# Qubit™ RNA BR Assay Kits

For use with the Qubit® 2.0 Fluorometer

**Table 1.** Contents and storage information.

| Material | Amount | | Concentration | Storage | Stability |
|---|---|---|---|---|---|
| | Q10210 (100 assays) | Q10211 (500 assays) | | | |
| Qubit™ RNA BR Reagent (Component A) | 250 µL | 1.25 mL | 200X concentrate in DMSO | • ≤ 25°C<br>• Desiccate<br>• Protect from light | When stored as directed, kits are stable for 6 months. |
| Qubit™ RNA BR Buffer (Component B) | 50 mL | 250 mL | Not applicable | ≤ 25°C | |
| Qubit™ RNA BR Standard #1 (Component C) | 1 mL | 5 mL | 0 ng/µL in TE buffer | • 2–6°C<br>• Do not freeze | |
| Qubit™ RNA BR Standard #2 (Component D) | 4 × 250 µL | 10 × 500 µL | 100 ng/µL in TE buffer | | |

## Introduction

The Qubit™ RNA BR (Broad-Range) Assay Kits for use with the Qubit® 2.0 Fluorometer make RNA quantitation easy and accurate. The kit provides concentrated assay reagent, dilution buffer, and prediluted RNA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µL and 20 µL is acceptable), and read the concentration using the Qubit® 2.0 Fluorometer. The assay is highly selective for RNA over double-stranded DNA (dsDNA) (*Appendix*, Figure 1) and is accurate for initial sample concentrations from 1 ng/µL to 1 µg/µL providing an assay range from 20–1,000 ng. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants, such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (*Appendix*, Table 2). In addition to the Qubit™ RNA BR Assay Kits described here, we also offer other kits for assaying DNA and protein (*Appendix*, Table 3).

To determine the purity of your sample, use the Qubit™ RNA BR Assay Kit together with the Qubit™ dsDNA BR Assay Kit. These measurements give you a much better indication of sample purity than that produced by measuring the $A_{260}/A_{280}$ ratio. To measure protein contamination in nucleic acid samples, simply run 1–20 µL of the sample in the Qubit™ protein assay.

**Note:** All Qubit™ assay kits can also be used with the Qubit® 1.0 Fluorometer.

## Before You Begin

### Materials Required but Not Provided

- Plastic container (disposable) for mixing the Qubit™ working solution (step 1.3)
- Qubit® assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830)

### Storing the Qubit™ RNA Assay Kits

The Qubit™ RNA reagent and buffer are designed for room temperature storage. The Qubit™ RNA reagent is supplied in DMSO, which freezes at temperatures lower than room temperature. Store the RNA standards at 4°C.

The Qubit™ RNA reagent is sensitive to light. Store the vial **in the dark** when not in use.

### Critical Assay Parameters

#### Assay Temperature

The Qubit™ RNA assay for the Qubit® 2.0 Fluorometer delivers optimal performance when all solutions are at room temperature (22–28˚C). The Qubit™ assays are designed to be performed at room temperature, and temperature fluctuations can influence the accuracy of the assay (*Appendix*, Figure 2). To minimize temperature fluctuations, store the Qubit™ RNA reagent and the Qubit™ RNA buffer 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, because the Qubit® 2.0 Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading, as this will warm the solution and result in a low reading.

#### Incubation Time

To allow the Qubit™ assay to reach optimal fluorescence, incubate the tubes for the DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature.

#### Photobleaching of the Qubit™ Reagent

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 *Appendix*, Figure 2). 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, 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, determine the level of comfort you have using the calibration data stored in from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing standards and the samples is stable for not longer than 3 hours. See Figure 3 in the *Appendix* for an example of the calibration curve used to generate the quantitation results.

### RNAse-free Handling

The calibration standards included in the Qubit™ RNA Assay Kit are high-quality rRNA standards. The integrity and concentration of these standards is critical to the optimal performance of the Qubit™ RNA BR assay. As such, 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.

