1. Formulate Qubit Working Solution Formulation

NOTE:	The **Qubit dsDNA HS Assay** is a useful tool for quantifying cfDNA samples after **cfDNA Extraction**, whereas the **Qubit dsDNA BR Assay** is more appropriate for quantifying pools after **Barcoding**. Please see the Qubit: dsDNA Broad Range Quantitation bench worksheet for the latter procedure.
NOTE:	Qubit dsDNA HS Assay reagents are very sensitive to light--keep in the dark whenever possible. Qubit Reagent and Buffer are stored at Room Temperature in the dark. Qubit Standards are stored at 4 °C in the dark.
____ 1.	Warm Qubit HS Standards to room temperature (15°C to 25°C) protected from light for 30 minutes.
	Start time: _____ End time: _____
____ 2.	Vortex cfDNA sample tubes for 5 seconds and centrifuge for 5 - 10 seconds.
____ 3.	Vortex Qubit dsDNA HS Buffer and Qubit dsDNA HS Standards 1 and 2.
____ 4.	Label two Qubit assay tubes for standards "S1" and "S2."
____ 5.	Label each Qubit sample tube <Sample ID>
____ 6.	Prepare Qubit Working Solution in a LoBind 1.5 mL microcentrifuge tube according to the following formulation:

Reagent	µL/sample	x	Total # of samples (incl. 2 standards)	+ 20% Overage			Total µL
Qubit dsDNA HS Buffer	195-199	x		x	1.2	=	
Qubit dsDNA HS Reagent	1-5						

____ 7.	Vortex Qubit Working Solution for 5 seconds and centrifuge for 5 - 10 seconds.

2. Prepare Qubit Standards and Samples

____ 1.	Dispense 190 µL of Qubit Working Solution into each Qubit standard tube.
____ 2.	Dispense 199 µL of Qubit Working Solution to each Qubit sample tube.
____ 3.	Dispense 10 µL of Qubit dsDNA HS Standard 1 into the S1 tube and 10 µL of Standard 2 into the S2 tube.
____ 4.	Dispense 1 µL of each sample to its respective Qubit sample tube.
NOTE:	Ensure that bubbles are not introduced to any of the standards or samples.
NOTE:	Hold the Qubit assay tubes from the top, as temperature fluctuations will affect the fluorescence measurements.
____ 5.	Vortex for 5 seconds, then centrifuge for 5 to 10 seconds.
____ 6.	Confirm that each Qubit standard/sample tube is free of bubbles and particulate, or if present, remove them.
____ 7.	Incubate at room temperature (15°C to 25°C) in the dark for 5 minutes.
	Start time: _____ End time: _____
NOTE:	Do not store Qubit assay samples or standards for more than 3 hours.

3. Measuring Qubit Standards and Samples	
____ 1.	Turn on Qubit (eqpt. ID:_____), press the **Home** button, and select the substance you wish to measure (e.g. **DNA**).
____ 2.	Select the **dsDNA HS** quantitation kit.
____ 3.	Select **Yes** to **Read New Standards?**
____ 4.	Load the S1 tube, close the lid and press **Read Sample**; repeat for Standard 2.
____ 5.	Press **Check Standards** and record results for each standard on the chart below.
____ 6.	Select **No** to **Read New Standards?**
____ 7.	Load the <Sample ID> tube, close the lid and press **Read Sample.**
____ 8.	Press **Stock Concentration**, adjust **Sample Volume** to 1 **µL**; adjust **Concentration** to **ng/µL**, and record result on the chart below.

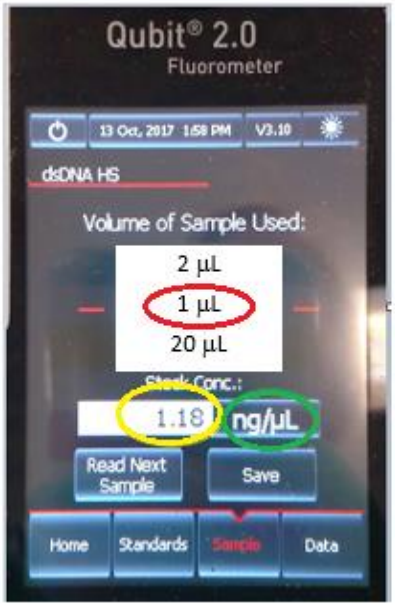
Sample ID:	**Stock Conc. (ng/µL)**
Std 1	
Std 2	

Sample ID:	**Stock Conc. (ng/µL)**

Qubit 2.0 Screen Examples

- After inserting a sample tube and clicking “Read Next Sample”, below is what the Qubit screen will look like. This is the Raw Qubit value (circled in orange)
- To calculate the ng/µL stock concentration click “Calculate Stock Conc.” (circled in green)

- After clicking “Calculate Stock Conc.”, the screen will look as below
- Adjust “Volume of Sample Used” (circled in red) to 1 µL. 1 µL should line up the red dashes on the sides.
- Select ng/µL as the units (circled in green)
- Screen will now display your ng/µL concentration (circled in yellow)



Qubit 3.0 Screen Examples

- Before inserting a sample, below is what the screen will look like
- Select 1 µL as Sample Volume (green box)
- Select ng/µL as Output Sample Units (yellow box)
- Insert Sample tube and Select "Read tube"

- Qubit concentration in ng/µL will be shown in the bottom circle (red box)