molecular probes
life technologies

Quant-iT™ RNA Assay Kit

Catalog no. Q33140

Table 1. Contents and storage

Material	Amount	Concentration	Storage*	Stability
Quant-iT™ RNA reagent (Component A)	1.0 mL	200X in DMSO	• Room temperature • Protect from light • Desiccate	When stored as directed, kit contents are stable for at least 6 months.
Quant-iT™ RNA buffer (Component B)	250 mL	NA	≤6°C	
E. coli rRNA standards (Component C)	set of 8 (500 µL each)	0, 0.5, 1, 2, 4, 6, 8, and 10 ng/µL	• ≤6°C • Avoid freeze/thaw cycles	

*The Quant-iT™ RNA buffer (Component B) may be left at room temperature for short-term storage (days); however, for longer periods we recommend storage at ≤6°C to prevent microbial contamination.

Number of labelings: 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.

Approximate fluorescence excitation/emission maxima: 644/673 nm (see Figure 1, page 2)

Introduction

The Quant-iT™ RNA Assay Kit 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, then read the fluorescence. The assay is highly selective for RNA over double-stranded DNA, and in the range of 5–100 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 (see *Contaminating substances*, page 7). The Quant-iT™ RNA Assay Kit 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, we have included instructions under *Using the Quant-iT™ RNA Assay Kit with the Qubit® Fluorometer* (page 4)

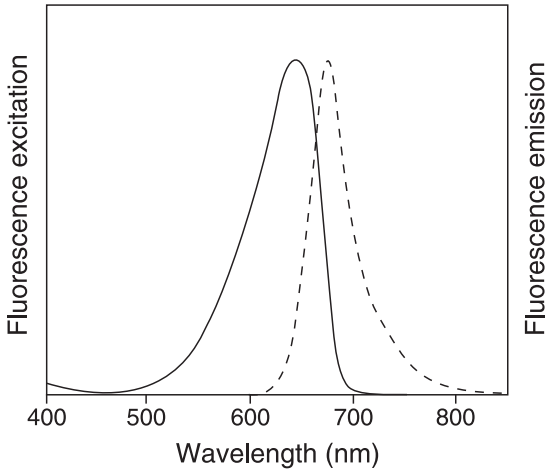

Figure 1. Excitation and emission maxima for the Quant-iT™ RNA reagent bound to RNA.

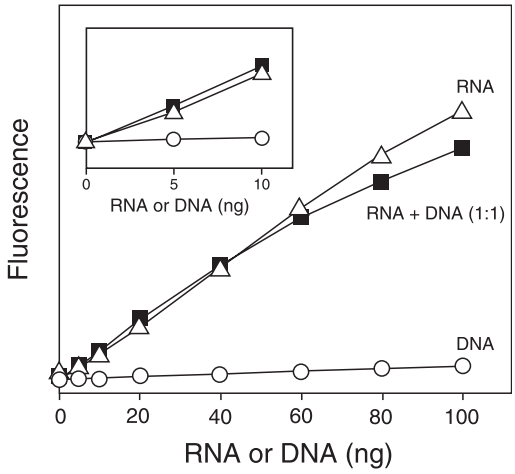

Figure 2. RNA selectivity and sensitivity of the Quant-iT™ RNA assay. Triplicate 10 µL samples of *E. coli* rRNA (△), λ DNA (○), or a 1:1 mixture of RNA and DNA (■) were assayed in the Quant-iT™ RNA assay. Fluorescence was measured at 630/680 nm and plotted versus the mass of nucleic acid for the RNA 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.

Before You Begin

Handling the Quant-iT™ reagent

There is no data are available addressing the mutagenicity or toxicity of the Quant-iT™ RNA reagent. 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. Dispose of the dye in accordance with local regulations.

