Effects of contaminating substances

A number of common contaminants have been tested with the Qubit™ dsDNA BR Assay, and most are well tolerated. For untested contaminating substances, and, in general, for highest accuracy, the standards should be assayed under the same conditions as the experimental samples. For example, if the experimental samples are in an unusual buffer and 10 µL of each sample is used, add 10 µL of the unusual buffer (lacking dsDNA) to each standard.

Table 1 Effect of contaminants in the Qubit™ dsDNA BR Assay, tested over a range of 0.01–5 µg/mL.

DNA standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 20-µL or 10-µL sample volumes are also listed. In all cases, results are given as OK, usually less than 10% perturbation.

Contaminant	Final concentration in the assay	Concentration in 20-µL sample	Concentration in 10-µL sample	Result
Sodium chloride	10 mM	100 mM	200 mM	OK[1]
Magnesium chloride	2 mM	20 mM	40 mM	OK[1]
Sodium acetate	10 mM	100 mM	200 mM	OK
Ammonium acetate	10 mM	100 mM	200 mM	OK[1]
Potassium phosphate, pH 7.4	5 mM	50 mM	100 mM	OK[1]
Ethanol	1%	10%	20%	OK
Phenol	0.1%	1%	2%	OK
Chloroform[2]	0.2%	2%	4%	OK
SDS	0.01%	0.1%	0.2%	OK
Triton™ X-100	0.001%	0.01%	0.02%	OK[1]
dNTPs[3]	100 µM	1 mM	2 mM	OK
BSA	20 µg/mL	200 µg/mL	400 µg/mL	OK[1]
IgG	10 µg/mL	100 µg/mL	200 µg/mL	OK
RNA	6X	6X	6X	OK
ssDNA	1X	1X	1X	OK
Oligos	3X	3X	3X	OK

[1] An acceptable result, but with some distortion of the standard curve; for best results, add the same amount of contaminant to the standard samples.
[2] Immiscible.
[3] A mixture of dATP, dCTP, dGTP, and dTTP.

Prepare samples and standards

This protocol assumes that you are preparing standards for calibrating the Qubit™ Fluorometer. If you plan to use the last calibration performed on the instrument, fewer tubes (step 1) and less working solution (step 3) will be needed (see "Calibrate the Qubit™ Fluorometer" on page 2).

IMPORTANT! For best results, ensure that all materials and reagents are at room temperature.

1. Set up the required number of Qubit™ tubes for standards and samples. The Qubit™ dsDNA BR Assay requires 2 standards.

   Note: Use only thin-wall, clear, 0.5-mL PCR tubes (Cat. No. Q32856) for the Qubit™ 4 Fluorometer and 8 x 200-µL tube strips (Cat. No. Q33252) for the Qubit™ Flex Fluorometer.

2. Label the tube lids.

   Note: Do not label the side of the tube as this could interfere with the sample read. Label the lid of each standard tube correctly. Calibration of the Qubit™ Fluorometer requires the standards to be inserted into the instrument in the right order.

3. Prepare the Qubit™ working solution by diluting the Qubit™ dsDNA BR Reagent 1:200 in Qubit™ dsDNA BR Buffer. Use a clean plastic tube each time you prepare the Qubit™ working solution.

   IMPORTANT! Do not mix the working solution in a glass container.

4. Add the Qubit™ working solution to each tube such that the final volume is 200 µL.

	Standard assay tubes	User sample assay tubes
Volume of working solution	190 µL	180–199 µL
Volume of standard	10 µL	—
Volume of user sample	—	1–20 µL
Total volume in each assay tube	200 µL	200 µL

Note: The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit™ working solution, and each sample tube requires anywhere from 180–199 µL. Prepare sufficient Qubit™ working solution to accommodate all standards and samples.

For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit™ reagent plus 1990 µL of Qubit™ buffer).

Qubit™ Fluorometers provide a reagent calculator, which quickly computes the necessary volume of working solution needed.

5. Add 10 µL of each Qubit™ standard to the appropriate tube.

6. Add 1-20 µL of each user sample to the appropriate tube.

   Note: If you are adding 1–2 µL of sample, use a 2-µL pipette for best results.

7. Vigorously vortex for 3–5 seconds. Be careful not to create bubbles.

8. Allow all tubes to incubate at room temperature for 2 minutes, then proceed to read standards and samples (next section).

