RediPlate™ 96 RiboGreen® RNA Quantitation Kit (R-32700)

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Desiccate
- Protect from light

Ex/Em: 500/525 nm

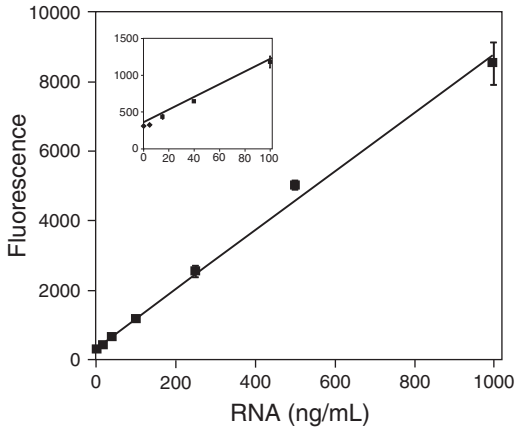

Figure 1. Dynamic range and sensitivity of the RediPlate 96 RiboGreen RNA Quantitation assay. The provided RNA standards were added in quadruplicate to assay wells as described in the text, and fluorescence was measured in a fluorescence microplate reader using excitation at 485 ± 12.5 nm and fluorescence detection at 530 ± 15 nm. The fluorescence signals were plotted against the RNA concentration with no background subtraction. The inset shows the sensitivity of the assay at very low levels of RNA.

Introduction

The RediPlate™ 96 RiboGreen® RNA Quantitation Kit provides materials for a rapid, simple and highly sensitive fluorescence-based RNA-quantitation assay. The microplate included with the kit has been preloaded with our patented RiboGreen reagent, which shows bright green fluorescence upon binding to RNA or DNA.[1] For an RNA determination, the user simply adds buffer and samples to the micro-plate wells, waits 10 minutes, and then reads the fluorescence in any standard fluorescence- based microplate reader. The fluorescence of the sample is compared to that of a standard curve of RNA, prepared from rRNA prealiquoted into one column of the plate. Pretreatment of mixed samples with DNase can be used to generate an RNA-selective assay [1] (See *Eliminating DNA from Samples* at the end of this document). The versatile RediPlate 96 design is ideal for either high-throughput quantitation or the measurement of a small number of samples. The RediPlate 96 RiboGreen RNA Quantitation Kit includes one microplate in a reseal-able foil packet and a bottle of RNase-free reaction buffer. The microplate consists of twelve removable strips, each with eight wells. Eleven of the strips (88 wells) are preloaded with the RiboGreen reagent. The remaining strip, marked with black tabs, contains a series of RNA standards for generating standard curves.

Advantages

- **Simple.** A 10-minute incubation time with no separation steps makes the assay ideal for automated, high-throughput measurements.
- **Sensitive and quantitative**. The assay has a linear range of ~15–1000 ng/mL (~3–200 ng in a 200 µL assay volume) with a single dye concentration (Figure 1).
- **Accurate.** Unlike measurements of UV absorbance, the assay is not affected by the presence of proteins or free nucleotides. The assay remains linear in the presence of many compounds commonly found to contaminate nucleic acid preparations, including salts, urea, ethanol, chloroform, detergents, proteins and agarose.[1]
- **Precise.** The average coefficient of variation for replicate assays is ≤5% using a robotic liquid handling system.
- **Convenient.** The RediPlate 96 RiboGreen RNA Quantitation Kit is ready for immediate use. Each plate includes premeasured RNA standards.
- **Easy to Read.** The assay can be performed using a fluorescence-based microplate reader with standard filters optimized for fluorescein-like dyes.
- **Safe.** There is no need to handle the RiboGreen reagent, a potentially mutagenic compound.

Applications

The RediPlate 96 RiboGreen RNA Quantitation Kit is a new, convenient packaging of our standard RiboGreen RNA quantitation reagent. The RiboGreen assay is ideal for measuring yields of RNA preparations for microarray experiments,[2] reverse transcription PCR (RT-PCR),[3] differential display PCR, Northern blot analysis, S1 nuclease assays, RNase protection assays [4] and cRNA library preparation.

Materials

Kit Contents

- RediPlate 96 RiboGreen RNA quantitation microplate (Component A), one microplate
- RediPlate TE buffer (Component B), 28 mL of 10 mM Tris-HCl, 1mM EDTA, pH 7.5

Storage and Handling

Store the RediPlate 96 RiboGreen RNA Quantitation Kit at ≤–20°C, desiccated and protected from light. 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 and solutions. All solutions should be prepared in sterile disposable plasticware or nuclease-free glassware, using nuclease-free pipettes. When stored and handled properly, the kit components should remain stable for at least six months.

