Quant-iT™ RNA XR Assay Kit

Catalog Number Q33225

Publication Number MAN0017581 Revision B.0

Product description

The Quant-iT™ RNA XR (Extended Range) Assay Kit makes quantification of total RNA, rRNA, or large mRNA easy and accurate. The kits include concentrated assay reagent, dilution buffer, and prediluted RNA standards. To perform the assay, dilute the reagent 1:200 in the buffer provided, load 200 µL into the wells of a microplate, add your sample (any volume from 1–20 µL is acceptable), mix, then read the fluorescence. The assay is highly selective for RNA over doublestranded DNA (dsDNA) and the fluorescence signal is linear with RNA in the range of 200 ng to 10 µg (Figure 2). 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 (Table 3).

The Quant-iT™ RNA XR 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).

Note: If you would like to use this kit with the Qubit™ Fluorometer, see "Assay procedure with the Qubit™ Fluorometer" on page 6.

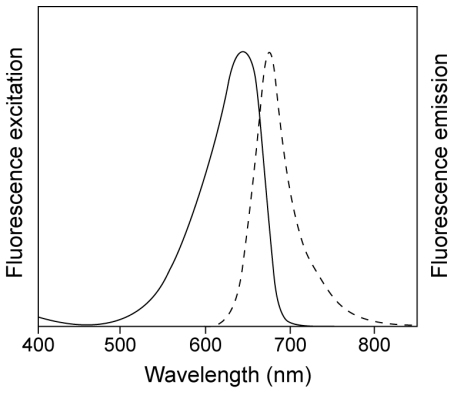

Figure 1 Excitation and emission maxima for the Quant-iT™ RNA XR Reagent bound to RNA.

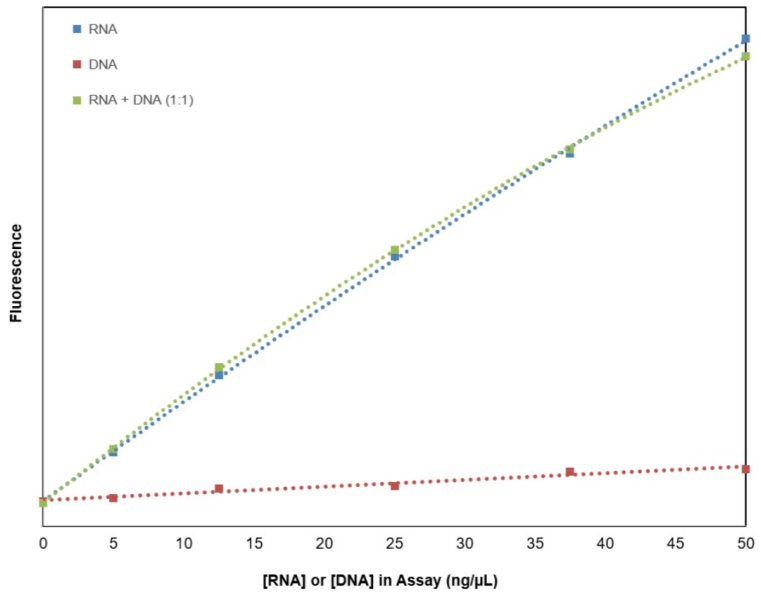

Figure 2 RNA selectivity of the Quant-iT™ RNA XR Assay Kit.

Triplicate 10-µL samples of Yeast tRNA (Blue), Calf Thymus DNA (Red) or a 1:1 mixture of RNA and DNA (Green) were assayed using the Quant-iT™ RNA XR Assay Kit. Fluorescence was measured at 630/660 nm and plotted versus the mass of nucleic acid for the RNA 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%.

Contents and storage

Table 1

Material	Amount	Concentration	Storage[1]
Quant-iT™ RNA XR Reagent (Component A)	1 mL	200X in DMSO	• < –20°C • Protect from light • Desiccate
Quant-iT™ RNA XR Buffer (Component B)[2]	225 mL	—	< –20°C or < –4°C
Yeast tRNA Standards (Component C)[3]	Set of 8 (500 µL each)	0, 50, 100, 200, 400, 600, 800, 1000 ng/µL	• < –80°C • Avoid freeze-thaw cycles

[1] When stored as directed, the kits are stable for at least 6 months from the date of receipt.

[2] The Quant-iT™ RNA XR Buffer is designed for refrigerator storage, although short-term storage (1 week) at room temperature is acceptable.

