Quant-iT™ RNA Assay Kit

Catalog Number Q33140

Pub. No. MAN0002343 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 Quant-iT™ RNA Assay Kit makes RNA quantification easy and accurate. 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 ("Assay selectivity" on page 1). Common contaminants, such as salts, solvents, detergents, or protein are well tolerated in the assay (see "Effects of contaminating substances" on page 3). 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).

Contents and storage

Component	Amount	Concentration	Storage[1]
Quant-iT™ RNA reagent (Component A)	1.0 mL	200X in DMSO	2–8°C[2] Protect from light Desiccate
Quant-iT™ RNA buffer (Component B)	250 mL	NA	≤30°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	2–8°C[3] Avoid freeze/thaw cycles
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 ("Spectral data" on page 2)			

[1] When stored as directed, kit contents are stable for at least 6 months.
[2] For long-term storage, the Quant-iT™ 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

- Nuclease-free pipettors and tips
- Microplates for Fluorescence-based Assays, 96-well (Cat. No. M33089)

Critical assay parameters

Assay selectivity

The Quant-iT™ RNA assay is highly selective for RNA over double-stranded DNA (Figure 1).

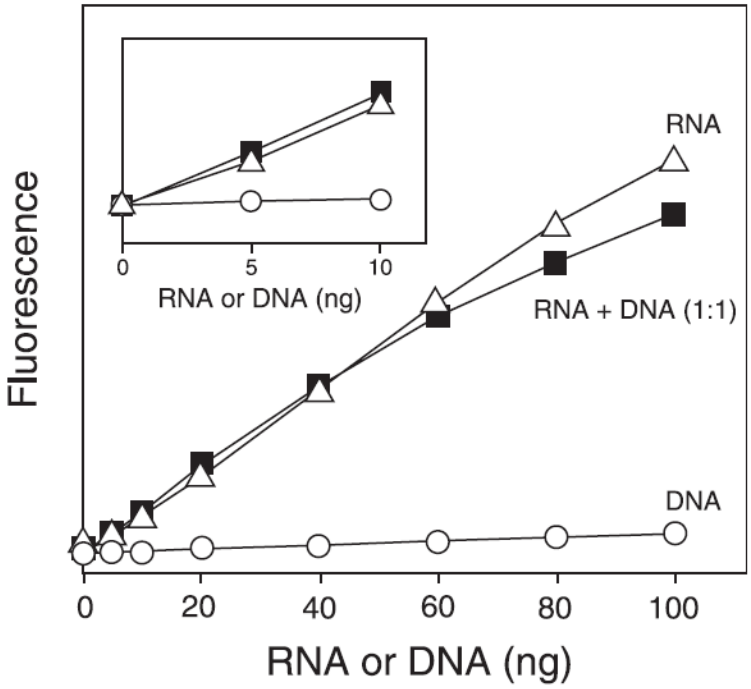

Figure 1 RNA selectivity and sensitivity of the Quant-iT™ RNA assay.
Triplicate 10 µL samples of *E. coli* rRNA (Δ), λ DNA (O), or a 1:1 mixture of RNA and DNA (■) were assayed in the Quant-iT™ RNA assay. Fluorescence was measured at 630/680 nm and plotted versus the mass of nucleic acid for the RNA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

Assay temperature

Quant-iT™ assays deliver optimal performance when all solutions are at room temperature; temperature fluctuations can influence the accuracy of the assay.

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.

Spectral data

The Quant-iT™ RNA reagent has an excitation/emission maxima of 644/673 nm when bound to RNA (Figure 2).

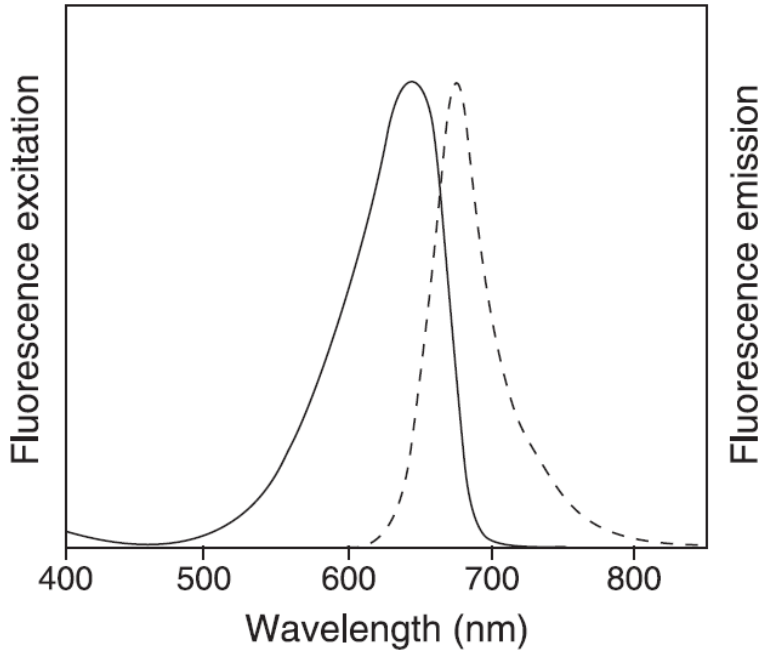

Figure 2 Excitation and emission maxima for the Quant-iT™ RNA reagent bound to RNA.