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 and disposed of properly.

Experimental Protocol

The following protocol describes the RNA quantitation assay in total volumes of 200 µL per microplate well. Each RediPlate 96 RiboGreen RNA Quantitation Kit contains one 96-well plate with 88 wells (11 strips) intended for assays, and 8 wells (1 strip, with black tabs) containing RNA for generating standard curves. Because each strip is removable, one can perform as many or as few assays as needed.

RediPlate 96 Microplate and Buffer Preparation

1.1 Allow the kit components to warm to room temperature. Remove the RediPlate 96 RiboGreen RNA Quantitation Kit from the freezer and allow it to warm to room temperature. DO NOT OPEN THE FOIL PACKET UNTIL IT IS WARM. The plate (Component A) will typically take about 20 minutes to warm. Remove the plate from the foil packet by tearing or cutting above the resealable seal. Because the TE buffer may take longer than 20 minutes to thaw at room temperature, place the vial of buffer in a warm water bath to accelerate thawing. After thawing, the buffer may be stored at 2–6°C, for convenience.

1.2 Remove any extra strips. Determine the number of strips required and carefully cut through the self-adhesive sealing film using a razor blade and remove any extra strips that are to be used at a later date. Store the extra strips at ≤–20°C in the foil packet with the included desiccator pack until future use. All of the strips, with the exception of the control strip with black tabs, contain equivalent amounts of the RiboGreen reagent. Empty strip holders from previously purchased RediPlate 96 kits are useful for storing extra assay strips.

RediPlate 96 RiboGreen RNA Quantitation Assay

The RediPlate RiboGreen RNA assay is easy to use with RNA samples from almost any source — simply add buffer and the appropriate amount of the RNA sample to each well. The order of addition is not critical; it is only important that the final volume of solution in each well be 200 µL and that the amount of RNA added be within the linear range of the assay (~15–1000 ng/mL or ~3–200 ng in a 200 µL assay volume). The assay can thus be easily adapted for different types of liquid handlers and different concentrations of RNA samples, as described in the protocol below. Please consult Application Tips at the end of this document for further suggestions on optimizing the RediPlate 96 RiboGreen RNA assay.

Regardless of the method used, an RNA standard curve must be employed to convert the observed fluorescence into RNA concentration units. The first strip of 8 wells (column 1, with black tabs) contains varying amounts of rRNA to use for the standard curve. Sufficient RNA is provided to generate up to 4 strips of standards, to use in making duplicate or triplicate readings for the standard curve or to use in different assays on different days.

2.1 Prepare the RNA standard samples. Add 100 µL of RediPlate TE buffer (Component B) to each well in column 1 (with black tabs) and mix by pipetting up and down ~10–15 times. It is important to wait ≥5 minutes before use to completely rehydrate the RNA. After rehydration, mix thoroughly again.

2.2 Add TE buffer to the assay wells. Add 180 µL of the provided RediPlate TE buffer (Component B) to as many assay wells as will be needed for all experimental samples and standard curve samples. Mix well.

- In order for very dilute RNA solutions to fall within the linear range of the assay, it may be necessary to decrease the amount of TE buffer added in this step to accommodate larger volumes of the RNA samples (see step 2.4).
- The RiboGreen reagent is not stable in stored aqueous solutions. Therefore, use wells containing the redissolved RiboGreen reagent within a few hours of preparation; do not save them for another day.

2.3 Add the RNA standards to the assay wells. Pipet 20 µL of RNA from each of the standard RNA wells (prepared in step 2.1) into the assay wells. Mix well. Use one strip of wells for a single determination. If desired, use a second or third strip for duplicate or triplicate determinations. Unused diluted RNA standards may be removed from the plate and stored at 4°C for use in a later experiment. The amounts of RNA provided in the standard curve strip and the final concentrations of the diluted RNA standards in the assay wells can be found in Table 1. Note that the last RNA standard (well H) contains no RNA and will serve as the no-RNA control to measure background fluorescence.

2.4 Add the experimental samples to the assay wells. Add 5–20 µL of the RNA samples to the assay wells. Mix well.

Table 1. RNA standards provided in column 1 of the RediPlate 96 RiboGreen RNA quantitation microplate.

