

AccuGreen High Sensitivity dsDNA Quantitation Instructions

ALL HIGH SENSITIVITY dsDNA COMPONENTS ARE MARKED WITH YELLOW LAB TAPE.

1. The AccuGreen High Sensitivity dsDNA Quantification Kit and assay tubes are in the drawer labeled “Biotium”. The AccuGreen Standard 1 (0 ng/μl) and AccuGreen Standard 2 (10 ng/μl) are in the refrigerator. Both standards are labeled “HS” on the top and marked with yellow tape.



2. Warm all components to room temperature before starting.
3. Prepare the working solution by diluting the AccuGreen Dye (200X) 1:200 with DNA Quantitation Buffer. Calculate the amount of working solution you will need. Include 10% overage.

Calculate amount of DNA Quantitation Buffer needed and add to appropriately sized tube:
[199 μl x (# of samples + 2 standards)] x 1.10 = volume Quantitation Buffer

Calculate amount of AccuGreen Dye (200X) needed and add to Quantitation Buffer from above:
[1 μl x (# of samples + 2 standards)] x 1.10 = volume AccuGreen Dye (200X)

Vortex tube containing working solution to thoroughly mix.

4. Label assay tubes. You will have one tube for each sample, one tube for Standard 1, and one tube for Standard 2.
5. For Standard 1 (0 ng/μl) and Standard 2 (10 ng/μl), add 190 μl of working solution to each tube. Then add 10 μl of the respective standard to each tube.
6. For each sample, add 199 μl of working solution to the assay tube. Then add 1 μl of sample.
 - a. If needed, a higher volume of sample can be used to obtain a measurement. For example, if using 2 μl of sample, then reduce the working solution to 198 μl.
7. Mix samples thoroughly by vortexing.
8. Incubate samples at room temperature for 2 minutes. Use timer that is available.
9. On the Qubit instrument, press **GO** to turn on. Use the arrows to navigate to **Quant-iT dsDNA, HS** then press **GO**.
10. Run a new calibration following the instructions on the screen.
11. Insert sample assay tube into Qubit and close the lid. Press **GO** to measure the sample. Arrow down to **Calculate sample concentration**, press **GO**, use the down arrow to select the volume of sample added to the assay tube (typically 1 μl), then press **GO**. The concentration of your sample will be displayed on the screen. Manually record the sample concentration.
12. Repeat step 11 until you have measured the concentration of all samples.
13. When finished, turn off the Qubit. (Press **HOME**, use the arrows to navigate to **Power OFF**, then press **GO**).
14. Record your Qubit usage on the Qubit log on the table by the door. Include the standards with the number of samples that you measured.