

# Quant-iT™ RNA Assay Kit, Broad Range

Catalog no. Q10213

Table 1. Contents and storage

Material	Amount	Concentration	Storage	Stability
Quant-iT™ RNA BR reagent (Component A)	1.0 mL	200X in DMSO	<ul style="list-style-type: none"> <li>• Room temperature</li> <li>• Desiccate</li> <li>• Protect from light</li> </ul>	When stored as directed, kit contents are stable for at least 6 months.
<i>E. coli</i> rRNA standards (Component C)	set of 8 (500 µL each)	0, 5, 10, 20, 40, 60, 80, and 100 ng/µL	<ul style="list-style-type: none"> <li>• ≤6°C</li> <li>• Avoid freeze/thaw cycles</li> </ul>	
Quant-iT™ RNA BR buffer (Component B)	250 mL	Not applicable	<ul style="list-style-type: none"> <li>• ≤6°C*</li> <li>• Protect from light</li> </ul>	
* For short-term storage (days), the buffer may be stored at room temperature; however, for longer periods we recommend storage at ≤6°C to prevent microbial contamination.				
<b>Number of labelings:</b> 1,000, with a 200 µL assay volume in a 96-well microplate format. The Quant-iT™ RNA assay can be adapted for use in cuvettes or 384-well microplates.				
<b>Approximate fluorescence excitation/emission maxima:</b> 644/673 nm (see Figure 1, page 2)				

## Introduction

The Quant-iT™ RNA Assay Kit, Broad Range makes RNA quantification easy and accurate. The kit provides concentrated assay reagent, dilution buffer, and pre-diluted RNA standards. Simply dilute the reagent 1:200, load 200 µL into the wells of a microplate, add 1–20 µL sample volumes, mix, and read the fluorescence. The assay is highly selective for RNA over double-stranded DNA, and in the range of 20–1,000 ng, the fluorescence signal is linear with RNA (Figure 2, page 2). The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants, such as salts, solvents, detergents, or protein are well tolerated in the assay. The Quant-iT™ RNA Assay Kit, Broad Range is intended for total RNA, rRNA, or large mRNA. For small RNA (~20 nt or bp), we recommend the Quant-iT™ microRNA Assay Kit (Cat. no. Q32882).

If you would like to use this kit with the Qubit® Fluorometer, see *Using the Quant-iT™ RNA Assay Kit, Broad Range with the Qubit® Fluorometer* (page 4).

For Research Use Only. Not for use in diagnostic procedures.

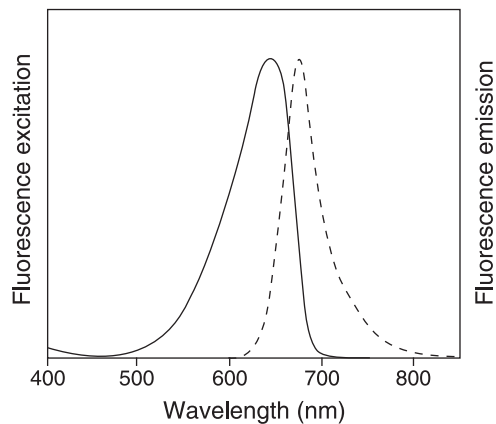


Figure 1. Excitation and emission maxima for the Quant-iT™ RNA BR reagent bound to RNA.

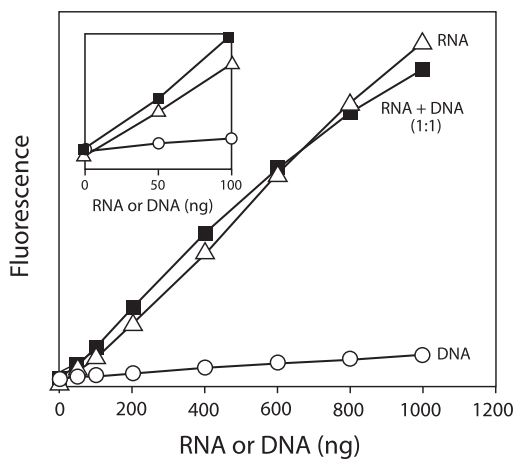


Figure 2. RNA selectivity and sensitivity of the Quant-iT™ RNA Broad Range assay. Triplicate 10  $\mu$ L samples of *E. coli* rRNA ( $\Delta$ ),  $\lambda$  DNA (O), or a 1:1 mixture of RNA and DNA ( $\blacksquare$ ) were assayed in the Quant-iT™ RNA Broad Range assay. Fluorescence was measured at 630/660 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  $\leq 10\%$ . The inset is an enlargement of the graph to show the sensitivity of the assay for RNA. Background fluorescence has not been subtracted.