Well	Mass of dried RNA standard in well	Concentration of reconstituted RNA standard (step 2.1)	Concentration of RNA standard in assay (step 2.3)
A	1000 ng	10,000 ng/mL	1000 ng/mL
B	500 ng	5000 ng/mL	500 ng/mL
C	250 ng	2500 ng/mL	250 ng/mL
D	100 ng	1000 ng/mL	100 ng/mL
E	40 ng	400 ng/mL	40 ng/mL
F	15 ng	150 ng/mL	15 ng/mL
G	5 ng	50 ng/mL	5 ng/mL
H	0 ng	0 ng/mL	0 ng/mL

- In order for very concentrated RNA samples to fall within the linear range of the assay, it may be necessary to dilute a portion of the RNA sample before adding it to the assay.
- In order for very dilute RNA solutions to fall within the linear range of the assay, it may be necessary to add a larger volume of the RNA sample solution in this step and decrease the amount of TE buffer added to the assay wells (in step 2.2) accordingly.
- To achieve the highest accuracy, the final volume in each well should be as close to 200 µL as possible. The volume can be adjusted by adding varying amounts of TE buffer.

2.5 Incubate the samples. Incubate the loaded microplate for 10 minutes at room temperature, protected from light. The samples may be incubated between 5 and 20 minutes. However, it is important to incubate all samples for approximately the same length of time.

2.6 Read the fluorescence. Use a fluorescence-based microplate reader with excitation light and filter settings set for standard fluorescein wavelengths (excitation ~480 nm, emission ~520 nm). The RiboGreen reagent bound to RNA has excitation/emission maxima of approximately 500/525 nm.

2.7 Correct for background fluorescence. For each value of sample fluorescence, subtract the value derived from the no-RNA control.

2.8 Determine the amounts of RNA. Using the data from the RNA standards, plot the amount of RNA versus the fluorescence intensity and fit a line to the data points. Use the standard curve to determine the amount of RNA from the fluorescence intensity measured for each sample.

Application Tips

Working within the Dynamic Range of the Assay

In order for the RediPlate 96 RiboGreen assay to be accurate, the amount of RNA in the assay well must fall within the linear range of the assay (~15–1000 ng/mL or ~3–200 ng in the 200 µL assay volume). If the approximate RNA concentration is not known, use a few different dilutions of the sample to ensure that one of the dilutions will fall within the linear range of the assay. Figure 1 shows a typical standard curve produced using the standards provided with the RediPlate 96 RiboGreen microplate. Note that although ≤15 ng/mL is the lowest concentration of RNA that can be detected in this assay, it may not be possible to reliably attain this limit of sensitivity with all microplate readers.

Optimizing the Fluorescence Signal

To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples. To ensure that the sample readings remain in the detection range of the fluorometer, the instrument's gain should be set so that the sample containing the highest RNA concentration yields a fluorescence intensity near the fluorometer's maximum.

Accurate RNA Quantitation

The standards included in the kit are ribosomal RNA (rRNA) from *E. coli*. This standard is ideal for measuring total RNA samples. However, the RiboGreen reagent does show some sequence specificity, and poly(A)+ RNA generally shows fluorescence signals that are ~19–26% lower than those of total RNA from the same source.[1] The assay is relatively independent of fragment size, however the fluorescence signal starts to drop with fragment sizes of ~100 bases or less.[1] To attain the most accurate RNA quantitations, use a standard curve derived from a dilution series of RNA that closely matches the RNA present in the experimental samples, rather than from the provided RNA standards. See also *Correcting for Contaminants in the Sample*, below.

Table 2. 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 *
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
Other Compounds		
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 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.

Correcting for Contaminants in the Sample

The RiboGreen assay remains linear in the presence of several compounds that commonly contaminate nucleic acid preparations, although the signal intensity may be affected (Table 2). 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. Note that a higher dilution of the experimental sample will minimize the interfering effect of certain contaminants, although extremely small sample volumes should be avoided because they are difficult to pipet accurately.

Eliminating DNA from Samples

The RiboGreen quantitation reagent binds to both RNA and DNA. Fluorescence due to the RiboGreen reagent binding to DNA can be eliminated by treating the sample with RNasefree DNase I (~5 units of DNase for every 1 µg thought to be in the sample) in 20 mM Tris-HCl, 10 mM $MgCl_2$, 2 mM $CaCl_2$, pH 7.5 at 37°C for 90 minutes.[1] Dilute the sample at least 10-fold into TE to diminish effects of the digestion buffer salts on the RiboGreen assay procedure. For the most accurate readings, the RNA standards should be treated in the same way.

References

1. Anal Biochem 265, 368 (1998); **2.** Nucl Acids Res 29, e41 (2001); **3.** Biochem J 359, 507 (2001); **4.** J Immunol 167, 2869 (2001).

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

Cat #	Product Name	Unit Size
R-32700	RediPlate™ 96 RiboGreen® RNA Quantitation Kit *one 96-well microplate*	1 kit

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.

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