### Handling and Disposal

No data are currently available addressing the mutagenicity or toxicity of the Qubit™ RNA BR reagent (Component A). This reagent is known to bind nucleic acid and is provided as a solution in DMSO. Treat the Qubit™ RNA BR reagent with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

## Experimental Protocol

### Performing the Qubit™ RNA BR Assay

The protocol below assumes you are preparing standards for calibrating the Qubit® 2.0 Fluorometer. If you plan to use the last calibration performed on the instrument, you need fewer tubes (step 1.1) and less working solution (step 1.3). More detailed instructions on the use of the Qubit® 2.0 Fluorometer (corresponding to steps 1.9–1.15 and 2.1–2.6) can be found in the user manual accompanying the instrument. For sample purity determinations, it is possible to use the Qubit® 2.0 Fluorometer to calculate the amount of dsDNA and RNA in the same sample. Simply perform each assay for your sample.

**1.1** Set up the required number of 0.5 mL tubes you need for standards and samples. The Qubit™ RNA BR assay requires 2 standards.

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

**1.2** Label the tube lids.

**1.3** Make the Qubit™ working solution by diluting the Qubit™ RNA BR reagent 1:200 in Qubit™ RNA BR buffer. Use a clean plastic tube each time you make Qubit™ working solution. **Do not mix the working solution in a glass container.**

**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 µL to 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 1,990 µL of Qubit™ buffer).

**1.4** Load 190 µL of Qubit™ working solution into each of the tubes used for standards.

**1.5** Add 10 µL of each Qubit™ RNA BR standard to the appropriate tube and mix by vortexing 2–3 seconds, being careful not to create bubbles.

**Note:** Careful pipetting is critical to ensure that exactly 10 µL of each Qubit™ RNA BR standard is added to 190 µL of Qubit™ working solution. It is also important to label the lid of each standard tube correctly as calibration of the Qubit® 2.0 Fluorometer requires that the standards be introduced to the instrument in the right order.

**1.6** Load Qubit™ working solution into individual assay tubes so that the final volume in each tube after adding sample is 200 µL.

**Note:** Your sample can be anywhere between 1 µL and 20 µL, therefore, load each assay tube with a volume of Qubit™ working solution anywhere between 180 µL and 199 µL.

**1.7** Add each of your samples to assay tubes containing the correct volume of Qubit™ working solution (prepared in step 1.6) and mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.

**1.8** Allow all tubes to incubate at room temperature for 2 minutes.

**1.9** On the Home Screen of the Qubit® 2.0 Fluorometer, press **RNA**, and then select **RNA Broad Range** as the assay type. The Standards Screen is automatically displayed.

**Note:** If you have already performed a calibration for the selected assay, Qubit® 2.0 Fluorometer will prompt you to choose between reading new standards and using the previous calibration. See *Calibrating the Qubit® 2.0 Fluorometer* above for calibration guidelines.

**1.10** On the Standards Screen, press **Yes** to run a new calibration or press **No** to use the last calibration.

**1.11** If you pressed **No** on the Standards Screen, proceed to step 1.12. If you selected **Yes** to a run new calibration, follow instructions below.

#### Running a New Calibration

Insert the tube containing Standard #1 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**. The reading will take approximately 3 seconds.
Remove Standard #1.
Insert the tube containing Standard #2 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.
Remove Standard #2.

**1.12** If you pressed **No** on the Standards Screen, the Sample Screen will be automatically displayed. Insert a sample tube into the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.

**1.13** Upon the completion of the measurement, the result will be displayed on the screen.

**Note:** The value given by the Qubit® 2.0 Fluorometer at this stage corresponds to the concentration after your sample was diluted into the assay tube. You can record this value and perform the calculation yourself to find out the concentration of your original sample (see *Calculating the Concentration of Your Sample*, next page) or the Qubit® 2.0 Fluorometer performs this calculation for you (see *Dilution Calculator*, next page).

**1.14** To read the next sample, remove the sample from the Qubit® 2.0 Fluorometer, insert the next sample, and press **Read Next Sample**.

