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LiQuant™ RiboGreen RNA Assay Kit

Cat. #: M0135 Size: 1000 assays

Introduction

LiQuant™ RiboGreen RNA Assay Kit is one of the most sensitive detection kits for quantitation of RNA in solution, with linear fluorescence detection in the range of 1-200 ng of RNA. The LiQuant™ 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. The linear range of the LiQuant™ RiboGreen RNA Reagent extends over three orders of magnitude in RNA concentration (1 ng/mL to 1 µg/mL) using two dye concentrations. 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. The assay kit contains RiboGreen RNA Reagent, assay buffer, and RNA standard. The assay is well tolerated to common contaminants such as proteins, salts, nucleotides, urea, ethanol, chloroform, detergents, and agarose.

Package Information

Components	M0135
LiQuant™ RiboGreen RNA Reagent	1 ml, Solution in DMSO
LiQuant™ RNA Buffer	200 ml
RNA Standard	1 ml, 100 ng/µl in TE buffer

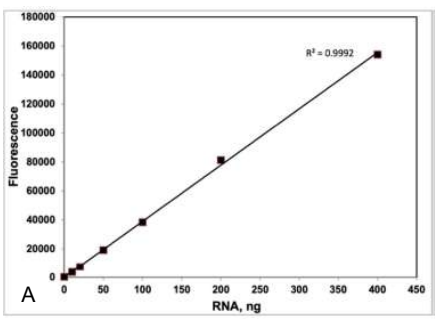
Approximate fluorescence excitation/emission maxima, in nm: 500/525, bound to RNA

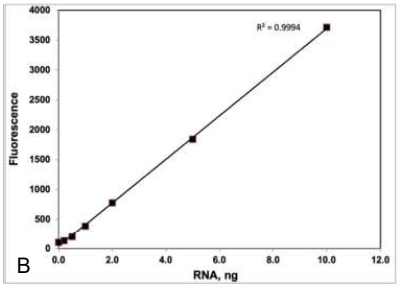
Storage

Store at 2-8°C and protect from light.

Handling and Disposal

There is no safety data available for LiQuant™ RiboGreen RNA reagent. Treat the LiQuant™ RiboGreen RNA reagent with the safety precautions as other potentially harmful reagents and to dispose of the reagent in accordance with local regulations. Centrifuge the LiQuant™ RiboGreen RNA reagent and theRNA standard before opening vials to minimize loss on the cap. Use properly calibrated pipettes for best accuracy.





Dynamic range and sensitivity of the LiQuant™ RiboGreen RNA Assay

For the high-range assay (Figure A), the LiQuant™ RiboGreen RNA Reagent was diluted 200-fold with assay buffer.

For the low-range assay (Figure B), the LiQuant™ RiboGreen RNA Reagent was diluted 2,000-fold with assay buffer.

Protocol

Measure RNA samples using a Fluorescence Microplate Reader

1. Prepare the reagent

Two different dye concentrations are required to achieve the full linear dynamic range of the LiQuant™ RiboGreen RNA Assay. Before preparing the working solution of the LiQuant™ 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 LiQuant™ RiboGreen RNA Reagent to warm to room temperature before opening the vial, then prepare an aqueous working solution of the LiQuant™ RiboGreen RNA Reagent by diluting the concentrated DMSO stock solution with RNA Buffer, 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 LiQuant™ 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 LiQuant™ RiboGreen RNA Reagent to 9.95 mL RNA Buffer for the high-range assay or add 5 µL LiQuant™ RiboGreen RNA Reagent to 9.995 mL RNA Buffer for the low-range assay.

Note: Allow the LiQuant™ RiboGreen RNA Reagent to warm to room temperature before opening the vial. 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 LiQuant™ RiboGreen RNA Reagent is susceptible to photodegradation. For best results, use the working solution within a few hours of preparation.

2. Prepare the RNA standard curve

2.1 Prepare a 2 µg/mL solution of RNA in TE using nuclease-free plasticware. Dilute the RNA standard 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.

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2.2 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 100 ng/mL RNA stock	Volume of 2000-fold diluted RiboGreen Reagent	Final RNA concentration
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	0 ng/mL

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

Volume of TE buffer	Volume of 100 ng/mL RNA stock	Volume of 2000-fold diluted RiboGreen Reagent	Final RNA concentration
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	0 ng/mL

2.3 Add 100 µL of the appropriate aqueous working solution of LiQuant™ RiboGreen RNA Reagent (prepared in “Prepare the reagent”) 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 lowrange assay. Mix well and incubate for 2-5 minutes at room temperature, protected from light.

2.4 Measure the fluorescence using a fluorescence microplate reader (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.

2.5 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.

3. Analyze samples

3.1 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.

3.2 Add 100 µL of the aqueous working solution of the LiQuant™ RiboGreen RNA Reagent (prepared in “Prepare the reagent”) to each sample. Incubate for 2-5 minutes at room temperature, protected from light.

3.3 Measure the fluorescence of the samples using the same instrument parameters used to generate the standard curve. To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

3.4 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”.

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

Measure RNA samples using the Qubit® Fluorometer

1. Prepare standards and samples

1.1 Prepare RNA standards as shown in Table 3.

Table 3. Standard preparation

Standard	Volume of TE buffer	Volume of 100 ng/µL RNA stock	Final RNA concentration
Standard #1	1000 µL	0 µL	0 ng/µL
Standard #2	800 µL	200 µL	20 ng/µL

1.2 Set up the required number of LiQuant tubes for standards and samples, and label the tube lids.

1.3 Prepare the LiQuant working solution by diluting the LiQuant ™ RiboGreen RNA Reagent 1:200 in LiQuant™ RNA Buffer. Use a clean plastic tube each time you prepare the LiQuant working solution.

1.4 Add the LiQuant working solution to each tube such that the final volume is 200 µL.

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	Standard assay tubes	Sample assay tubes
Volume of working solution	190 µL	180-199 µL
Volume of standard	10 µL	-
Volume of samples	-	1-20 µL
Total volume in each assay tube	200 µL	200 µL

Note: The final volume in each tube should be 200 µL. Each standard tube requires 190 µL of LiQuant working solution, and each sample tube requires anywhere from 180-199 µL. Prepare sufficient LiQuant working solution to accommodate all standards and samples.

1.5 Add 10 µL of Standard #1 and #2 to the appropriate tube.

1.6 Add 1-20 µL of each sample to the appropriate tube.

1.7 Vortex for 3-5 seconds, and incubate at room temperature for 2 minutes.

2. Read standards and samples

Before reading the standards and samples, the LiQuant™ RiboGreen RNA Assay file need be uploaded to your Qubit 3 or 4 Fluorometer. The LiQuant™ RiboGreen RNA Assay file can be downloaded from our website.

Once imported New Assay, on the home screen, select RNA RiboGreen Assay as the assay type. Then, follow the instruction to run standard calibration and measure samples.