# Quant-iT™ RiboGreen™ RNA Reagent and Kit

Catalog Numbers R11490, R11491, T11493

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**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™ RiboGreen™ RNA Reagent is an ultrasensitive fluorescent nucleic acid stain for quantitating RNA in solution. Detecting and quantitating small amounts of RNA is important in many applications including measuring yields of *in vitro* transcribed RNA and measuring RNA concentrations before performing Northern blot analysis, S1 nuclease assays, RNase protection assays, cDNA library preparation, reverse transcription PCR, and differential display PCR.

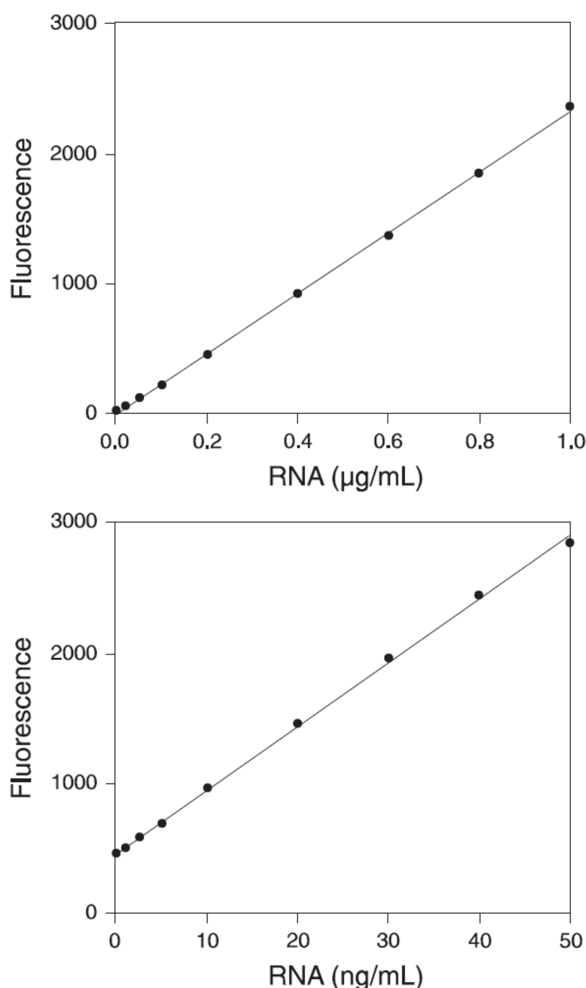


**Figure 1 Dynamic range and sensitivity of the Quant-iT™ RiboGreen™ RNA Assay.**
For the high-range assay (top panel), the Quant-iT™ RiboGreen™ RNA Reagent was diluted 200-fold into 10 mM Tris-HCl, 1 mM EDTA, pH 7.5 (TE) and 100 µL of the reagent solution was added to microplate wells containing 100 µL of ribosomal RNA in TE. For the low-range assay (bottom panel), the Quant-iT™ RiboGreen™ RNA Reagent was diluted 2,000-fold into TE and 100 µL of the reagent solution was added to microplate wells containing 100 µL of ribosomal RNA in TE. Samples were excited at 485 ± 10 nm and the fluorescence emission intensity was measured at 530 ± 12.5 nm using a fluorescence microplate reader. Fluorescence emission intensity was then plotted versus RNA concentration.

The Quant-iT™ RiboGreen™ RNA Reagent enables quantitation of as little as 1 ng/mL RNA (200 pg RNA in a 200 µL assay volume) with a fluorescence microplate reader using fluorescein excitation and emission wavelengths. The linear range of the Quant-iT™ RiboGreen™ RNA

Reagent extends over three orders of magnitude in RNA concentration (1 ng/mL to 1 µg/mL) using two dye concentrations (Figure 1). The high-range assay allows quantitation of 20 ng/mL to 1 µg/mL RNA, and the low-range assay allows quantitation of 1 ng/mL to 50 ng/mL RNA. This linearity is maintained in the presence of several compounds commonly found to contaminate nucleic acid preparations, including nucleotides, salts, urea, ethanol, chloroform, detergents, proteins, and agarose. Although the Quant-iT™ RiboGreen™ RNA Reagent also binds to DNA, pretreatment of mixed samples with DNase can be used to generate an RNA-selective assay (see "Eliminate DNA from samples" on page 5).

