NOTE: For best results, store the dye and the buffer at room temperature. Store the DNA, RNA, and protein standards at 4°C. Ensure that all assay reagents are at room temperature before you begin.

1. Set up two Assay Tubes for the standards (three for the protein assay) and one tube for each user sample.

2. Prepare the Qubit™ Working Solution by diluting the Qubit™ reagent 1:200 in Qubit™ buffer. Prepare 200 μL of Working Solution for each standard and sample.

3. Prepare the Assay Tubes* according to the table below.

<table>
<thead>
<tr>
<th></th>
<th>Standard Assay Tubes</th>
<th>User Sample Assay Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of Working Solution (from step 2) to add</td>
<td>190 μL</td>
<td>180–199 μL</td>
</tr>
<tr>
<td>Volume of Standard (from kit) to add</td>
<td>10 μL</td>
<td>—</td>
</tr>
<tr>
<td>Volume of User Sample to add</td>
<td>—</td>
<td>1–20 μL</td>
</tr>
<tr>
<td>Total Volume in each Assay Tube</td>
<td>200 μL</td>
<td>200 μL</td>
</tr>
</tbody>
</table>

* Use only thin-wall, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit® assay tubes (set of 500, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830).

4. Vortex all tubes for 2–3 seconds.

5. Incubate the tubes for 2 minutes at room temperature (15 minutes for the Qubit™ protein assay).

6. Insert the tubes in the Qubit® 2.0 Fluorometer and take readings. For detailed instructions, refer to the Qubit® 2.0 Fluorometer manual.

7. Optional: Using the Dilution Calculator feature of the Qubit® 2.0 Fluorometer, determine the stock concentration of your original sample.
Qubit™

Reagent

Qubit™

Buffer

Qubit™

Working

Solution

Standards from kit

User Samples

Final volume is 200 µL

180–199 µL

1 × n µL*

199 × n µL*

where n = number of Standards plus number of Samples

Standards from Kit

Final volume is 200 µL

190 µL

1–20 µL

Final volume is 200 µL

1–20 µL

Ensure all reagents are at room temperature

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