Photostability of the Quant-iT™ reagent

Avoid multiple sequential fluorescence reads to minimize reagent photobleaching.

Effects of contaminating substances

A number of common contaminants have been tested in the Quant-iT™ RNA assay, and most are well tolerated (Table 1). 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 1 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[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	1%	10%	20%	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
NTPs[5]	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

[1] *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.

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

Prepare and read samples and standards 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). For an overview of this procedure, see Figure 3.

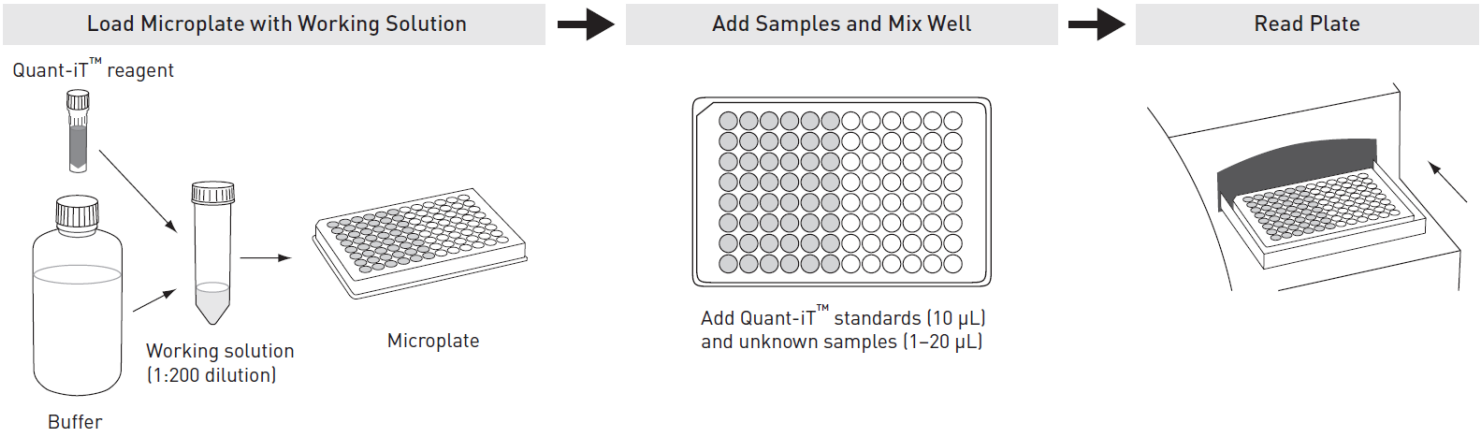

Figure 3 The Quant-iT™ RNA assay.

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

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.

   IMPORTANT! Do not use glass containers. Do not use buffers other than the Quant-iT™ RNA buffer to make the working solution.

2. 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.

3. 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 “Effects of contaminating substances” on page 3.

4. 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.

5. Measure the fluorescence using a microplate reader (excitation/emission maxima are 644/673 nm). The fluorescence signal is stable for 3 hours at room temperature.

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 up to 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 (e.g., 2 µL volumes for a standard curve ranging from 0–20 ng).

Related products

Table 2 Bulk Reagents and Kits

Product	Quantity	Cat. No.
Quant-iT™ PicoGreen™ dsDNA Assay Kit	1 mL assay kit 10 x 100 µL	P7589 P11496
Quant-iT™ PicoGreen™ dsDNA Reagent	1 mL reagent 10 x 100 µL	P7581 P11495
TE Buffer (20X), RNase-free	100 mL	T11493
Quant-iT™ RiboGreen™ RNA Assay Kit	1 mL assay kit	R11490
Quant-iT™ RiboGreen™ RNA Reagent	1 mL reagent	R11491
Quant-iT™ RediPlate™ 96 RiboGreen™ RNA Quantitation Kit	1 plate	R32700
Quant-iT™ OliGreen™ ssDNA Assay Kit	1 mL assay kit	O11492
Quant-iT™ OliGreen™ ssDNA Assay Reagent	1 mL reagent	O7582

Table 3 Microplate Reader Assays

Product	Dynamic Range	Quantity	Cat. No.
Quant-iT™ 1X dsDNA Assay Kit, High Sensitivity	200 pg–100 ng	1,000 reactions	Q33232
Quant-iT™ 1X dsDNA Assay Kit, Broad-Range	4 ng–2 µg	1,000 reactions	Q33267
Quant-iT™ DNA Assay Kit, High Sensitivity	200 pg–100 ng	1,000 reactions	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	4 ng–1 µg	1,000 reactions	Q33130
Quant-iT™ RNA Assay Kit	5–100 ng	1,000 reactions	Q33140
Quant-iT™ RNA Reagent	5–100 ng	1,000 reactions	Q32884
Quant-iT™ RNA Assay Kit, Broad Range	20 ng–1 µg	1,000 reactions	Q10213
Quant-iT™ RNA XR Assay Kit	200 ng–10 µg	1,000 reactions	Q33225
Quant-iT™ microRNA Assay Kit	1–100 ng	1,000 reactions	Q32882
Quant-iT™ Protein Assay Kit	250 ng–5 µg	1,000 reactions	Q33210
Microplates for Fluorescence-based Assays, 96-well	—	10 plates	M33089

Limited product warranty

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Revision history: Pub. No. MAN0002343

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