[3] The Quant-iT™ RNA XR Assay Kit standards are stable for shipment and short-term storage at ≤–20°C; long-term storage at ≤–80°C is recommended.

Before you begin

Materials required but not provided

- Sterile or nuclease-free plastic container (disposable) for mixing the Quant-iT™ working solution (Step 2, "Assay procedure with the Qubit™ Fluorometer" on page 6)
- Nuclease-free pipettors and tips
- 96-well microplate suitable for fluorescence-based assays (e.g., Cat. No. M33089)

Handling and disposal

No data are currently available that address the mutagenicity or toxicity of the Quant-iT™ RNA XR 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 XR Assay Kit from storage and allow the components to equilibrate to room temperature. During all steps, protect the Quant-iT™ RNA XR Reagent concentrate and the working solution from light as much as possible.

Storage

The Quant-iT™ RNA XR Buffer is designed for refrigerator storage, although short-term storage (1 week) at room temperature is acceptable. The Quant-iT™ RNA XR Reagent is supplied in DMSO, which freezes at temperatures lower than room temperature. Store the RNA standards at ≤–80°C. The Quant-iT™ RNA XR Reagent is sensitive to light. Store the vial in the dark when not in use.

RNase-free handling

The calibration standards included in the Quant-iT™ RNA XR Assay Kit are high quality RNA standards. The integrity and concentration of these standards is critical to the optimal performance of this kit. As such, treat the tRNA standards as you would any other precious RNA. Use appropriate RNase-free handling techniques, including RNase-free gloves, filtered 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. Thaw the RNA standards on ice, and return the RNA standard to the freezer as soon as possible after use. As a precaution against a possible RNase contamination of a standard vial, the RNA standards are supplied pre-aliquotted into multiple vials. If RNase contamination is suspected, discard the vial in question and use a new standard vial.

Use the Quant-iT™ RNA XR Assay Kit with a fluorescence microplate reader

This protocol describes the use of the Quant-iT™ RNA XR Assay Kit with a fluorescence microplate reader 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 "Contaminants tolerated by the Quant-iT™ RNA XR Assay" on page 8 for more information. For an overview of this procedure, see Figure 3.

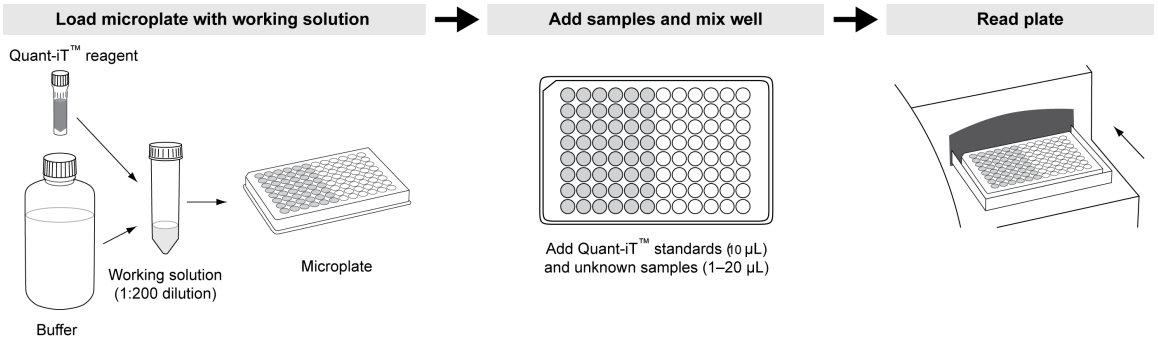

Figure 3 Overview of the Quant-iT™ RNA XR assay used with a microplate plate reader.

Assay procedure with a microplate reader

1. Prepare a working solution by diluting the Quant-iT™ RNA XR Reagent (Component A) 1:200 in Quant-iT™ RNA XR Buffer (Component B).

   For example, for ~100 assays, mix 100 µL of Quant-iT™ RNA XR Reagent and 20 mL of Quant-iT™ RNA XR Buffer in a disposable plastic container and mix well. Do not use glass containers. Do not use buffers other than the Quant-iT™ RNA XR Buffer to make the working solution.

2. Load 200 µL of the working solution into each microplate well. Diluted Quant-iT™ RNA XR Reagent is stable for at least 3 hours at room temperature, protected from light.

3. Add 10 µL of each Yeast tRNA 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.

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 "Contaminants tolerated by the Quant-iT™ RNA XR Assay" on page 8

5. Measure the fluorescence using a microplate reader (excitation/emission maxima are 644/673 nm; see Figure 1). The fluorescence signal is stable for 3 hours at room temperature.

