

## RiboGreen® RNA Quantitation Reagent And Kit

R-11491 RiboGreen® RNA Quantitation Reagent

R-11490 RiboGreen® RNA Quantitation Kit

### Quick Facts

#### Storage upon receipt:

- -20°C
- Desiccate
- Protect from light

**Ex/Em:** 500/525 nm, bound to nucleic acid

#### Number of assays:

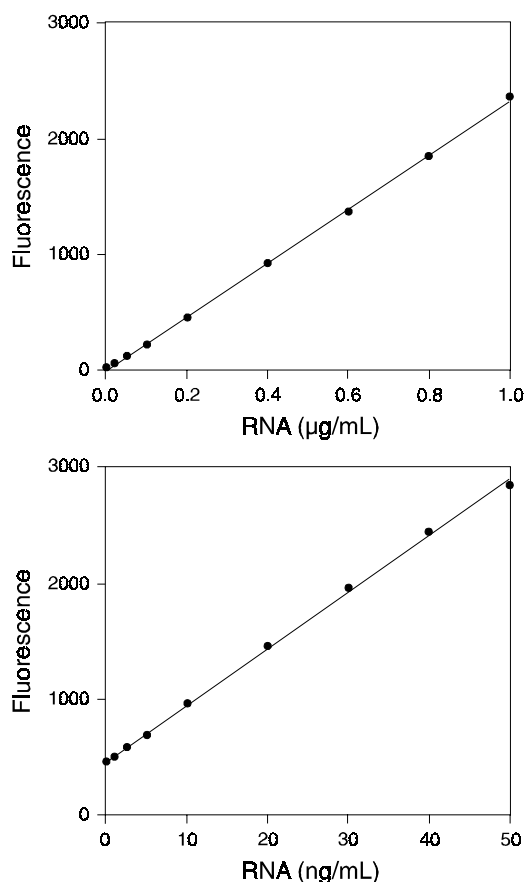
- 200–2000 cuvette assays
- 2000–20,000 microplate assays

### Introduction

RiboGreen® RNA quantitation reagent is an ultrasensitive fluorescent nucleic acid stain for quantitating RNA in solution. Molecular Probes offers the RiboGreen reagent either as a stand-alone product (R-11491) or as part of a convenient kit (R-11490), which also includes concentrated assay buffer and a ribosomal RNA standard. Detecting and quantitating small amounts of RNA is extremely important for a wide variety of molecular biology procedures. These include 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.

The most commonly used technique for measuring nucleic acid concentration is the determination of absorbance at 260 nm ( $A_{260}$ ). The major disadvantages of the absorbance-based method are the large relative contribution of proteins and free nucleotides to the signal, the interference caused by contaminants commonly found in nucleic acid preparations and the relative insensitivity of the assay (an  $A_{260}$  of 0.1 corresponds to a 4 µg/mL RNA solution). The use of sensitive, fluorescent nucleic acid stains alleviates many of these problems.

Our RiboGreen RNA quantitation reagent enables researchers to quantitate as little as 1 ng/mL RNA with a fluorescence microplate reader, standard spectrofluorometer or filter fluorometer, using fluorescein excitation and emission wavelengths.<sup>1</sup> The excitation maximum for RiboGreen reagent bound to RNA is ~500 nm and the emission maximum is ~525 nm. Using a fluorescence microplate reader, as little as 200 pg RNA can be detected in a 200 µL assay volume.<sup>1</sup> This sensitivity exceeds that



**Figure 1.** Dynamic range and sensitivity of the RiboGreen RNA quantitation assay. For the high-range assay (panel A), RiboGreen 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 ribosomal RNA in TE. For the low-range assay (panel B), RiboGreen reagent was diluted 2000-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  $485 \pm 10$  nm and the fluorescence emission intensity was measured at  $530 \pm 12.5$  nm using a fluorescence microplate reader. Fluorescence emission intensity was then plotted versus RNA concentration.

achieved with ethidium bromide-based assays<sup>2</sup> by 200-fold and exceeds that achieved with ultraviolet absorbance determination by 1000-fold. The linear range for quantitation with RiboGreen reagent extends over three orders of magnitude in RNA concentration — from 1 ng/mL to 1 µg/mL RNA — using two dye concentrations<sup>1</sup> (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.<sup>1</sup> In addition, although the RiboGreen reagent also binds to DNA, pretreatment of mixed samples with DNase can be used to generate an RNA-selective assay<sup>1</sup> (See *Eliminating DNA from Samples* at the end of this document).

