Qubit™ RNA BR Assay Kits

Catalog Numbers Q10210, Q10211

Pub. No. MAN0001987 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Qubit™ RNA BR (Broad-Range) Assay Kits make RNA quantitation easy and accurate. The assay is highly selective for RNA over double-stranded DNA (dsDNA) (“Assay selectivity” on page 2). Depending on sample volume, the assay is accurate for initial sample concentrations from 0.5 ng/µL to 1200 ng/µL, providing an assay range of 10–1,200 ng. Common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (“Effects of contaminating substances” on page 4). The Qubit™ RNA BR assay is intended for total RNA, rRNA, or large mRNA. For small RNA (~20 nt or bp), we recommend the Qubit™ microRNA Assay Kit (Cat. Nos. Q32880, Q32881). In addition to the Qubit™ RNA BR Assay Kits described here, we also offer other kits for assaying DNA and protein (“Related products” on page 7).

Note: This Qubit™ assay kit can be used with any Qubit™ Fluorometer.

Contents and storage

Component	Cat. No. Q10210 (100 assays)	Cat. No. Q10211 (500 assays)	Concentration	Storage[1]
Qubit™ RNA BR Reagent (Component A)	250 µL	1.25 mL	200X in DMSO	Room temperature[2] Desiccate Protect from light
Qubit™ RNA BR Buffer (Component B)	50 mL	250 mL	Not applicable	≤30°C
Qubit™ RNA BR Standard #1 (Component C)	1 mL	5 mL	0 ng/µL in TE buffer	2–8°C[3] Avoid freeze/thaw cycles
Qubit™ RNA BR Standard #2 (Component D)	4 × 250 µL	10 × 500 µL	100 ng/µL in TE buffer	

[1] When stored as directed, kits are stable for 6 months.
[2] For long-term storage, theQubit™ RNA reagent can be stored at ≤–20°C.
[3] For long-term storage, store the rRNA standards at ≤–20°C or –70°C.

Required materials not supplied

- Plastic container (disposable) for mixing the Qubit™ working solution (step 3)
- Qubit™ Assay Tubes (500 tubes, Cat. No. Q32856) or Qubit™ Flex Assay Tube Strips (Cat. No. Q33252)

RNase-free handling

The calibration standards included in the Qubit™ RNA BR 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 RNA, including:

- 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 pipette to the inside wall of the tube when withdrawing a sample
- Return the rRNA standard to the refrigerator as soon as possible after use

Critical assay parameters

Assay temperature

Qubit™ assays delivers optimal performance when all solutions are at room temperature; temperature fluctuations can influence the accuracy of the assay.

To minimize temperature fluctuations, insert all assay tubes into the Qubit™ Fluorometer only for as much time as it takes for the instrument to measure the fluorescence; the Qubit™ 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 because this warms the solution and results 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.

Calibrate the Qubit™ Fluorometer

For each assay, you have the option to run a new calibration or use values from the previous calibration. To minimize variables that can affect performance, performing a new calibration for every new assay run is strongly recommended. See Figure 1 for an example of the calibration curve used to generate the quantification results.

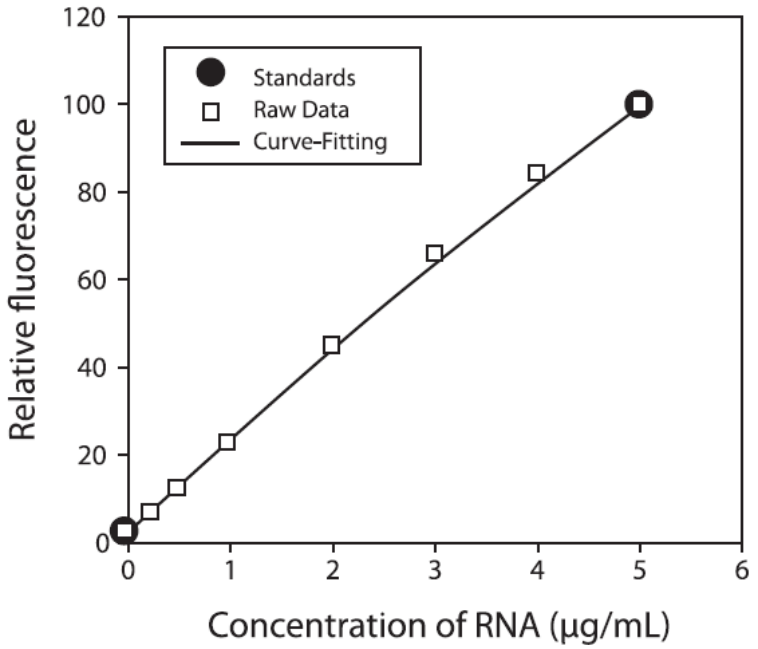

Figure 1 Sample calibration curve.
The Qubit™ Fluorometer generates concentration data based on the relationship between the two standards used in calibration. This plot shows the line corresponding to the curve-fitting algorithm used in the calculation of concentration data for the Qubit™ RNA BR assay. 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.