Read standards and samples

Follow the procedure appropriate for your instrument.

Read samples and standards with the Qubit™ 4 Fluorometer

For a more complete overview on using the Qubit™ 4 Fluorometer, please refer to the *Qubit™ 4 Fluorometer User Guide* (Pub. No. MAN0017209), available for download at **thermofisher.com/qubit**.

1. On the **Home** screen, touch **dsDNA**, then select **dsDNA Broad Range** as the assay type. Touch **Read standards** to proceed.

   Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, skip to step 4. Otherwise, continue with step 2.

2. Insert the tube containing Standard #1 into the sample chamber, close the lid, then touch **Read standard**. When the reading is complete (~3 seconds), remove Standard #1.

3. Insert the tube containing Standard #2 into the sample chamber, close the lid, then touch **Read standard**. When the reading is complete, remove Standard #2.

   Note: The instrument displays the results on the Read Standards screen. For information on interpreting the calibration results, refer to the *Qubit™ 4 Fluorometer User Guide* (Pub. No. MAN0017209), available for download at **thermofisher.com/qubit**.

4. Touch **Run samples**.

5. On the assay screen, select the **Sample volume** and units.
   a. Touch the + or – buttons on the wheel, or anywhere on the wheel itself, to select the sample volume added to the assay tube (1–20 µL).
   b. From the **Unit** dropdown menu, select the units for the output sample concentration.

6. Insert a sample tube into the sample chamber, close the lid, then touch **Read tube**. When the reading is complete (~3 seconds), remove the sample tube. The top value (in large font) is the concentration of the original sample and the bottom value is the dilution concentration. For information on interpreting the sample results, refer to the *Qubit™ 4 Fluorometer User Guide* (Pub. No. MAN0017209).

7. Repeat step 6 until all samples have been read.

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Read standards and samples with the Qubit™ Flex Fluorometer

For a more complete overview on using the Qubit™ Flex Fluorometer, please refer to *Qubit™ Flex Fluorometer User Guide* (Pub. No. MAN0018186), available for download at **thermofisher.com/qubit**.

1. On the **Home** screen, select **dsDNA Broad Range (BR)** as the assay type. Touch **Read standards & run samples** to proceed.

   Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, press **Run samples** and skip to step 4. Otherwise, continue with step 2.

2. Insert the tube strip containing Standard #1 into the sample chamber, close the lid, then touch **Run standards**. When the reading is complete (~3 seconds), remove Standard #1.

3. Insert the tube strip containing Standard #2 into the sample chamber, close the lid, then touch **Run standards**. When the reading is complete, remove Standard #2.

   Note: The instrument displays graphical results on the Standards complete screen. For information on interpreting the calibration results, refer to the *Qubit™ Flex Fluorometer User Guide* (Pub. No. MAN0018186), available for download at **thermofisher.com/qubit**.

4. Press **Next** from the Standards complete screen. When prompted, load the tube strips with your samples as shown in the Insert samples screen. If you have fewer than 8 samples, touch to deselect the tube positions that do not contain a sample.

5. Select the units for the output sample concentration, then touch **Next**.

6. (Optional) Select **More options** to add the assay kit lot #, tags, or sample IDs. For information on using these options, refer to the *Qubit™ Flex Fluorometer User Guide*.

7. In the **Sample volume** screen, enter the sample volume added to the assay tube (1–20 µL). Enter the volume directly in the **Sample volume** text box, use the + and – buttons, or adjust the sample volume wheel to select the **Sample volume** added to the assay tube.

   Note: The sample volume used (1–20 µL) changes the assay accuracy range. A different sample volume or assay may be required if the sample concentration is outside of what the assay can accurately quantify.

8. Insert a sample tube strip into the sample chamber, close the lid, then touch **Run samples**. When the reading is complete (~3 seconds), remove the sample tube strip.

   Standards and sample measurements are displayed on a graph with the results in a list below it.

   Touch the graph icon to switch to the results list-only view. The values listed are the concentrations of the original samples. For information on interpreting the sample results, refer to the *Qubit™ Flex Fluorometer User Guide* (Pub. No. MAN0018186).

9. Select **Add samples** and repeat step 8 to read more samples.