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## Contents and Storage

### RiboGreen RNA Quantitation Reagent (R-11491)

RiboGreen RNA quantitation reagent is provided as a 1 mL concentrated dye solution in high-quality, anhydrous dimethylsulfoxide (DMSO). The DMSO stock solution should be stored frozen at -20°C and protected from light. Allow the reagent to warm to room temperature before opening vial. When stored properly, the RiboGreen reagent is stable for at least one year.

Sufficient RiboGreen reagent is supplied for 200 high-range assays or 2000 low-range assays with a sample volume of 2 mL. With a microplate reader and a 96-well microplate, the assay volume is reduced to 200 µL and 2000 high-range assays or 20,000 low-range assays can be performed.

### RiboGreen RNA Quantitation Kit (R-11490)

- **RiboGreen RNA quantitation reagent** (Component A), 1 mL solution in DMSO
- **20X TE** (Component B), 25 mL of 200 mM Tris-HCl, 20 mM EDTA, pH 7.5 (20X TE) in DEPC-treated water
- **Ribosomal RNA standard** (16S and 23S rRNA from *E. coli*; Component C), five vials, each containing 200 µL of a 100 µg/mL solution in TE

The RiboGreen reagent supplied in the kit is exactly the same as the reagent sold separately; as described above, the DMSO stock solution should be stored frozen at -20°C and protected from light. The ribosomal RNA standard should be stored at 4°C for daily use and frozen at -20°C or -70°C for long-term storage. Avoid repeated freeze-thaw cycles, which can result in strand scission. The 20X TE should be stored at room temperature. Sufficient RiboGreen reagent is supplied for 200 to 2000 assays using a 2 mL assay volume or 2000 to 20,000 assays using a 200 µL assay volume in 96-well microplates. Sufficient 20X TE assay buffer is supplied for 200 assays using a 2 mL assay volume or 2000 assays using a 200 µL assay volume.

### Handling and Disposal

Care should be taken to prevent RNase contamination of the RiboGreen reagent and kit components. Clean disposable gloves should be worn while handling all materials. **Caution:** no data are available addressing the mutagenicity or toxicity of RiboGreen RNA quantitation reagent. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling the DMSO stock solution. As with all nucleic acid reagents, solutions of RiboGreen reagent should be poured through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dye.

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## Experimental Protocol

### Overview

Two different dye concentrations are required to achieve the full linear dynamic range of the RiboGreen RNA quantitation assay. Before preparing the working solution of RiboGreen reagent (see *Reagent Preparation* below), 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. Described below are protocols for performing both procedures. In addition, the procedure below has 2 mL assay volumes and is designed for use with standard fluorescence cuvettes. To perform microplate assays, reduce the indicated volumes appropriately. For example, 200 µL assay volumes are recommended for use with 96-well microplates.

### Assay Buffer Preparation

TE assay buffer, 10 mM Tris-HCl, 1 mM EDTA, pH 7.5, is used for diluting the RiboGreen reagent and for diluting RNA samples. It is imperative that the TE solution used is free of contaminating nucleases and nucleic acids. Clean disposable gloves should be worn during handling and preparation of all materials and solutions. All solutions should be prepared in sterile disposable plasticware or nuclease-free glassware, using nuclease-free pipettes. The 20X TE buffer that is included in the RiboGreen RNA Quantitation Kit is nuclease-free and nucleic acid-free. Prepare the 1X TE working solution by diluting the concentrated buffer (Component B) 20-fold with nuclease-free water. Nuclease-free water should be prepared 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.