## Contents and storage

| Component | Quant-iT™ RiboGreen™ RNA Reagent[1] | Quant-iT™ RiboGreen™ RNA Assay Kit | Concentration | Storage[2] |
|---|---|---|---|---|
| | Cat. No. R11491 | Cat. No. R11490 | | |
| Quant-iT™ RiboGreen™ RNA Reagent (Component A) | 1 mL | 1 mL | Solution in DMSO | 2°C to 8°C[3]<br>Desiccate<br>Protect from light |
| 20X TE Buffer, RNase-free (Component B) | Not applicable | 25 mL | 200 mM Tris-HCl, 20 mM EDTA, pH 7.5 in DEPC-treated water | ≤30°C |
| Ribosomal RNA standard, 16S and 23S rRNA from *E. coli* (Component C) | Not applicable | 5 × 200 µL | 100 µg/mL in TE buffer | 2°C to 8°C[4]<br>Avoid freeze-thaw cycles |
| **Number of labelings:** 2,000 to 20,000 with an assay volume of 200 µL in a 96-well microplate format. The Quant-iT™ RiboGreen™ RNA Assay can be adapted for use in cuvettes or 384-well microplates. | | | | |
| **Approximate fluorescence excitation/emission maxima:** 500/525 nm, bound to nucleic acid. | | | | |

[1] Stand-alone reagent does not include Components B and C.
[2] When stored as directed, products are stable for at least 6 months.
[3] For long-term storage, the Quant-iT™ RiboGreen™ RNA Reagent can be stored at ≤–20°C
[4] For long-term storage, store the rRNA standards at ≤–20°C or –70°C.

## Required materials not supplied

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

## Prepare the assay buffer

Prepare the 1X TE working solution by diluting the concentrated buffer (Component B) 20-fold with nuclease-free water. Prepare nuclease-free water by treating distilled, deionized water with 0.1% diethyl pyrocarbonate (DEPC), incubating for several hours at 37°C, and autoclaving for at least 15 minutes at 15 lbs/sq. inch to sterilize the water and eliminate DEPC.

**IMPORTANT!** TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) is used to prepare the Quant-iT™ RiboGreen™ RNA Reagent and for diluting RNA standards and samples. Make sure the TE solution is free of contaminating nucleases and nucleic acids. The 20X TE buffer included in the Quant-iT™ RiboGreen™ RNA Assay Kit is nuclease-free and nucleic acid–free.

## Prepare the reagent

Two different dye concentrations are required to achieve the full linear dynamic range of the Quant-iT™ RiboGreen™ RNA Assay. Before preparing the working solution of the Quant-iT™ RiboGreen™ RNA Reagent, decide whether you wish to perform the **high-range** assay (20 ng/mL to 1 µg/mL RNA), **low-range** assay (1 ng/mL to 50 ng/mL RNA), or both.

On the day of the experiment, allow the Quant-iT™ RiboGreen™ RNA Reagent to warm to room temperature before opening the vial, then prepare an aqueous working solution of the Quant-iT™ RiboGreen™ RNA Reagent by diluting the concentrated DMSO stock solution (Component A) into TE, 200-fold for the **high-range** assay or 2,000-fold for the **low-range** assay. For microplate assays of a total 200 µL assay volume, you need 100 µL of the Quant-iT™ RiboGreen™ RNA Reagent working solution per sample.

**For example**, to prepare enough working solution to assay 100 samples in 200 µL volumes, add 50 µL Quant-iT™ RiboGreen™ RNA Reagent to 9.95 mL TE for the **high-range** assay or add 5 µL Quant-iT™ RiboGreen™ RNA Reagent to 9.995 mL TE for the **low-range** assay.

**Note:** Allow the Quant-iT™ RiboGreen™ RNA Reagent to warm to room temperature before opening the vial. Cold DMSO solutions absorb moisture from warmer, room temperature air, resulting in loss of efficacy for the reagent. Always store the DMSO stock solution in the

presence of desiccant when not in use. We recommend preparing the working solution in sterile, disposable polypropylene plasticware rather than glassware, as the reagent may adsorb to glass surfaces. Protect the working solution from light, as the Quant-iT™ RiboGreen™ RNA Reagent is susceptible to photodegradation. **For best results, use the working solution within a few hours of preparation.**

## Prepare the RNA standard curve

1. Prepare a 2 µg/mL solution of RNA in TE using nuclease-free plasticware. Determine the RNA concentration on the basis of absorbance at 260 nm ($A_{260}$) in a cuvette with a 1 cm pathlength; an $A_{260}$ of 0.05 corresponds to 2 µg/mL RNA.

