Qbit 4.0 Fluorometer protocol using dsDNA BR broad spectrum assay kit (Q32853)

Set up

1. Obtain the Qubit dsDNA BR assay kit and allow components to come to room temperature (22-28°C). Standards #1 and #2 are kept in the refrigerator.
    a. dsDNA reagent (component A)
    b. dsDNA buffer (component B)
    c. Standard #1 (component C)
    d. Standard #2 (component D)

2. Obtain 0.5 mL Qubit tubes to use for each standard and each DNA sample to be quantified. Label the lids of tubes STD 1, STD 2, and sample identifier. Do not label the sides.

Preparation of working solution

1. Dilute the dsDNA BR reagent (component A) 1:200 in the dsDNA BR buffer (component B) in a clean plastic tube. Do not use a glass container.
    a. Prepare enough working solution for both standards and the amount of DNA samples being quantified. Total volume in each 0.5 mL Qubit tube will be 200 µL.
    b. Each standard tube requires 190 µL of working solution. Each sample tube requires 180-199 µL of working solution depending on the volume of DNA being quantified (1-20 µL).
        - Example for 2 DNA samples of 2 µL volume: (190 µL x 2 standards) + (198 µL x 2 samples) = 776 µL working solution. It is recommended to make a little more than required to account for error, in this case 800 µL would be sufficient.
            - *Note:* 800 µL working solution would be comprised of 4 µL dsDNA reagent and 796 µL of dsDNA buffer to obtain to 1:200 dilution ratio.

Preparation of samples and standards

1. To the 0.5 mL Qubit tube labeled STD 1, add 190 µL of working solution and 10 µL of the Standard #1 (component C) from the assay kit. Close the lid of the tube and spin for 2-3s to eliminate bubbles. Set aside and keep at room temperature.

2. To the 0.5 mL Qubit tube labeled STD 2, add 190 µL of working solution and 10 µL of the Standard #2 (component D) from the assay kit. Close the lid of the tube and spin for 2-3s to eliminate bubbles. Set aside and keep at room temperature.

3. To each 0.5 mL Qubit sample tube, add 180-199 µL of working solution and corresponding amount of DNA sample (1-20 µL). Close the lid and vortex 2-3s to eliminate bubbles. Set aside and keep at room temperature.
    - *Note*: Total volume in sample tube must be 200 µL.

4. Incubate all tubes at room temperature for 2 minutes.

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Reading Concentrations with Qubit

1. Plug in and turn on Qubit 4.0 Fluorometer.

2. Calibrate the Qubit using the standards

    a. From the initial home screen, select “DNA”, then select the assay type “dsDNA Broad Range”. Select the “Read Standards” option.

    b. Insert the tube STD 1 into the chamber. Close the lid and select “Read standard.” When reading is complete, remove the tube from the chamber and set aside.

    c. Insert the tube STD 2 into the chamber. Close the lid and select “Read standard.” When the reading is complete, remove the tube from the chamber and set aside.

    - *Note:* After becoming accustomed to using the Qubit, or if standards were recently read, the previous standard readings can be used as the calibration setting when collecting sample data in future uses.

3. Read the DNA samples for concentration values

    a. From the current screen (post calibration), select “Run samples”. Select the sample DNA volume (1-20 µL) using the +/- options and the units (ng/µL) from the dropdown menu.

    b. Insert the DNA sample tube into the chamber. Close the lid and select “Read standard.” When reading is complete, remove the sample tube from the chamber and set aside. Record the concentration in ng/mL.

    c. Repeat the previous step with remaining DNA sample tubes.

4. When complete and all samples have been quantified, select the home screen option in the top left-hand corner of the screen. Unplug the Qubit 4.0 Fluorometer and return to storage. Dispose of all tubes. Return assay kit components to proper storage.

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