**1.15** Repeat sample readings until all samples have been read.

## Calculating the Concentration of Your Sample

The Qubit® 2.0 Fluorometer gives values for the Qubit™ RNA BR assay in µg/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times \left(\frac{200}{x}\right)$$

*where* QF value = the value given by the Qubit® 2.0 Fluorometer
x = the number of microliters of sample you added to the assay tube

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer (*i.e.*, if the Qubit® 2.0 Fluorometer gave a concentration in µg/mL, the result of the equation will be in µg/mL).

## Dilution Calculator

The "Dilution Calculator" feature of the Qubit® 2.0 Fluorometer calculates the concentration of your original sample based on the volume of sample you have added to the assay tube. To have the Qubit® 2.0 Fluorometer perform this calculation for you, follow the instruction below.

**2.1** Upon completion of the sample measurement, press **Calculate Stock Conc.** The Dilution Calculator Screen containing the volume roller wheel is displayed.

**2.2** Using the volume roller wheel, select the volume of your original sample that you have added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.

**2.3** To change the units in which the original sample concentration is displayed, press **ng/mL**. A pop-up window showing the current unit selection (as indicated by an adjacent red dash) opens.

**2.4** Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up.

The Qubit® 2.0 Fluorometer automatically converts the units to your selection once the unit selection pop-up window is closed.

**Note:** The unit button next to your sample concentration reflects the change in the units (e.g., if you change the unit to pg/µL, the button will display pg/µL).

**2.5** To save the data from your calculation to the Qubit® 2.0 Fluorometer, press **Save** on the Dilution Calculator screen. The last calculated value of your measurement will be saved in the .CSV file and tagged with a time and date stamp.

**2.6** To exit the Dilution Calculator Screen, press any navigator button on the bottom of the screen or Read Next Sample.

**Note:** When you navigate away from the Dilution Calculator screen, the Qubit® 2.0 Fluorometer saves the last values for the sample volume and the units in the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

# Appendix

### Selectivity of the Qubit™ RNA BR Assay

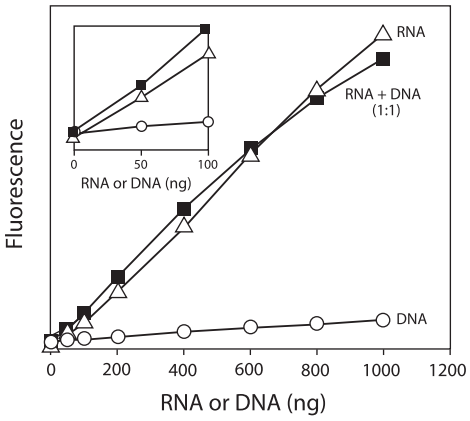


**Figure 1.** RNA selectivity and sensitivity of the Qubit™ RNA BR assay (Q10210, Q10211). Triplicate 10 µL samples of *E. coli* rRNA (△), λ DNA (○), or a 1:1 mixture of RNA and DNA (■) were assayed in the Qubit™ RNA BR assay. Fluorescence was measured at 630/660 nm and plotted versus the mass of nucleic acid for the RNA alone or DNA alone, or versus the mass of the RNA component in the 1:1 mixture. The variation (CV) of replicate RNA determinations was ≤10%. The inset is an enlargement of the graph to show the sensitivity of the assay for RNA. Background fluorescence has not been subtracted.

### Effect of Temperature on the Qubit™ RNA Assay

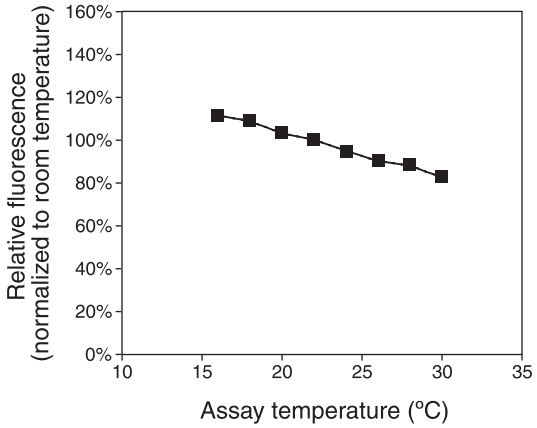


**Figure 2.** Plot of fluorescence vs. temperature for the Qubit™ RNA assay. The Qubit™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

