

# AccuBlue Broad Range RNA Quantitation Instructions

**ALL BROAD RANGE RNA COMPONENTS ARE MARKED WITH RED LAB TAPE.**

1. The AccuBlue Broad Range RNA Quantification Kit is in the refrigerator. The assay tubes (0.5 ml tubes) are in the drawer labeled “Biotium”. The RNA Dilution Buffer (0 ng/μl), which is standard 1, is in the refrigerator. The RNA BR Standard 2 (100 ng/μl) is in the freezer.



2. Warm all components to room temperature before starting.
3. Prepare the working solution by diluting the RNA Broad Range Dye (200X) 1:200 in RNA Broad Range Buffer. Calculate the amount of working solution you will need. Include 10% overage.  
Calculate amount of working solution needed and add to appropriately sized tube:  
 $[199 \mu\text{l} \times (\# \text{ of samples} + 2 \text{ standards})] \times 1.10 = \text{volume RNA Broad Range Buffer}$   
Calculate amount of RNA Broad Range Dye needed and add to RNA Broad Range Buffer from above:  
 $[1 \mu\text{l} \times (\# \text{ of samples} + 2 \text{ standards})] \times 1.10 = \text{volume RNA Broad Range Dye (200X)}$   
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 RNA Dilution Buffer (0 ng/μl) (i.e. standard 1) and RNA BR Standard 2 (100 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, reduce the working solution to 198 μl.
7. Mix standards and 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 RNA, BR** 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.