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 tubes for the standards (three for the protein assay) and one tube for each user sample.

2. Prepare the Quant-iT™ **Working Solution** by diluting the Quant-iT™ reagent 1:200 in Quant-iT™ buffer. 200 μL of **Working Solution** is required for each sample and standard.

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>

4. Vortex all tubes for 2–3 seconds.

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

6. Insert the tubes in the Qubit® fluorometer and take readings.

7. Multiply the reading by the dilution factor (see Manual) to determine concentration of your original sample. Alternatively, choose **Calculate sample concentration** to have the Qubit® fluorometer perform this multiplication for you.

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

www.invitrogen.com/qubit
Ensure all reagents are at room temperature

Vortex all assay tubes for 2–3 seconds

Incubate at room temperature for 2 minutes (15 minutes for Quant-iT™ protein assay)

Read tubes in Qubit® fluorometer

* where n = number of Standards plus number of Samples

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