

## Guidelines for Using the Qubit® 2.0 Fluorometer

To obtain the best results, follow the recommendations below. For more information, see Critical Qubit™ Assay Considerations in the Appendix, pages 33–35. Manual is on the desktop of the Nanospec computer.

- Do not operate the instrument in direct sunlight.
- Lower lights and shades when working with the kits to reduce photobleaching.
- Wear gloves during sample handling.
- Use the Qubit® 2.0 Fluorometer at room temperature only (22–28°C).
- Store all kit reagents at room temperature and insert all assay tubes into the Qubit® 2.0 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence.
- Do not hold the assay tubes in your hand before performing a measurement.
- Make sure that you have calibrated the Qubit® 2.0 Fluorometer using the appropriate standards.
- Incubate the tubes for the Qubit™ DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution.
  - \***Note**- 7 minutes seems to work better for RNA. This is the time you are giving your fluorescent tag to bind to your sample.
- Incubate the tubes for the Qubit™ protein assays for 15 minutes after mixing the sample or standard with the working solution.
- If you are performing multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.
- Visit [www.invitrogen.com/qubit](http://www.invitrogen.com/qubit) for additional application notes, technical notes, citations, firmware updates, and a list of validated Qubit™ assays that have been tested using the Qubit® 2.0 Fluorometer.

### Photobleaching of Qubit™ Reagents

The Qubit™ reagents exhibit high photostability in the Qubit® 2.0 Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit® 2.0 Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (see Figure 2 in Assay Temperature, previous page). Note that the temperature inside the Qubit® 2.0 Fluorometer may be as much as 3°C above room temperature after 1 hour. For this reason, if you want to perform multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

### Calibrating the Qubit® 2.0 Fluorometer

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, you should perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you should determine the level of comfort you have using the calibration data stored from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing the standards and the samples is stable for not longer than 3 hours. See Figure 1 in How the Qubit® 2.0 Fluorometer Calculates Concentration (page 33) for an example of the calibration curve used to generate the quantitation results.

### Checking Standards

The Check Standards Screen displays the raw fluorescence values for Standard #1, Standard #2, Standard #3 (if applicable), and the last measurement taken for a given assay. These values can assist you in making a judgment regarding the performance of the Qubit® 2.0 Fluorometer and whether or not the samples you used were within range.

- For the Qubit™ dsDNA BR, Qubit™ dsDNA HS, Qubit™ ssDNA, Qubit™ RNA, and Qubit™ RNA BR assays, the reading given by Standard #2 should be much higher than that of Standard #1, and the reading of your sample should fall somewhere between that of the two Standards.
- For the Qubit™ protein assay, the reading given by Standard #3 should be much higher than that of Standard #2, and the reading of your sample should be no more than 50% greater than Standard #3.
- To access the Check Standards Screen, press Check Stds. on the Standards Screen. The raw fluorescence values for the standards and the last sample will be displayed on the screen.

### Handling Samples

The calibration standards included in the Qubit™ RNA and Qubit™ RNA BR Assay Kits are high-quality rRNA standards. The integrity and concentration of these standards is critical to the optimal performance of the Qubit™ RNA assay. We highly recommend treating the rRNA standards as you would any other precious RNA. Use appropriate RNase-free handling techniques, including RNase-free gloves, pipette tips, and tubes. Keep the tube lids closed whenever possible; do not touch the pipet to the inside wall of the tube when withdrawing a sample, and return the rRNA standard to the refrigerator as soon as possible after use.

- Ensure that the assay tubes are at room temperature at the time the reading is taken. Do not hold assay tubes in your hand and do not leave assay tubes in the Qubit® 2.0 Fluorometer for longer than it takes to read the fluorescence. See Assay Temperature on page 34.
- Be careful not to spill sample into the sample chamber. Promptly wipe any spills.
- The Qubit™ assays are very sensitive to even traces of material from a previous sample. Use a clean 0.5 mL PCR tube for each reading.
- The tube must be clean and dry on the outside when taking readings. Moisture and condensation on the tube surface can lead to reading errors.
- Minute bubbles in samples will cause errors in readings. Be sure not to introduce bubbles into samples. Slight tapping on the tube wall or brief centrifugation will often help dissipate bubbles.

Use a combination of kits to determine contamination if it is critical to know. For instance if your DNA experiment will be horribly hindered by excess RNA or protein in your sample. You will need to take readings using the various kits to determine contamination levels.

If your DNA is supercoiled, it is recommended that you either nick it, or use supercoiled DNA for your Std. #2.