6. Use a standard curve to determine the RNA amounts. For the Yeast tRNA standards, plot amount vs. fluorescence, and fit a straight line to the data points.

Use the Quant-iT™ RNA XR Assay Kit with the Qubit™ Fluorometer

The Quant-iT™ RNA XR Assay Kit 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 thermofisher.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 for an overview of the procedure.

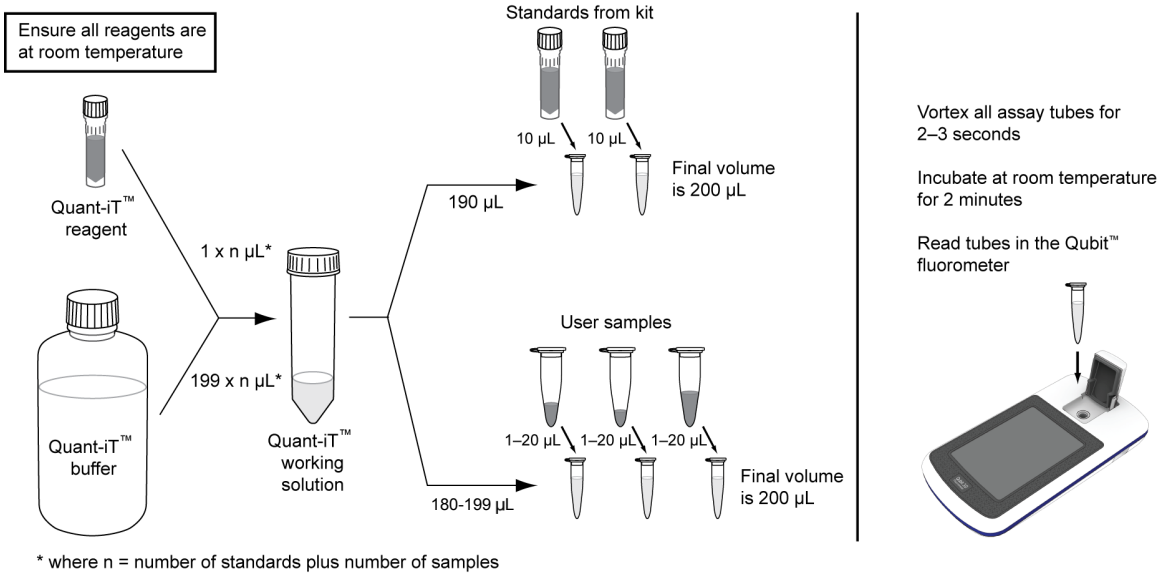

Figure 4 Overview of the Quant-iT™ RNA XR assay used with a Qubit™ Fluorometer.

Note: This assay is designed for use with Qubit™ 4 and Qubit™ Flex Fluorometers. These instruments may require a firmware update, for more information visit: thermofisher.com/qubit

Assay procedure with the Qubit™ Fluorometer

IMPORTANT! Ensure all assay reagents are at room temperature before you begin.

Use thin-wall, clear, 0.5-mL PCR tubes (Cat. No. Q32856) for the Qubit™ 4 and 8 x 200-µL tube strips (Cat. No. Q33252) for the Qubit™ Flex instrument.

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

   Note: The Quant-iT™ RNA XR Assay Kit requires two standards for the calibration of the Qubit™ Fluorometer. Prepare a dilution of the 0 mg/mL Yeast tRNA standard from the Component C set to generate Standard #1, and a dilution of the 1 mg/mL Yeast tRNA standard from the Component C set to generate Standard #2.

2. Prepare the Quant-iT™ RNA working solution by diluting the Quant-iT™ RNA XR Reagent 1:200 in Quant-iT™ RNA XR Buffer.

3. Prepare assay tubes according to Table 2.

   Table 2 Tube setup

	Standard Assay Tubes	User Sample Assay Tubes
Volume of working solution (Step 2, "Assay procedure with the Qubit™ Fluorometer" on page 6)	190 µL	180–199 µL
Volume of standard (from kit)[1]	10 µL	—
Volume of user sample to add	—	1–20 µL
Total volume in each assay tube	200 µL	200 µL

[1] Prepare Standard #1 by diluting 10 µL of the 0 ng/µL standard, and Standard #2 by diluting 10 µL of the 1,000 ng/µL standard.