## Before you begin

### Handling the Quant-iT™ reagent

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

Remove the Quant-iT™ RNA Assay Kit, Broad Range from storage and allow the components to equilibrate to room temperature. During all steps, protect the Quant-iT™ RNA BR reagent concentrate and the working solution from light as much as possible.

## Using the Quant-iT™ RNA BR Assay Kit with a Fluorescence Microplate Reader

This protocol describes the use of the Quant-iT™ RNA Assay Kit, Broad Range with a fluorescence microplate reader that is equipped with excitation and emission filters appropriate for the excitation/emission maxima of 644/673 nm. Some contaminating substances may interfere with the assay. See *Contaminating substances* (page 7) for more information. For an overview of this procedure, see Figure 2, below.

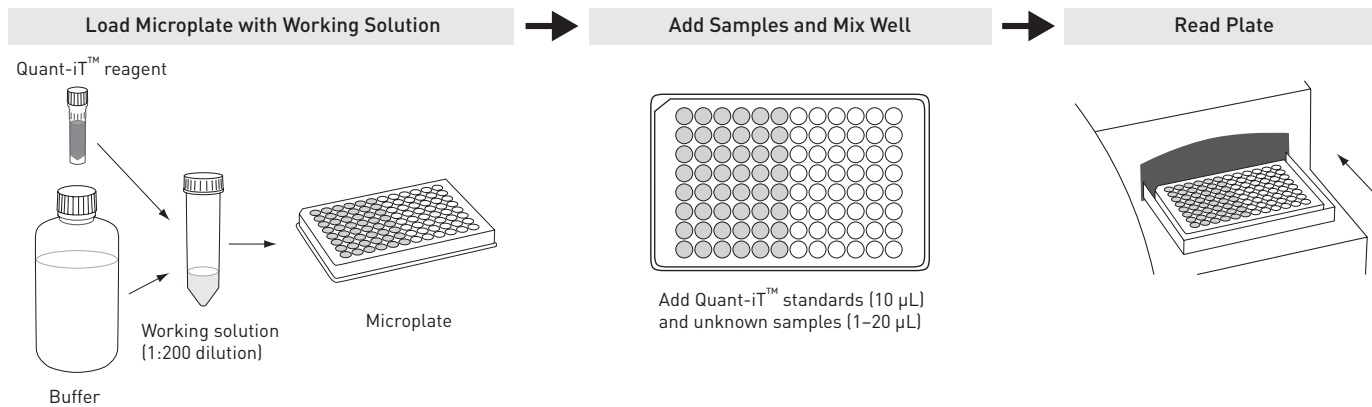


Figure 3. The Quant-iT™ RNA Broad Range assay.

### Assay procedure

- 1.1 Prepare a working solution by diluting Quant-iT™ RNA BR reagent 1:200 in Quant-iT™ RNA BR buffer. For example, for ~100 assays mix 100 µL of Quant-iT™ RNA BR reagent (Component A) and 20 mL of Quant-iT™ RNA BR buffer (Component B) in a disposable plastic container and mix well. **Do not use glass containers.** Do not use buffers other than the Quant-iT™ RNA BR buffer to make the working solution.
- 1.2 Load 200 µL of the working solution into each microplate well. Diluted Quant-iT™ RNA BR reagent is stable for at least 3 hours at room temperature, protected from light.
- 1.3 Add 10 µL of each *E. coli* rRNA standard (Component C) to separate wells and mix well. Take care not to introduce nucleases into the tubes of RNA standard as you remove aliquots for the assay. Duplicates or triplicates of the standards are recommended.
- 1.4 Add 1–20 µL of each unknown RNA sample to separate wells and mix well. Duplicates or triplicates of the unknown samples are recommended. Some contaminating substances may interfere with the assay, see *Contaminating substances* on page 7 for more information.
- 1.5 Measure the fluorescence using a microplate reader (excitation/emission maxima are 644/673 nm, see Figure 1 on page 2). The fluorescence signal is stable for 3 hours at room temperature.
- 1.6 Use a standard curve to determine the RNA amounts. For the *E. coli* rRNA standards, plot amount vs. fluorescence, and fit a straight line to the data points.

Data analysis considerations –  
standard curves and extended  
ranges

The fluorescence of the Quant-iT™ RNA BR reagent bound to RNA is extremely linear from 0–100 ng. For best results at the low end of the standard curve, the line should be forced through the background point (or through zero, if background has been subtracted). When 10 µL volumes of the standards are used, the lowest RNA-containing standard represents 5 ng of DNA.

To assess the reliability of the assay in the low range, use smaller volumes of the standards, e.g. 2 µL volumes for a standard curve ranging from 0–20 ng. During development of the Quant-iT™ RNA BR assay, we were able to detect 2 ng of *E. coli* rRNA under ideal experimental circumstances (using calibrated pipettors, octuplicate determinations, the best microplate readers, and Z-factor<sup>1</sup> analysis). Your results may vary.

