

Bracken-Grissom Lab Qubit Protocol

Materials:

- xDNA samples
- Qubit sample tubes
- Qubit dsDNA HS Assay kit
 - Qubit dsDNA HS Buffer
 - Qubit dsDNA HS Reagent Concentrate in DMSO
 - Qubit dsDNA Standards (1 & 2)
- Qubit Machine
- Pipettes
- Pipette Tips
- Qubit sample tube tray
- 15mL Falcon Tube

Protocol

1. Remove Qubit dsDNA HS Standards from refrigerator. Bring to room temperature.
(Bottom left drawer in fridge)
2. Procure 1 Qubit sample tube for every xDNA sample to be tested, and the 2 standards.
(On the wall shelf opposite the Qubit machine)
3. Label each tube sufficiently to correspond with the xDNA samples and the 2 standards. Use Qubit sample tube tray to hold the sample tubes
4. From the Qubit dsDNA HS Assay Kit, create a mastermix solution using the 15mL Falcon Tube and the dsDNA HS Buffer and Reagent. Calculate the volume of each by the following formulas:

$$\text{dsDNA Buffer} = 199\mu\text{L} \times (\# \text{ of xDNA samples} + 3)$$
$$\text{dsDNA Reagent} = 1\mu\text{L} \times (\# \text{ of xDNA samples} + 3)$$
5. Vortex mastermix thoroughly.
6. Aliquot 190uL of mastermix into each Qubit Standard sample tube.
Aliquot 198uL of mastermix into each Qubit xDNA sample tube.
7. Aliquot 10uL of each standard into each Qubit Standard sample tube.
Aliquot 2uL of each xDNA sample into each Qubit xDNA sample tube.
8. Thoroughly vortex each Qubit sample tube.

9. Incubate for 2 minutes at room temperature.
10. Turn on Qubit machine by touching the screen.
11. Select DNA > dsDNA High Sensitivity.
12. You will be asked if new standards should be run. Select "Yes."
13. Open black cap and insert **Standard 1 sample tube**. Select "Read."

When prompted, remove Standard 1 and insert **Standard 2 sample tube**.
Select "Read."

14. Remove Standard 2 sample tube. Insert first **xDNA sample tube**. Select "Read."

A value will be presented in ng/mL. After performing Step 15 for the first xDNA sample, every other sample concentration can be recorded in ng/uL by simply moving the decimal point of this output to the left 1 decimal place.

15. Select "Calculate Stock Conc." Set "Volume of Sample Used" to 2uL, and ensure the units of measure are in ng/uL. Record the presented value as your xDNA concentration
16. Remove sample, and insert next xDNA sample. Select "Read Next Sample"
17. Repeat steps 14 and 16 (Omit step 15) for every remaining sample.
18. Upon completion:
 - dispose of Qubit sample tubes in trash
 - rinse mastermix falcon tube with DI water and reuse when next performing Qubit analysis
 - Return Qubit standards to fridge
 - Qubit machine will automatically turn off after a period of time.

END