Qubit Fluorometer Protocol

For use with the Invitrogen Qubit Fluorometer.

Note: Qubit buffer is light sensitive. Store the buffer in a dark place, and avoid leaving it out for long periods of time. The longer the buffer is exposed to light, the less accurate the Qubit readings become.

Qubit assays are also temperature sensitive, and temperature fluctuations can influence the accuracy of the assay. If Qubit buffer is refrigerated, allow time for the buffer to come to room temperature before performing a reading.

Equipment:
→ QuBit fluorometer
→ Tube Centrifuge
→ Vortexer
→ P10, P20, P200 pipettes

Consumables:
→ QuBit assay tubes
→ Pipette tips: P20, P200

Reagents:
→ Qubit High Sensitivity DNA Kit

Protocol:

1. In a **clean area**, label one Qubit Assay tube per sample that needs to be measured, plus one tube each for Standard 1 (“S1”) and Standard 2 (“S2”). Avoid labeling the side of the assay tube (label the top instead), since this could interfere with optical readings.
2. Add 190µl of Qubit Buffer to the tube labeled “S1” and 190µL of Qubit Buffer to the tube labeled “S2”.
3. Add 199µl of Qubit Buffer to each sample tube.
4. Add 10µl of Standard 1 to the tube labeled “S1” and 10µl of Standard 2 to the tube labeled “S2”. Vortex both tubes and spin down.
5. Turn on the Qubit Fluorometer and select *dsDNA* > *dsDNA High Sensitivity* > *Read Standards*
6. Insert the tube labeled “S1”, close the lid, and select *Read Standard*. Once the reading is complete, replace “S1” with the tube labeled “S2”, close the lid, and select *Read Standard*. Once this is complete, the fluorometer is calibrated. You should perform this calibration once each day you use the Qubit.
7. Take the sample tube(s) with buffer added to the **post-PCR area** and add 1µl of sample DNA to each corresponding tube.
8. Insert the first sample tube into the Qubit Fluorometer, select *Run Samples*, set sample volume as 1µl and output units as ng/µl. Select *Read Tube* and record the results. Repeat for all samples.