## Using the Quant-iT™ RNA BR Assay Kit with the Qubit® Fluorometer

The Quant-iT™ RNA Assay Kit, Broad Range can easily be adapted for use with the Qubit® Fluorometer. The protocol below is abbreviated from the Qubit® Fluorometer user guide, which is available at [www.lifetechnologies.com/qubit](http://www.lifetechnologies.com/qubit). Although a step-by-step protocol and critical assay parameters are given here, more detail is available in the Qubit® Fluorometer user guide and you are encouraged to familiarize yourself with this manual before you begin your assay. See Figure 4, below, for an overview of the procedure.

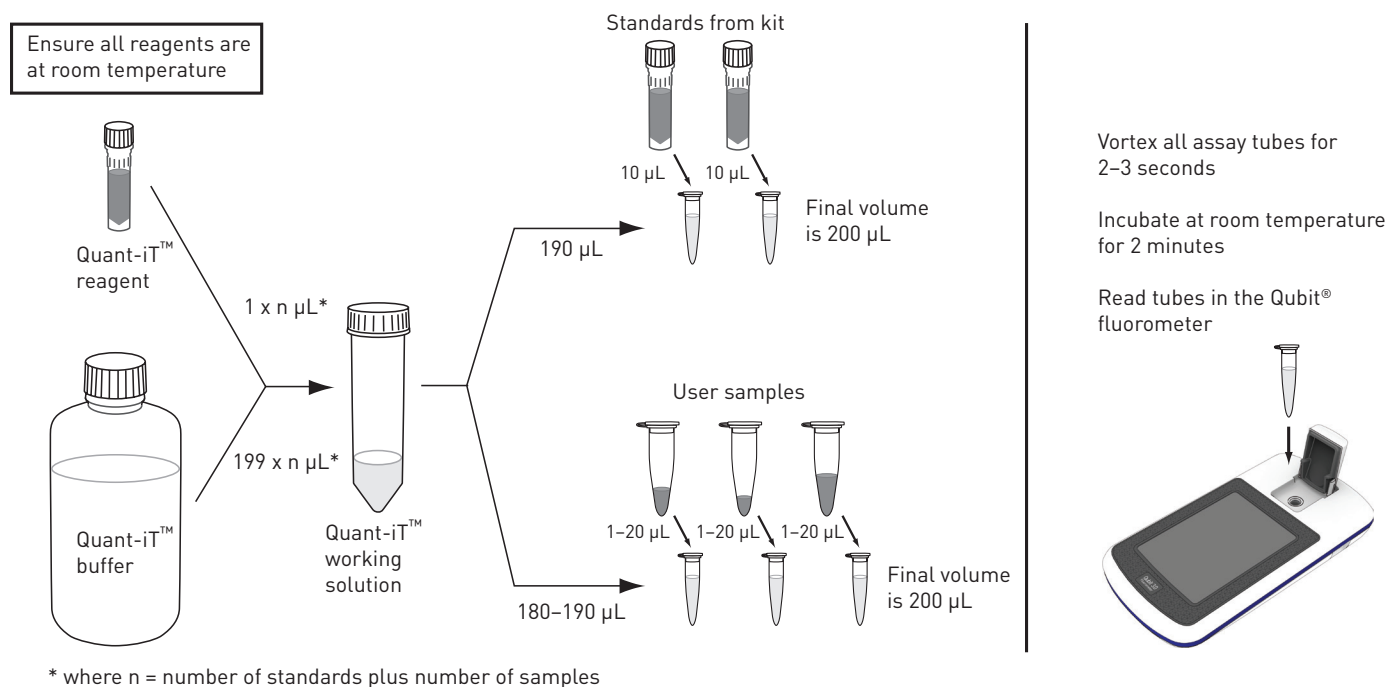


Figure 4. Overview for using the Quant-iT™ RNA BR assay in the Qubit® Fluorometer.

**Assay procedure**     **IMPORTANT!** Ensure all assay reagents are at room temperature before you begin. Use only thin-wall, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit® assay tubes (500 tubes, Cat. no. Q32856) or Axygen® PCR-05-C tubes (VWR, part no. 10011-830).

**2.1** Label the lids of the assay tubes you need for the standards and samples.

**Note:** The Quant-iT™ RNA Assay Kit, Broad Range requires two standards for the calibration of the Qubit® Fluorometer. Prepare a dilution of the 0 ng/μL *E. coli* rRNA standard from the Component C set to generate Standard #1, and a dilution of the 10 ng/μL *E. coli* rRNA standard from the Component C set to generate Standard #2 (see step 2.3).

**2.2** Prepare the Quant-iT™ RNA working solution by diluting the Quant-iT™ RNA BR reagent 1:200 in Quant-iT™ RNA BR buffer.

