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iQuant™ RiboGreen RNA Assay Kit

(1 – 200 ng)

Catalog Number: N025

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
iQuant™ RiboGreen RNA Reagent (Component A)	1 mL	Solution in DMSO	2-8 °C Protect from light	The product is stable for at least 6 months when stored as directed.
iQuant™ RNA Buffer (Component B)	200 mL	1X		
RNA Standard (Component C)	1 mL	100 ng/µL in TE buffer		

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

Product Description

The iQuant™ 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 iQuant™ 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 iQuant™ 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. 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.

Handling and Disposal

There is no safety data available for iQuant™ RiboGreen RNA reagent. Treat the iQuant™ 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 iQuant™ RiboGreen RNA reagent and the RNA standard before opening vials to minimize loss on the cap. Use properly calibrated pipettes for best accuracy.

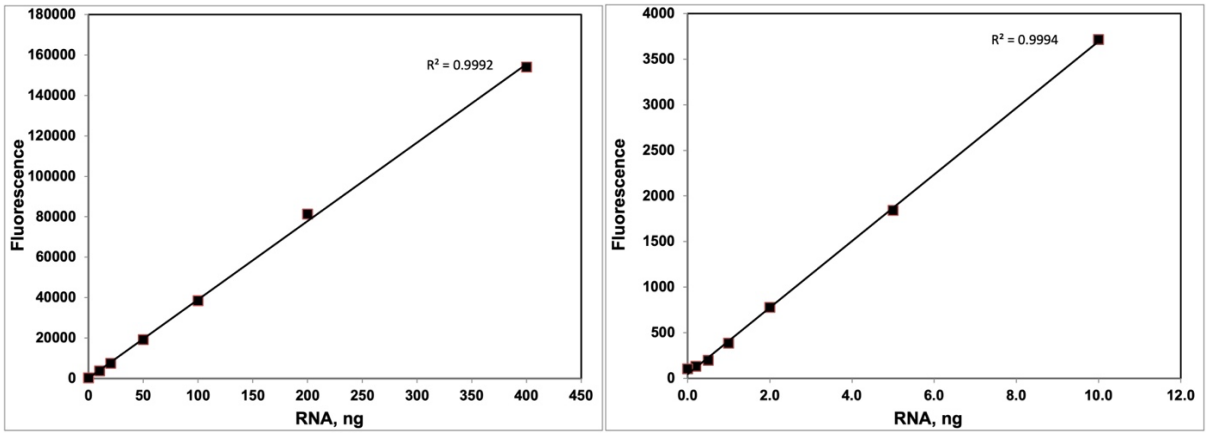

Figure 1. Dynamic range and sensitivity of the iQuant™ RiboGreen RNA Assay .
For the high-range assay (left panel), the iQuant™ RiboGreen RNA Reagent was diluted 200-fold with assay buffer.
For the low-range assay (right panel), the iQuant™ RiboGreen RNA Reagent was diluted 2,000-fold with assay buffer.

General Protocol

Prepare the reagent

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

Note: Allow the iQuant™ 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 iQuant™ 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. Dilute the RNA standard (Component C) 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.
2. For the **high-range** standard curve, dilute the 2 µg/mL RNA solution into microplate wells as shown in Table 2. 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 3.

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

Volume of TE buffer	Volume of 2 µg/mL RNA stock	Volume of 200-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 3. 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

3. Add 100 µL of the appropriate aqueous working solution of iQuant™ 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 low-range assay. Mix well and incubate for 2-5 minutes at room temperature, protected from light.
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.
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.

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.
2. Add 100 µL of the aqueous working solution of the iQuant™ RiboGreen RNA Reagent (prepared in "Prepare the reagent") 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. 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".
5. The assay can be repeated using a different dilution of the sample to confirm the quantitation results.

Appendix

Effects of common contaminants

The iQuant™ RiboGreen RNA Assay remains linear in the presence of several compounds that commonly contaminate nucleic acid preparations, although the signal intensity may be affected (Table 4). For the highest accuracy, the standards should be prepared under the same conditions as the experimental samples and contain similar levels of contaminants.

Table 4. Effects of common contaminants on the signal intensity of the assay.

Contaminant	Maximum acceptable concentration	% Signal change
Sodium Chloride	20 mM	15% decrease
Ammonium Acetate	20 mM	5% decrease
Sodium Acetate	20 mM	11% decrease
Magnesium chloride	0.5 mM	9% decrease
Guanidinium thiocyanate	10 mM	9% decrease
Urea	3 M	13% decrease
Ethanol	20%	10% decrease
Chloroform	2%	15% decrease
Phenol	0.5%	5% decrease
Sodium Dodecyl Sulfate	0.05%	10% decrease
Triton X-100	0.5%	8% decrease
BSA	0.2%	11% decrease
Formamide	10%	12% decrease
Polyethylene glycol	10%	10% decrease
Agarose	0.2%	3% increase

Related Products

Cat. No.	Product Name	Unit Size
N010	iQuant™ dsDNA HS Assay Kit	200 assays
N011	iQuant™ dsDNA HS Assay Kit	1000 assays
N012	iQuant™ dsDNA BR Assay Kit	200 assays
N013	iQuant™ dsDNA BR Assay Kit	1000 assays
N014	iQuant™ ssDNA Assay Kit	200 assays
N015	iQuant™ ssDNA Assay Kit	1000 assays
N016	iQuant™ RNA HS Assay Kit	200 assays
N017	iQuant™ RNA HS Assay Kit	1000 assays
N018	iQuant™ RNA BR Assay Kit	200 assays
N019	iQuant™ RNA BR Assay Kit	1000 assays
N020-1	iQuant™ 1X dsDNA HS Assay Kit	200 assays
N020-2	iQuant™ 1X dsDNA HS Assay Kit	500 assays
N022	iQuant™ Assay Tubes	500 tubes
N023	iQuant™ microRNA Assay Kit	200 assays
N024	iQuant™ microRNA Assay Kit	1000 assays
N025	iQuant™ RiboGreen RNA Assay Kit	1000 assays