**Qubit Broad Range RNA Quantification Protocol**

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1. Thaw Qubit Standards 1 and 2 at room temperature for 30-60min.
2. Label the required number of 0.5 mL Qubit tubes, including 2 tubes for standards.
3. Prepare the Qubit working solution by diluting the BR Reagent 1:200 in Buffer. To calculate the total volume required, multiply the number of tubes by 200 µL. For 24 samples (a full round of extractions), add 27 µL reagent to 5373 µL buffer.
4. Optional: Use the **Reagent Calculator** on the Qubit 4 to determine the Qubit working solution dilution and total volume.
5. Add 190 µL of Qubit working solution to each of the 2 standard tubes.
6. Add 10 µL of each Qubit standard to the respective tube, then mix by vortexing 2-3 sec.
7. Add 198 µL of Qubit working solution to each of the sample tubes.
8. Add 2 µL of each sample extract to the respective tube, then mix by vortexing 2-3 sec.
9. Incubate all tubes at room temperature for 2 min.
10. On the Qubit, select the **assay type** (RNA: Broad range).
11. Select **Read standards**, insert Standard 1, the select Read standard. Repeat for Standard 2.
12. Select **Run samples**, select a sample volume of 2 µL, and set the output concentration to ng/µL.
13. Insert a sample tube and select **Read tube**. The screen will display the original concentration, as well as the diluted concentration. Repeat for all remaining samples.
14. Wait 30-60 min, then repeat steps 11-14, for a total of 3 sample runs.
15. With the USB inserted, select **Data**, then select and **Export** the datasets.

**Qubit Tube Prep:**

0.5 mL Qubit assay tubes (+2 for standards)

15 mL Falcon tube for Qubit working solution