**2.3** Prepare assay tubes according to Table 2, below.

**Table 2.** Tube setup.

	Standard assay tubes	User Sample assay tubes
Volume of working solution (from step 2.2)	190 μL	180–199 μL
Volume of Standard (from kit)*	10 μL	—
Volume of User Sample to add	—	1–20 μL
Total volume in each assay tube	200 μL	200 μL
* Prepare Standard #1 by diluting 10 μL of the 0 ng/μL standard, and Standard #2 by diluting 10 μL of the 10 ng/μL standard.		

**2.4** Vortex all tubes for 2–3 seconds.

**2.5** Incubate the tubes for 2 minutes at room temperature.

**2.6** Calibrate the Qubit® Fluorometer using Standard #1 and Standard #2.

**2.7** Read your samples in the Qubit® Fluorometer.

**2.8** For Qubit® 2.0 Fluorometer users: Multiply by the value given by the dilution factor to determine concentration of your original sample. Alternatively, choose **Calculate Sample Concentration** to have the Qubit® 2.0 Fluorometer perform this multiplication for you. For more information, refer to the Qubit® 2.0 Fluorometer user manual.

**Note:** The Qubit® 3.0 Fluorometer performs this calculation automatically.

## Appendix: Critical Assay Parameters

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Assay temperature	The Quant-iT™ RNA BR assay for the Qubit® Fluorometer delivers optimal performance when all solutions are at room temperature. The Quant-iT™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay. To minimize temperature fluctuations, store the Quant-iT™ RNA BR reagent and the Quant-iT™ RNA BR buffer at room temperature and insert all assay tubes into the Qubit® Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, because the Qubit® Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. <b>Do not hold the assay tubes in your hand before reading, as this warms the solution and result in a low reading.</b>
Incubation time	To allow the Quant-iT™ RNA BR assay to reach maximum fluorescence, incubate the assay tubes 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 Quant-iT™ reagent	The Quant-iT™ RNA BR reagent exhibits high photostability in the Qubit® 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® Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature. 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.
Assay tubes to use with the Qubit® Fluorometer	Use only thin-wall, clear 0.5 mL PCR tubes with the Qubit® Fluorometer. Acceptable tubes include Qubit® assay tubes (Cat. no. Q32856, 500 tubes) or Axygen® PCR-05-C tubes (VWR, part number 10011-830). The assay volume must be 200 µL for an accurate read.
Calibrating the Qubit® Fluorometer	When quantifying your samples using the Qubit® Fluorometer, you have the choice to calibrate the instrument using freshly prepared calibration solutions or to apply the values from a previously run calibration. <i>Using the Quant-iT™ RNA BR Assay Kit with the Qubit® Fluorometer</i> , page 4, describes the preparation of fresh calibration standards. Consult the user manual for the Qubit® Fluorometer for guidance on choosing a calibration mode.

## Contaminating substances

A number of common contaminants have been tested in the Quant-iT™ RNA BR assay, and most are well tolerated (Table 3, below). For untested contaminating substances and in general, the standards should be assayed under the same conditions as the unknowns for highest accuracy. For example, if the experimental samples are in an unusual buffer and if 10 µL volumes of these samples are used, then add 10 µL volumes of the unusual buffer (lacking RNA) to the assays of the standards.

**Table 3.** Effect of Contaminants in the Quant-iT™ 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
NTPs**	1X	1X	1X	OK
BSA	20 µg/mL	200 µg/mL	400 µg/mL	OK
IgG	10 µg/mL	100 µg/mL	200 µg/mL	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.

\*\* A mixture of ATP, CTP, GTP, and UTP. 1X indicates a concentration equal to the concentration of rRNA.

## Reference

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1. J Biomol Screen 4, 67-73 (1999).

## Product List

Current prices may be obtained from our website or from our Customer Service Department.

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Cat. no.	Product Name	Unit Size
Q10213	Quant-iT™ RNA Assay Kit, Broad Range, 1000 assays *20–1000 ng* . . . . .	1 kit
<b>Related products</b>		
Q33120	Quant-iT™ dsDNA Assay Kit, High Sensitivity, 1000 assays *0.2–100 ng* . . . . .	1 kit
Q33130	Quant-iT™ dsDNA Assay Kit, Broad Range, 1000 assays *2–1000 ng* . . . . .	1 kit
Q33140	Quant-iT™ RNA Assay Kit, 1000 assays *5–100 ng* . . . . .	1 kit
Q32882	Quant-iT™ microRNA Assay Kit, 1000 assays *5–500 ng* . . . . .	1 kit
Q33210	Quant-iT™ Protein Assay Kit, 1000 assays *0.25–5 µg* . . . . .	1 kit
O11492	Quant-iT™ OliGreen® ssDNA Assay Kit *2000 assays* . . . . .	1 kit

## Purchaser Notification

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16 February 2015