## How the Qubit® 2.0 Fluorometer Calculates Concentration

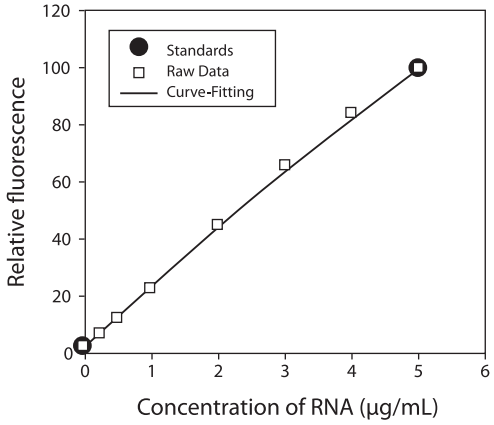


**Figure 3.** The curve-fitting algorithm used to determine concentration in the Qubit™ RNA BR assay. The Qubit® 2.0 Fluorometer generates concentration data based on the relationship between the two standards used in calibration. This plot shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ RNA BR assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation.

## Contaminants Tolerated by the Qubit™ RNA BR Assay

**Table 2.** Effect of contaminants in the Qubit™ RNA BR assay, tested over the range 500–5,000 ng/mL.*

| 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 |
| Magnesium chloride | 2 mM | 20 mM | 40 mM | OK† |
| Sodium acetate | 10 mM | 100 mM | 200 mM | OK† |
| Ammonium acetate | 10 mM | 100 mM | 200 mM | OK |
| Potassium phosphate, pH 7.4 | 5 mM | 50 mM | 100 mM | OK |
| Ethanol | 0.1% | 1% | 2% | OK |
| Phenol | 0.1% | 1% | 2% | OK† |
| Chloroform‡ | 0.2% | 2% | 4% | OK |
| SDS | 0.01% | 0.1% | 0.2% | NR |
| Triton® X-100 | 0.001% | 0.01% | 0.02% | OK |
| dNTPs§ | 100 µM | 1 mM | 2 mM | OK |
| BSA | 20 µg/mL | 200 µg/mL | 400 µg/mL | OK |
| IgG | 10 µg/mL | 100 µg/mL | 200 µg/mL | OK |
| ssDNA | 1X | 1X | 1X | OK |
| Oligos | 1X | 1X | 1X | OK |
| dsDNA | 1X | 1X | 1X | OK |

**E.coli* rRNA 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. Results are given either as OK, usually less than 10% perturbation, or as NR, not recommended. †An acceptable result, but with some distortion of the standard curve; for best results, add the same amount of contaminant to the standard samples. ‡Immiscible. §A mixture of dATP, dCTP, dGTP, and dTTP.

## Qubit™ Assay Kits Compatible with the Qubit® 2.0 Fluorometer

A number of fluorescence-based quantitation kits are available for use with the Qubit® 2.0 Fluorometer. Table 3 helps you choose a kit based on the target molecule being measured and the number of assays you require.