   The ribosomal RNA standard (Component C), provided at 100 µg/mL in the Quant-iT™ RiboGreen™ RNA Assay Kit, is diluted 50-fold in TE to make the 2 µg/mL working solution. For example, 4 µL of the RNA standard mixed with 196 µL of TE is sufficient for the standard curve described in step 2.

   **Note:** For a standard curve, we commonly use 16S and 23S ribosomal RNA, although any purified RNA preparation may be used. It is sometimes preferable to prepare the standard curve with RNA similar to the type being assayed. However, most single-stranded RNA molecules yield approximately equivalent signals.

   **Note:** The RNA solution used to prepare the standard curve should be treated the same way as the experimental samples and should contain similar levels of contaminants. See “Effects of common contaminants” on page 4 for a list of contaminants tested in the Quant-iT™ RiboGreen™ assay.

2. For the **high-range** standard curve, dilute the 2 µg/mL RNA solution into microplate wells as shown in Table 1. For the **low-range** standard curve, dilute the 2 µg/mL RNA solution 20-fold into TE to make a 100 ng/mL RNA stock solution, then prepare the dilution series shown in Table 2.

   **Table 1 Protocol for preparing a high-range standard curve.**

| Volume of TE buffer | Volume of 2 µg/mL RNA stock | Volume of 200-fold diluted Quant-iT™ RiboGreen™ RNA Reagent | Final RNA concentration in the assay |
|---|---|---|---|
| 0 µL | 100 µL | 100 µL | 1 µg/mL |
| 50 µL | 50 µL | 100 µL | 500 ng/mL |
| 90 µL | 10 µL | 100 µL | 100 ng/mL |
| 98 µL | 2 µL | 100 µL | 20 ng/mL |
| 100 µL | 0 µL | 100 µL | blank |

   **Table 2 Protocol for preparing a low-range standard curve.**

| Volume of TE buffer | Volume of 100 ng/mL RNA stock | Volume of 2,000-fold diluted Quant-iT™ RiboGreen™ RNA Reagent | Final RNA concentration in the assay |
|---|---|---|---|
| 0 µL | 100 µL | 100 µL | 50 ng/mL |
| 50 µL | 50 µL | 100 µL | 25 ng/mL |
| 90 µL | 10 µL | 100 µL | 5 ng/mL |
| 98 µL | 2 µL | 100 µL | 1 ng/mL |
| 100 µL | 0 µL | 100 µL | blank |

3. Add 100 µL of the appropriate aqueous working solution of Quant-iT™ RiboGreen™ RNA Reagent (prepared in “Prepare the reagent” on page 2) to each microplate well. Use the high-range working solution for performing the high-range assay, and use the low-range working solution for performing the low-range assay. Mix well and incubate for 2–5 minutes at room temperature, protected from light.

4. Measure the sample fluorescence using a fluorescence microplate reader and standard fluorescein wavelengths (excitation ~480 nm, emission ~520 nm).

   **Note:** To ensure that the sample readings remain in the detection range, set the instrument's gain so that the sample containing the highest RNA concentration yields a fluorescence intensity near the microplate reader’s maximum. For optimal detection sensitivity, the instrument gain can be increased for the low-range assay relative to the high-range assay. To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

5. Subtract the fluorescence value of the reagent blank from that of each of the samples. Use corrected data to generate a standard curve of fluorescence versus RNA concentration (see Figure 1).

## Analyze samples

1. Dilute the experimental RNA solution in TE to a final volume of 100 µL in microplate wells.

   **Note:** You can alter the amount of sample diluted, provided that the final volume remains 100 µL. High dilutions of the experimental sample may serve to diminish the interfering effect of certain contaminants. However, extremely small sample volumes should be avoided because they are difficult to pipet accurately. In addition, the level of assay contaminants should be kept as uniform as possible throughout an experiment, to minimize sample-to-sample signal variation. For example, if a series of RNA samples contain widely differing salt concentrations, then they cannot be compared to a single standard curve. To avoid this problem, simply adjust the concentration of contaminants to be the same in all samples, if possible (see "Effects of common contaminants" on page 4).

2. Add 100 µL of the aqueous working solution of the Quant-iT™ RiboGreen™ RNA Reagent (prepared in "Prepare the reagent" on page 2) to each sample. Incubate for 2–5 minutes at room temperature, protected from light.