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

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

This protocol describes the use of the Quant-iT™ RNA Assay Kit with a fluorescence micro-plate reader equipped with excitation and emission filters appropriate for the Quant-iT™ RNA reagent (excitation/emission maxima 644/673 nm; see Figure 1, page 2). Some contaminating substances may interfere with the assay; see *Contaminating substances*, page 7, for more information. For an overview of this procedure, see Figure 3, below.

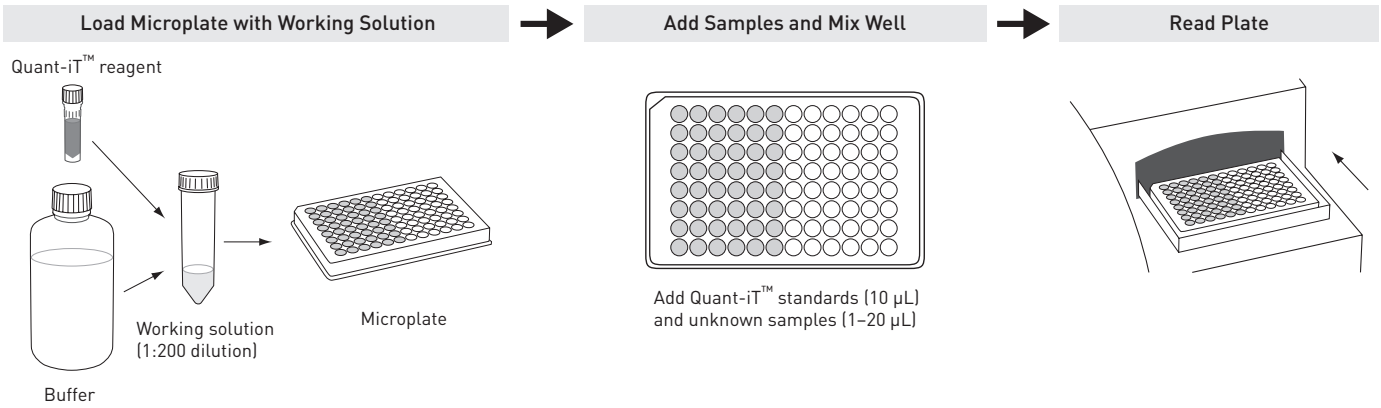

Figure 3. The Quant-iT™ RNA assay.

Assay procedure

1.1 Make a working solution by diluting Quant-iT™ RNA reagent 1:200 in Quant-iT™ RNA buffer. For example, for ~100 assays put 100 µL of Quant-iT™ RNA reagent (Component A) and 20 mL of Quant-iT™ RNA 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 buffer to make the working solution.

1.2 Load 200 µL of the working solution into each microplate well. Diluted Quant-iT™ RNA 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 the *Appendix*.

1.5 Measure the fluorescence using a microplate reader (excitation/emission maxima are 644/673 nm; see Figure 1, 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 reagent bound to RNA is extremely linear between 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 RNA.

To assess the reliability of the assay in the low range, use smaller volumes of the standards; for example, 2 µL volumes for a standard curve ranging from 0–20 ng. During development of the Quant-iT™ RNA 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[1] analysis). Your results may vary.

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

You can easily adapt the Quant-iT™ RNA Assay Kit 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**. 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.