**Caution: DEPC is a suspected carcinogen and should be handled with care.** Compounds containing amines, such as Tris, will react rapidly with DEPC and should be added to DEPC treated water only after DEPC is removed by heating. Removal of DEPC by heating is also important to prevent carboxyethylation of the RNA sample.<sup>3</sup>

### Reagent Preparation

On the day of the experiment, prepare an aqueous working solution of the RiboGreen reagent by diluting an aliquot of the concentrated DMSO stock solution into TE. If performing the **high-range** assay, dilute 200-fold. For example, to prepare enough working solution to assay 20 samples in 2 mL volumes, add 100 µL RiboGreen RNA quantitation reagent to 19.9 mL TE. If performing the **low-range** assay, dilute 2000-fold. For example, to prepare enough working solution to assay 20 samples in 2 mL volumes, add 10 µL RiboGreen RNA quantitation reagent to 20.0 mL TE. We recommend preparing these solutions in sterile, disposable, polypropylene plasticware rather than glassware, as the reagent may adsorb to glass surfaces. Protect the working solutions from light by covering them with foil or placing them in the dark, as the RiboGreen reagent is susceptible to photodegradation. *For best results, these solutions should be used within a few hours of their preparation.*

### RNA Standard Curves

**1.1** 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 path-length; an  $A_{260}$  of 0.05 corresponds to 2 µg/mL RNA. For a stan-

**Table 1.** Effects of several compounds that commonly contaminate nucleic acid preparations on the signal intensity of the RiboGreen RNA quantitation assay

Compound	Maximum Acceptable Concentration	% Signal Change *
<b>Salts</b>		
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
<b>Organic Solvents</b>		
Phenol	0.5%	5% decrease
Ethanol	20%	10% decrease
Chloroform	2%	15% increase
<b>Detergents</b>		
Sodium dodecyl sulfate	0.05%	10% decrease
Triton X-100	0.5%	8% decrease
<b>Proteins</b>		
Bovine serum albumin	0.2%	11% decrease
IgG	0.02%	4% decrease
<b>Other Compounds</b>		
Formamide	10%	12% decrease
Sucrose	>500 mM	4% decrease
Boric acid	100 mM	15% decrease
Poly(ethylene glycol)	10%	10% decrease
Agarose	0.2%	3% increase
* The compounds were incubated at the indicated concentrations with RiboGreen reagent in the presence of 1.0 mg/mL ribosomal RNA. All samples were assayed in a final volume of 200 $\mu$ L in 96-well microplates using a fluorescence microplate reader. Samples were excited at $485 \pm 10$ nm and fluorescence intensity was measured at $530 \pm 12.5$ nm.		

dard curve, we commonly use 16S and 23S ribosomal RNA, although any purified RNA preparation may be used. The ribosomal RNA standard, provided at 100  $\mu$ g/mL in the RiboGreen Kit, can simply be diluted 50-fold in TE to make the 2  $\mu$ g/mL working solution. For example, 40  $\mu$ L of the RNA standard mixed with 1.96 mL of TE will be sufficient for the standard curve described below. It is sometimes preferable to prepare the standard curve with RNA similar to the type being assayed. However, we have found that most single-stranded RNA molecules yield approximately equivalent signals. Our results have shown that the assay remains linear in the presence of several compounds that commonly contaminate nucleic acid preparations, although the signal intensity may be affected (Table 1).

Thus, to serve as an effective control, 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 such compounds.

**1.2** For the **high-range** standard curve, dilute the 2  $\mu$ g/mL RNA solution into disposable cuvettes (or nuclease-free plastic test tubes for transfer to quartz cuvettes or microplates) as shown in Table 2. For the **low-range** standard curve, dilute the 2  $\mu$ g/mL RNA solution 20-fold into TE to make a 100 ng/mL RNA stock solution, and then prepare the dilution series shown in Table 3.