Assay selectivity

The assay is highly selective for RNA over double-stranded DNA (dsDNA) (Figure 2).

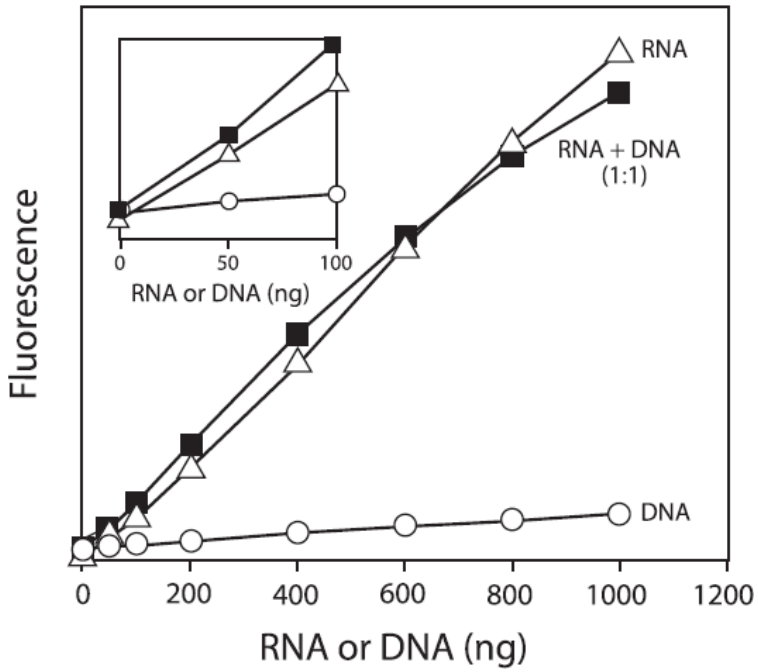

Figure 2 RNA selectivity and sensitivity of the Qubit™ RNA BR assay.
Triplicate 10-µL samples of *E. coli* rRNA (Δ), λ DNA (O), or a 1:1 mixture of RNA and DNA (■) were assayed in the Qubit™ RNA BR Assay. Fluorescence was measured at 630/660 nm and plotted versus the mass of nucleic acid for the RNA 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.

Photostability of the Qubit™ reagents

The Qubit™ reagents exhibit high photostability in the Qubit™ Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. However, if the assay tube remains in the Qubit™ Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (Figure 3). Note that the temperature inside the Qubit™ 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.

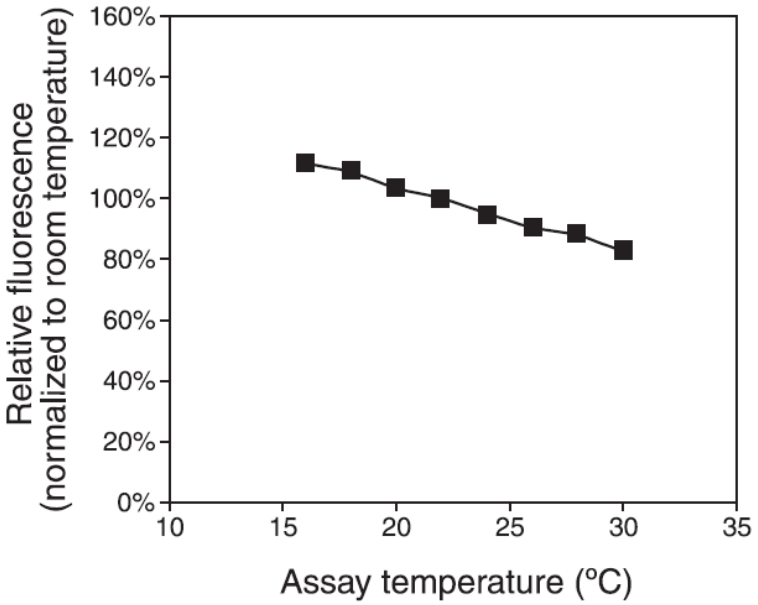

Figure 3 Plot of fluorescence versus temperature for the Qubit™ RNA BR assay.
The Qubit™ assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

Effects of contaminating substances

Table 1 Effect of contaminants in the Qubit™ RNA BR assay.

