Qubit Protocol

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Materials Needed:

Qubit Kit
Qubit Assay Tubes
Falcon Tube
Qubit Fluorometer
DNA or RNA Samples

IMPORTANT NOTE: The Qubit reagent is sensitive to light, so make sure that it is not exposed to light until you are ready to use it.

1. To make the master mix pipette 199 µL of Qubit buffer and 1 µL of Qubit reagent per sample into a Falcon tube, vortex for 15 seconds, and then place into a dark environment (e.g. a closed drawer). Note: You need to make enough master mix for all your samples AND the two calibrations AND two standards, while also accounting for pipetting error.
   (a) 6 DNA/RNA samples + 2 calibrations + 2 standards = 10 samples total
   (b) 199 µL of Qubit buffer x 10 samples x 1.1 = 2189 µL of total Qubit buffer
   (c) 1 µL of Qubit reagent x 10 samples x 1.1 = 11 µL of total Qubit reagent
2. Pipette 1 µL of DNA/RNA per sample into a Qubit assay tube and then add 199 µL of the master mix into each Qubit assay tube.
3. Prepare the two calibration tubes and two standard tubes:
   (a) Calibration 1 = 190 µL of the master mix + 10 µL of Standard 1
   (b) Calibration 2 = 190 µL of the master mix + 10 µL of Standard 2
   (c) Standard 3 = 195 µL of the master mix + 5 µL of Standard 2
   (d) Standard 2 = 199 µL of the master mix + 1 µL of Standard 2
4. Vortex each Qubit assay tube for 15 seconds and then flick the tube to ensure that all the liquid is uniformly at the bottom of the Qubit assay tube.
5. Select the setting on the Qubit fluorometer that reflects the Qubit kit.
6. Run calibrations one and two on the Qubit fluorometer.
7. Run standards three and four on the Qubit fluorometer correcting for the volume of standard two in each solution. Note: The readings for standards three and four should be 10 µg/mL +/- 5 µg/mL.
8. Run your samples on the Qubit fluorometer correcting for the volume of DNA/RNA (1 µL) in each sample.