

## SOP Title: Qubit Assay for Nucleic Acid Quantification

### 1.0 Purpose and scope

This SOP is for the quantification of nucleic acids including extracted and prepared sequencing libraries.

Related documents: [User Guide: Qubit dsDNA HS Assay Kits](#)

### 2.0 Materials

<i>Workspace</i>		
Library prep or extraction room for all the steps prior to PCR amplification		
Post-PCR area for PCR amplified products and all the subsequent steps		
<i>Equipment and associated consumables</i>		
ThermoFisher	Qubit 3.0 Fluormeter	Q33216
	Qubit RNA HS Assay Kit (5-100 ng/ul)	Q32852
	Qubit RNA BR Assay Kit (20-1000ng/ul)	Q10210
	Qubit dsDNA HS Assay Kit (0.2-100 ng/ul)	Q32854
	Qubit dsDNA BR Assay Kit (2-1000ng/ul)	Q32853
	Qubit Assay Tubes	Q32856

### 3.0 General Guidance

There are 4 main Qubit kits available from ThermoFisher. Each kit has a different dye and buffer component. Dyes are stored in a light protected box in a lab drawer. Buffers and standards are stored in the 4C fridge with the exception of the RNA kit, stored on the RNA bench, with dye in the drawers below the bench. Use Qubit assay tubes (Q32856).

Ideally, use High Sensitivity (HS) kits for DNA (0.2-100 ng/ul) and RNA (0.2-100 ng/ul) quantification. If samples are too concentrated the qubit may “error” and the stock DNA/RNA may need to be diluted. Never use broad range values for calculating input material for library prep. All steps are at room temperature. Broad range kits may be used to estimate an appropriate dilution for a DNA or RNA stock. Only use high sensitivity kits when submitting material to PM-OICR TGL for generation of sequencing libraries. Ideally, target stock concentrations of 10ng/ul for DNA, or 10-20 ng/ul for RNA; higher concentrations are acceptable. See our [website](#) for recommended submission amounts for DNA and RNA for your NGS assay. Please indicate if samples submitted to PM-OICR TGL are not quantified by Qubit or other fluorescence based method. Nanodrop is not a suitable method for nucleic acid quantification. Old quantification values may not be accurate if they were recorded >6months from date of submission, ideally verify stocks before submission. Stock DNA and RNA samples submitted to PM-OICR TGL will be verified with High Sensitivity assays prior to library synthesis.

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Approved by:	<b>Dax Torti</b>	
Date:	170523	

Use one dye buffer master mix for all quant, and/or make extra for repeat quantifications. If you do not have sufficient dye/buffer mix left for a repeat, you must also remake the standards again and create a new quant curve on the qubit, never use stored standard curves.

#### 4.0 Procedure

There are 2 standards for each kit, a 0 ng/ul and x ng/ul concentration.

- 1) Make a dye buffer mix that will be used with all standards, and all samples.
  - i. Each sample will require 199 ul of buffer/dye mix
  - ii. Each standard will require 190 ul of buffer/dye mix
  - iii. Calculate a total number of mixes required. For example, 18 samples +2 standards, 20 wells total, Make 1.15X more wells than required (23 wells)
  - iv. Make the following amount of dye buffer mix:
    - 1ul of kit specific dye
    - 200ul of kit specific buffer
 \*For 23 wells this would be 4600ul of buffer, 23 ul of dye, Vortex well, always make a master mix solution, do not make individual dye/mixes
  - v. Add 1 ul of sample to 199 ul of dye/buffer mix in qubit tube, vortex
  - vi. Add 10 ul of standard 0 ng/ul or standard X ng/ul to 190 ul of dye buffer mix in qubit tube, vortex
  - vii. Incubate samples for a minimum of 2 minutes, vortex again, and tap down before loading in Qubit reader
- 2) From the home screen select dsDNA or RNA Broad or High Sensitivity assays.
- 3) Select kit specific assay, and follow instructions on touch screen interface for standard calibration and sample quantification. If samples read as “out of range” they may be too concentrated or too diluted and may need to be re-diluted or more material requested.
- 4) When all assays are complete return to the Qubit home screen and select export data. Highlight all samples for export and press export icon. Data is exported as a CSV file. Open the log file on Qubit virtual drive on the connected PC. Copy the complete text file output into sample tracking sheet and associate specific sample names to txt output.

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