

Southeast Center for Integrated Metabolomics Clinical and Translational Science Institute

Title: Appendix C

Protein & DNA quantification using Qubit Analyzer

Date Effective: 04/09/15

Materials needed:

- Protein or DNA Kit (Life Technologies)
- 0.5 mL PCR tubes
- Qubit 2.0 Fluorometer

Protein or DNA quantification:

- 1. Set up 3 assay tubes (for standards) and 1 assay tube for each sample that will have quantification performed. Make sure labeling is on lid- as fluorometer reads through the walls of the tube.
- 2. Prepare Qubit working solution by making a reagent:buffer solution at a 1 μ L:200 μ L ratio. Keep in mind that 200 μ L of this working solution is needed for each standard and sample.
- 3. When making standards, mix 190 μL of working solution, prepared in step 2, and 10uL of standard (which come with the kit.) Perform for each of the standards.
- 4. Vortex standards for 2-3 seconds and incubate for 5 minutes at room temperature if performing DNA quantification. If performing protein quantification, incubate for 15 minutes at room temperature before obtaining reading(s).
- 5. Select Protein Assay or DNA Assay on the Qubit 2.0 Fluorometer. Place standards in fluorometer and select "read standard". Repeat this for all standards.
 - 5.1. Once standards are made and read, the Qubit stores that information.
 - 5.2. If your study needs fresh standards, go ahead and make them. If your study does not need fresh standards, you can use the information that is stored in Qubit.
- 6. Prepare assay tubes for samples by adding 180 μ L-199 μ L of working solution and mixing 1 μ L 20 μ L of sample. The total volume of working solution and sample should equal 200 μ L.
- 7. Vortex samples for 2-3 seconds and incubate for 5 minutes at room temperature if performing DNA quantification. If performing protein quantification, incubate for 15 minutes at room temperature before reading.
- 8. Select Protein Assay or DNA Assay on the Qubit 2.0 Fluorometer. Place samples in fluorometer and select read sample. Repeat this for all samples.