3. Measure the fluorescence of the samples using the same instrument parameters used to generate the standard curve (see step 4). To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

4. Subtract the fluorescence value of the reagent blank from that of each of the samples. Determine the RNA concentration of the sample from the standard curve generated in "Prepare the RNA standard curve" on page 3.

5. The assay can be repeated using a different dilution of the sample to confirm the quantitation results.

## Effects of common contaminants

The Quant-iT™ RiboGreen™ Assay remains linear in the presence of several compounds that commonly contaminate nucleic acid preparations, although the signal intensity may be affected (Table 3). For the highest accuracy, the standards should be prepared under the same conditions as the experimental samples and contain similar levels of contaminants.

**Table 3 Effects of common contaminants on the signal intensity of the assay.**

| Compound | Maximum acceptable concentration | % Signal change[1] |
|---|---|---|
| **Salts** | | |
| Ammonium acetate | 20 mM | 4% decrease |
| Sodium acetate | 20 mM | 11% decrease |
| Sodium chloride | 20 mM | 15% decrease |
| Zinc chloride | 1 mM | 9% decrease |
| Magnesium chloride | 0.5 mM | 9% decrease |
| Calcium chloride | 0.1 mM | 2% increase |
| Cesium chloride | 10 mM | 8% decrease |
| Guanidinium thiocyanate | 10 mM | 9% decrease |
| Urea | 3 M | 13% decrease |
| **Organic solvents** | | |
| Phenol | 0.5% | 5% decrease |
| Ethanol | 20% | 10% decrease |
| Chloroform | 2% | 15% increase |
| **Detergents** | | |
| Sodium dodecyl sulfate | 0.05% | 10% decrease |
| Triton™ X-100 | 0.5% | 8% decrease |
| **Proteins** | | |
| Bovine serum albumin | 0.2% | 11% decrease |
| IgG | 0.02% | 4% decrease |

| Compound | Maximum acceptable concentration | % Signal change[1] |
|---|---|---|
| **Other compounds** | | |
| Formamide | 10% | 12% decrease |
| Sucrose | >500 mM | 4% decrease |
| Boric acid | 100 mM | 15% decrease |
| Polyethylene glycol | 10% | 10% decrease |
| Agarose | 0.2% | 3% increase |

[1] The compounds were incubated at the indicated concentrations with the Quant-iT™ RiboGreen™ RNA Reagent in the presence of 1.0 mg/mL ribosomal RNA. All samples were assayed in a final volume of 200 µL in 96-well microplates using a fluorescence microplate reader. Samples were excited at 485 ± 10 nm and fluorescence intensity was measured at 530 ± 12.5 nm.

## Eliminate DNA from samples

The Quant-iT™ RiboGreen™ RNA Reagent also binds to DNA. Fluorescence in samples that is due to the Quant-iT™ RiboGreen™ RNA Reagent binding to DNA can be eliminated by pre-treating the sample with RNase-free DNase, ensuring that the entire sample fluorescence is due to dye bound to RNA.

1. Prepare 10X DNase digestion buffer: nuclease-free 200 mM Tris-HCl, pH 7.5, containing 100 mM $MgCl_2$ and 20 mM $CaCl_2$.
2. Add 0.11 volume of 10X DNase digestion buffer to each DNA-containing sample (for example, to a 9 µL sample, add 1 µL 10X buffer).
3. Add ~5 units of RNase-free DNase I per µg of DNA estimated to be in the sample.
4. Incubate the sample at 37°C for 90 minutes.
5. Dilute the sample at least 10-fold into TE to diminish effects of the digestion buffer salts on the Quant-iT™ RiboGreen™ assay procedure.
6. Perform the Quant-iT™ RiboGreen™ assay as described (see "Analyze samples" on page 4).

## Related products

**Table 4 Bulk Reagents and Kits**

| Product | Quantity | Cat. No. |
|---|---|---|
| Quant-iT™ PicoGreen™ dsDNA Assay Kit | 1 mL assay kit<br>10 x 100 µL | P7589<br>P11496 |
| Quant-iT™ PicoGreen™ dsDNA Reagent | 1 mL reagent<br>10 x 100 µL | P7581<br>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 5 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 |

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**Revision history:** Pub. No. MAN0002073

| Revision | Date | Description |
|---|---|---|
| A.0 | 15 March 2022 | The format and content were updated. The version numbering was reset to A.0 in conformance with internal document control. |
| 1.00 | 10 June 2008 | New document for the Quant-iT™ RiboGreen™ RNA Assay Kit and Quant-iT™ RiboGreen™ RNA Reagent. |

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