4. Vortex all tubes for 2–3 seconds.

5. Incubate the tubes for 2 minutes at room temperature.

6. Calibrate the Qubit™ Fluorometer using Standard #1 and Standard #2.

7. Read your samples in the Qubit™ Fluorometer.

Critical assay parameters

Assay temperature

The Quant-iT™ RNA XR Assay Kit delivers optimal performance when all solutions are at room temperature (22–28˚C). Temperature fluctuations can influence the accuracy of the assay (Figure 5). To minimize temperature fluctuations, store the Quant-iT™ RNA XR Buffer at room temperature and insert all assay tubes into the microplate reader or the Qubit™ Fluorometer only for as much time as it takes for the instrument to measure the fluorescence; the instruments 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 different reading.

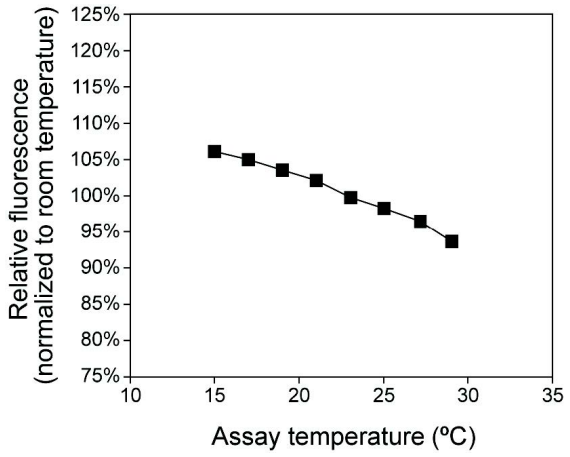

Figure 5 Plot of fluorescence vs. temperature for the Quant-iT™ RNA XR Assay Kit.

The Quant-iT™ assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

Incubation time

To allow the Quant-iT™ RNA XR Assay to reach optimal fluorescence, incubate the microplate or 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 XR Reagent exhibits high photostability, showing <0.3% drop in fluorescence after 9 readings and <1.5% drop in fluorescence after 40 readings on the Qubit™ Fluorometer. However, be careful to maintain temperature control over the assay plate or tubes, as the fluorescence output of the assay is sensitive to changes in temperature (Figure 5). If your plate reader does not possess temperature controls or is unable to indicate the temperature within the reader, store the microplate outside of the plate reader between reads if multiple readings are desired.

Contaminants tolerated by the Quant-iT™ RNA XR Assay

A number of common contaminants have been tested in the Quant-iT™ RNA XR Assay, and most are well tolerated (Table 3). For untested contaminating substances and in general, assay the standards 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 of these samples are used, then add 10 µL of the unusual buffer (lacking RNA) to the standards when performing the assay.

Table 3 Effect of contaminants in the Quant-iT™ RNA XR Assay

Contaminant	Final concentration in the assay	Concentration in 20-µL sample	Concentration in 10-µL sample	Result
Sodium chloride	50 mM	500 mM	1 M	OK
Magnesium chloride	1 mM	10 mM	20 mM	OK[1]
Sodium acetate	10 mM	100 mM	200 mM	OK
Ammonium acetate	50 mM	500 mM	1 M	OK
Potassium phosphate	10 mM	100 mM	200 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%	OK[1]
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

Contaminant	Final concentration in the assay	Concentration in 20-µL sample	Concentration in 10-µL sample	Result
NTPs	1X	1X	1X	OK
ssDNA	1X	1X	1X	OK
dsDNA	1X	1X	1X	OK

[1] At this concentration some distortion of the standard curve was noted, although results were within 10% of expected values. For higher accuracy either reduce the level of impurity present or add it at the same concentration to your standard solutions.

Note: RNA 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.

Related products

Product	Cat. No.
Quant-iT™ RNA XR Assay Kit, 1000 assays	Q33225
Qubit™ RNA XR Assay Kit, 500 assays	Q33224
Qubit™ RNA XR Standard	Q33236
Qubit™ RNA BR Assay Kit, 1000 assays	Q10210
Quant-iT™ RNA Assay Kit, broad range, 1000 assays	Q10213
Qubit™ Assay Tubes, 500 tubes	Q32856
Microplates for fluorescence-based assays, 96-well, black-walled, clear bottom	M33089
Qubit™ Flex Assay Tube Strips, 1 set	Q33252

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

Revision history: Pub. No. MAN0017581

Revision	Date	Description
B.0	14 May 2020	Adding edits and new images/figures.
A.0	24 January 2018	New user guide

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14 May 2020