Contaminant	Final concentration in the assay	Concentration in 20-µL sample	Concentration in 10-µL sample	Result[1]
Sodium chloride	10 mM	100 mM	200 mM	OK
Magnesium chloride	2 mM	20 mM	40 mM	OK[2]
Sodium acetate	10 mM	100 mM	200 mM	OK[2]
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[2]
Chloroform[3]	0.2%	2%	4%	OK
SDS	0.01%	0.1%	0.2%	NR
Triton™ X-100	0.001%	0.01%	0.02%	OK
dNTPs[4]	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
NTPs	1X[5]	1X[5]	1X[5]	OK
ssDNA	1X[5]	1X[5]	1X[5]	OK
Oligos	1X[5]	1X[5]	1X[5]	OK
dsDNA	1X[5]	1X[5]	1X[5]	OK

[1] *E.coli* rRNA standards, over a range of 500–5000 ng/mL, 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.

[2] An acceptable result, but with some distortion of the standard curve; for best results, add the same amount of contaminant to the standard samples.

[3] Immiscible.

[4] A mixture of dATP, dCTP, dGTP, and dTTP.

[5] 1X indicates a concentration equal to the concentration of rRNA.

Preparing 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 4) 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™ RNA 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 × 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™ RNA BR Reagent 1:200 in Qubit™ RNA BR Buffer. Use a clean plastic tube each time you prepare Qubit™ working solution.

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

4. Add Qubit™ working solution to individual assay tubes so that the final volume in each tube after adding sample 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. Ensure that you have 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.

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

Reading standards and samples

Read samples and standards with the Qubit™ 4 Fluorometer

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

1. On the **Home** screen, touch **RNA**, then select **RNA 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.

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 **RNA 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.

Related products

Table 2 Assays

Product	Quantitation range	Quantity	Cat. No.
Qubit™ Protein BR Assay Kit[1]	0.1–20 mg	100 reactions 500 reactions	A50668 A50669
Qubit™ Protein Assay Kit	12.5–5,000 µg	100 reactions 500 reactions	Q33211 Q33212
Qubit™ 1X dsDNA HS Assay Kit	0.1–120 ng	100 reactions 500 reactions	Q33230 Q33231
Qubit™ 1X dsDNA BR Assay Kit	4–4,000 ng	100 reactions 500 reactions	Q33265 Q33266
Qubit™ dsDNA HS Assay Kit	0.1–120 ng	100 reactions 500 reactions	Q32851 Q32854
Qubit™ dsDNA BR Assay Kit	4–2,000 ng	100 reactions 500 reactions	Q32850 Q32853
Qubit™ ssDNA Assay Kit	0.2–240 ng	100 reactions	Q10212
Qubit™ RNA IQ Assay Kit	N/A	75 reactions 275 reactions	Q33221 Q33222
Qubit™ RNA HS Assay Kit	4–200 ng	100 reactions 500 reactions	Q32852 Q32855
Qubit™ RNA BR Assay Kit	10–1,200 ng	100 reactions 500 reactions	Q10210 Q10211
Qubit™ RNA XR Assay Kit	100–20,000 ng	100 reactions 500 reactions	Q33223 Q33224
Qubit™ microRNA Assay Kit	0.5–150 ng	100 reactions 500 reactions	Q32880 Q32881
Qubit™ 4 System Verification Assay Kit	N/A	50 reactions	Q33237
Qubit™ Flex System Verification Assay Kit	N/A	50 reactions	Q33254

[1] Qubit™ Protein BR Assay Kit is designed for use with Qubit™ 4 only.

Table 3 Instruments

Product	Cat. No.
Qubit™ Flex Fluorometer	Q33327
Qubit™ Flex Fluorometer NGS Starter Kit	Q45893
Qubit™ Flex Fluorometer Quantitation Starter Kit	Q45894
Qubit™ 4 Fluorometer	Q33238
Qubit™ 4 NGS Starter Kit	Q33240
Qubit™ 4 Quantitation Starter Kit	Q33239
Qubit™ 4 RNA IQ Starter Kit	Q33241
Qubit™ 4 Protein BR Starter Kit	A51292

Table 4 Consumables/Accessories

Product	Quantity	Cat. No.
Qubit™ Flex Assay Tube Strips	125 tube strips	Q33252
Qubit™ Assay Tubes	500 tubes	Q32856
Qubit™ 4 Fluorometer International Power Supply (replacement)	1 each	A36204
Qubit™ 4 USB Flash Drive	1 each	Q46009

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision history: Pub. No. MAN0001987

Revision	Date	Description
B.0	8 March 2022	The format and content were updated.
A.0	February 2015	New document for Qubit™ RNA BR Assay Kit.

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