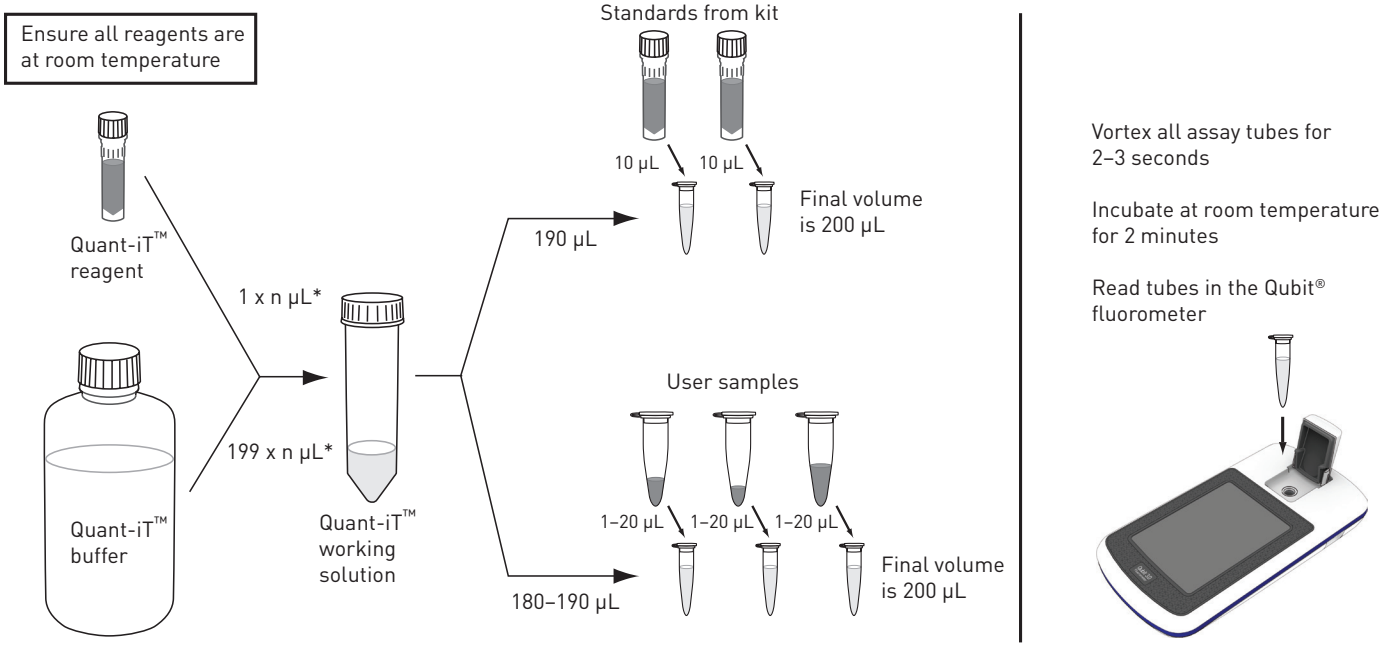

Figure 4. Overview for using the Quant-iT™ RNA assay in the Qubit® fluorometer.

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* for the standards and user samples.

Note: The Quant-iT™ RNA Assay Kit requires two standards for 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 prepare a dilution of the 10 ng/µL *E. coli* rRNA standard from the Component C set to generate Standard #2 (see step 2.3 below).

2.2 Prepare the Quant-iT™ RNA working solution by diluting the Quant-iT™ RNA reagent 1:200 in Quant-iT™ 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 the user 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 Flourometer performs this calculation automatically.

Appendix: Critical Assay Parameters

Assay temperature

The Quant-iT™ RNA 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 reagent and the Quant-iT™ RNA 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, as 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, as this will warm the solution and result in a low reading.**

Incubation time

To allow the Quant-iT™ RNA assay to reach maximum fluorescence, incubate the assay tubes for 2 minutes after mixing the sample or the 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 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, you should 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

Only thin-wall, clear 0.5 mL PCR tubes are appropriate for use in 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. *Using the Quant-iT™ RNA Assay Kit with the Qubit® Fluorometer* above describes the preparation of fresh calibration standards. Consult the instruction 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 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 Assay. *

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	1%	10%	20%	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

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

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

Cat. no.	Product name	Unit size
Q33140	Quant-iT™ RNA Assay Kit, 1000 assays *5–100 ng*	1 kit
Related products		
Q10213	Quant-iT™ RNA Assay Kit, Broad Range, 1000 assays *20–1000 ng*	1 kit
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
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
Q32856	Qubit® assay tubes	500 tubes

Purchaser Notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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