**1.3** Add 1.0 mL of the appropriate aqueous working solution of RiboGreen reagent (prepared in *Reagent Preparation*) to each cuvette. The **high-range** working solution should only be used for performing the **high-range** assay and the **low-range** working solution should only be used for performing the **low-range** assay. Mix well and incubate for 2 to 5 minutes at room temperature, protected from light.

**1.4** Measure the sample fluorescence using a spectrofluorometer or fluorescence microplate reader and standard fluorescein wavelengths (excitation  $\sim$ 480 nm, emission  $\sim$ 520 nm). To ensure that the sample readings remain in the detection range of the fluorometer, set the instrument's gain so that the sample containing the highest RNA concentration yields a fluorescence intensity near the fluorometer's maximum. For optimal detection sensitivity, the instrument gain may 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.

**1.5** 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.

### Sample Analysis

**2.1** Dilute the experimental RNA solution in TE to a final volume of 1.0 mL in disposable cuvettes or test tubes. You may wish to prepare more than one dilution of the experimental sample. 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

**Table 2.** Protocol for preparing high-range standard curve.

Volume ( $\mu$ L) of TE	Volume ( $\mu$ L) of 2 $\mu$ g/mL RNA Stock	Volume ( $\mu$ L) of 200-fold Diluted RiboGreen Reagent	Final RNA Concentration in RiboGreen Assay
0	1000	1000	1 $\mu$ g/mL
500	500	1000	500 ng/mL
900	100	1000	100 ng/mL
980	20	1000	20 ng/mL
1000	0	1000	blank

standard curve. To avoid this problem, simply adjust the concentration of contaminants to be the same in all samples, if possible. See *Eliminating DNA from Samples* for information on eliminating DNA from the sample.

**2.2** Add 1.0 mL of the aqueous working solution of the RiboGreen reagent (prepared in *Reagent Preparation*) to each sample. Incubate for 2 to 5 minutes at room temperature, protected from light.

**2.3** Measure the fluorescence of the sample using instrument parameters that correspond to those used when generating your standard curve (see step 1.4). To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

**2.4** 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 *RNA Standard Curves*.

**2.5** The assay may be repeated using a different dilution of the sample to confirm the quantitation results.

### ***Eliminating DNA from Samples***

RiboGreen reagent also binds to DNA. Fluorescence in samples that is due to RiboGreen 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.

**3.1** Prepare 10X DNase digestion buffer: nuclease-free 200 mM Tris-HCl, pH 7.5, containing 100 mM MgCl<sub>2</sub> and 20 mM CaCl<sub>2</sub>.

**Table 3.** Protocol for preparing low-range standard curve.

Volume (μL) of TE	Volume (μL) of 100 ng/mL RNA Stock	Volume (μL) of 2000-fold Diluted RiboGreen Reagent	Final RNA Concentration in RiboGreen Assay
0	1000	1000	50 ng/mL
500	500	1000	25 ng/mL
900	100	1000	5 ng/mL
980	20	1000	1 ng/mL
1000	0	1000	blank

**3.2** Add 0.11 volume of 10X DNase digestion buffer to each DNA-containing sample (for example, to a 9 μL sample, add 1 μL 10 X buffer).

**3.3** Add ~5 units of RNase-free DNase I per μg of DNA thought to be in the sample.

**3.4** Incubate the sample at 37°C for 90 minutes.

**3.5** Dilute the sample at least 10-fold into TE to diminish effects of the digestion buffer salts on the RiboGreen assay procedure.

**3.6** Perform the RiboGreen assay as described above.

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## ***References***

1. Anal Biochem 265, 368 (1998); 2. Anal Biochem 17, 100 (1966); 3. Sambrook, J., Fritsch, E.F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor Laboratory Press (1989).

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## ***Product List*** Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
R-11490	RiboGreen® RNA Quantitation Kit *200-2000 assays* .....	1 kit
R-11491	RiboGreen® RNA quantitation reagent *200-20,000 assays* .....	1 mL
T-11493	20X TE buffer *RNase free* .....	100 mL

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## Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — [www.probes.com](http://www.probes.com) — for the most up-to-date information

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