**Table 3.** Qubit™ Assay Kits for use with the Qubit® 2.0 Fluorometer.

| Product | Cat. no. | Number of Assays* | Target | Notes |
|---|---|---|---|---|
| Qubit™ dsDNA BR Assay Kit | Q32850 | 100 | dsDNA | • core range (high confidence): 0.01 µg/mL to 5 µg/mL†<br>• extended range (moderate confidence): 5 µg/mL to 10 µg/mL†<br>• useful for quantitation of genomic and miniprep DNA samples<br>• accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides |
| Qubit™ dsDNA BR Assay Kit | Q32853 | 500 | | |
| Qubit™ dsDNA HS Assay Kit | Q32851 | 100 | dsDNA | • core range (high confidence): 1 ng/mL to 500 ng/mL†<br>• extended ranges (moderate confidence): 0.5 ng/mL to 1 ng/mL and 500 ng/mL to 600 ng/mL†<br>• useful for quantitation of PCR products, viral DNA, and samples for subcloning<br>• accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides |
| Qubit™ dsDNA HS Assay Kit | Q32854 | 500 | | |
| Qubit™ ssDNA Assay Kit | Q10212 | 100 | ssDNA | • core range (high confidence): 5 ng/mL to 1,000 ng/mL†<br>• extended ranges (moderate confidence): 1 ng/mL to 5 ng/mL and 1,000 ng/mL to 1,200 ng/mL†<br>• useful for quantitation of oligos, primers, denatured DNA, PCR products<br>• accurate in the presence of salts, urea, solvents, proteins, ATP, and agarose |
| Qubit™ RNA Assay Kit | Q32852 | 100 | RNA | • core range (high confidence): 25 ng/mL to 500 ng/mL†<br>• extended ranges (moderate confidence): 20 ng/mL to 25 ng/mL and 500 ng/mL to 1,000 ng/mL†<br>• useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures<br>• accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides |
| Qubit™ RNA Assay Kit | Q32855 | 500 | | |
| Qubit™ RNA BR Assay Kit | Q10210 | 100 | RNA | • core range (high confidence): 0.1 µg/mL to 5 µg/mL†<br>• extended ranges (moderate confidence): 0.05 µg/mL to 0.1 µg/mL and 5–6 µg/mL†<br>• useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures<br>• accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides |
| Qubit™ RNA BR Assay Kit | Q10211 | 500 | | |
| Qubit™ Protein Assay Kit | Q33211 | 100 | protein | • core range (high confidence): 1.25 µg/mL to 25 µg/mL†<br>• extended ranges (moderate confidence): 1 µg/mL to 1.25 µg/mL and 25 µg/mL to 26 µg/mL†<br>• little protein-to-protein difference in signal<br>• accurate in the presence of DTT, β-mercaptoethanol, amino acids, and DNA<br>• signal is stable for 3 hours |
| Qubit™ Protein Assay Kit | Q33212 | 500 | | |

*Based on an assay volume of 200 µL. †Concentration ranges refer to the concentration of sample after dilution in the assay tube.

## Product List **Current prices may be obtained from our website or from our Customer Service Department.**

| Cat. no. | Product Name | Unit Size |
|---|---|---|
| Q10210 | Qubit™ RNA BR Assay Kit, 100 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer* | 1 kit |
| Q10211 | Qubit™ RNA BR Assay Kit, 500 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer* | 1 kit |
| *Related products* | | |
| Q32852 | Qubit™ RNA Assay Kit, 100 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer* | 1 kit |
| Q32855 | Qubit™ RNA Assay Kit, 500 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer* | 1 kit |
| Q10212 | Qubit™ ssDNA Assay Kit, 100 assays *1–200 ng* *for use with the Qubit® 2.0 Fluorometer* | 1 kit |
| Q32850 | Qubit™ dsDNA BR Assay Kit, 100 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer* | 1 kit |
| Q32853 | Qubit™ dsDNA BR Assay Kit 500 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer* | 1 kit |
| Q32851 | Qubit™ dsDNA HS Assay Kit, 100 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer | 1 kit |
| Q32854 | Qubit™ dsDNA HS Assay Kit, 500 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer* | 1 kit |
| Q33211 | Qubit™ Protein Assay Kit, 100 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer* | 1 kit |
| Q33212 | Qubit™ Protein Assay Kit, 500 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer* | 1 kit |
| Q32856 | Qubit™ assay tubes *set of 500* | 1 set |
| Q32866 | Qubit® 2.0 Fluorometer | each |
| Q32867 | Qubit® 2.0 Fluorometer USB | each |
| Q32868 | Qubit® 2.0 Fluorometer International Power Cord (replacement) | each |

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