well by inverting the bottle 2-3 times and mix HS reagent by tapping the bottom of the tube. Make enough solution for all the standards and samples. Each standard tube requires 190 µL of working solution, and each sample tube requires 180-199 µL, depending on sample dilution. To calculate amount of working solution to prepare use: 1 X n µL HS reagent and 199 X n µL HS Buffer, where n= number of standards plus number of samples. Mix in a PCR tube of appropriate size.

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5. CALIBRATION

5.1 Set up two assay tubes for the standards and a tube for each sample to be analyzed in triplicate. Label the lids with the name and dilution.

5.2 Add 190 µL of working solution to each standard tube.

5.3 Add 10 µL of each standard to the appropriate tube. Mix by vortexing for 2-3 seconds or tapping the bottom of the tube. Centrifuge briefly.

5.4 Incubate tubes at room temperature for 2 minutes.

5.5 Plug in the Qubit Fluorometer. On the Home screen, press **DNA**, and then select the **dsDNA High Sensitivity** assay.

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

6. MEASUREMENT

6.1 For each sample tube, add appropriate amount of working solution and sample for a total final volume of 200 µL. Sample volume can range from 1-20 µL. Mix by vortexing for 2-3 seconds or tapping the bottom of the tube. Incubate tubes at room temperature for 2 minutes.

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

6.3 Remove sample tube, insert the next sample and select **Read Next Sample**. When reading is completed, select **Calculate Stock Conc.** and select the volume of sample added to the assay tube. Select **Save**.

6.4 Repeat Step 6.3 until all samples are read. The graph will display each sample point as a black circle.

6.5 Select **Data** to display the samples 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.

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

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the data are transferred, clear the data from the Qubit and power off by selecting the power button.

8. MAINTENANCE

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

9. QUALITY CONTROL

9.1 Read standard #2 after all samples have been measured. The Relative Fluorescence Units (RFU) should not be more than 15% different from the original RFU amount. If it is more than 15% different, recalibrate the fluorometer and reread all the samples. Once prepared, the standards are stable for 3 hours.

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, Invitrogen Catalog No Q32866

11.2 Qubit dsDNA HS Assay Kits Product Insert

11.3 DEP Laboratory Quality Manual

11.4 Laboratory Health and Safety Plan

Appendix of 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.

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