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LiQuta™ RNA BR Assay Kit

Cat. #: M0069 Size: 200/1000 assays

Introduction

LiQuta™ RNA BR Assay Kit provides a simple, sensitive, and accurate quantitation for RNA. The kit contains concentrated assay reagent, dilution buffer, and pre-diluted RNA standards. The assay kit is highly selective for RNA over dsDNA, and highly reliable for initial sample concentrations from 1 ng/µL to 1000 ng/µL, and offers advantages in stability, linear dynamic range, and sensitivity over other traditional of RNA quantitation. The assay is performed at room temperature, and the signal is stable for 3 hours. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µl and 50 µl is acceptable), and read the fluorescence using fluorescence plate reader or Fluorometer such as Qubit® Fluorometer. The kit is well tolerated to common contaminants such as proteins, free nucleotides, salts, solvents and detergents.

Package Information

Components	M0069-02	M0069-10
LiQuta™ RNA BR Reagent	200 µl	1 ml
LiQuta™ RNA BR Buffer	50 ml	200 ml
RNA Standard #1	200 µl	1 ml
RNA Standard #2	200 µl	1 ml

Approximate fluorescence excitation/emission maxima, in nm: 640/660, bound to RNA

Storage

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

Handling and Disposal

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

Protocol

Measure RNA samples using a Fluorescence Microplate Reader

Note: For simplicity, the following protocol is written using 10 µL of RNA sample volume. In practice, the volume of RNA sample could be ranging from 1 µL to 50 µL depending on the concentration of RNA sample, then adjust the volume of LiQuta™ working solution to 200 µL.

1. Warm up the LiQuta™ RNA BR Assay Kit to room temperature.

2. Prepare the LiQuta™ working solution by diluting the LiQuta™ RNA BR reagent 1:200 in 1× LiQuta™ RNA BR Buffer. **IMMEDIATELY** before use. Use a clean plastic tube each time you make LiQuta™ working solution. For example, to measure 8 samples in duplicate, add 20 µL of LiQuta™ RNA BR reagent to 4 mL of 1× LiQuta™ RNA BR Buffer. Mix well and use immediately.

3. Add 190 µL of the LiQuta™ working solution to each well of a black 96-well microplate. Black plates such as Greiner or Corning black 96-well plates are recommended to minimize fluorescence bleed-through from other well.

4. Prepare a series of RNA standard dilutes from RNA Standard #2 or your known RNA sample.

5. Add 10 µL of each RNA standard dilutes and the unknown RNA samples in duplicate or triplicates into separated wells and mix well by pipetting up and down.

6. Incubate the microplate at room temperature for 2 minutes in the dark.

7. Measure the fluorescence using a microplate reader with 620 nm excitation and 660 nm emission, with the appropriate cut-off.

8. Generate a linear standard curve by plotting fluorescence versus RNA concentration of the RNA standards. Use the standard curve and the fluorescence of the unknown RNA samples to determine the unknown RNA concentration.

Measure RNA samples using the Qubit® Fluorometer from ThermoFisher

Note: For simplicity, the following protocol is written using 10 µL of RNA sample volume. In practice, the volume of RNA sample could be ranging from 1 µL to 50 µL depending on the concentration of RNA sample, then adjust the volume of LiQuta™ working solution to 200 µL.

1. Warm up the LiQuta™ RNA BR Assay Kit to room temperature.

2. Prepare the LiQuta™ working solution by diluting the LiQuta™ RNA BR reagent 1:200 in 1× LiQuta™ RNA BR Buffer **IMMEDIATELY** before use. Use a clean plastic tube each time you make LiQuta™ working solution. For example, to measure 8 samples in duplicate, add 10 µL of LiQuta™ RNA BR reagent to 2 mL of 1× LiQuta™ RNA BR Buffer. Mix well and use immediately.

3. Add 190 µL of the LiQuta™ working solution to each assay tube.

Note: Use only thin-wall, clear 0.5 mL PCR tubes. Axygen PCR-05-C tubes.

4. Add 10 µL of RNA standard #1, RNA standard #2, and the unknown RNA samples to the appropriate tubes and mix by vortexing 2-3 seconds, and label the lids of each RNA standard tube and unknown sample tubes correctly.

FOR RESEARCH USE ONLY

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5 Incubate all tubes at room temperature for 2 minutes in the dark.

6. Measure the fluorescence on the Qubit® fluorometer using the RNA: Broad Range program, according to the manufacture's recommendation.

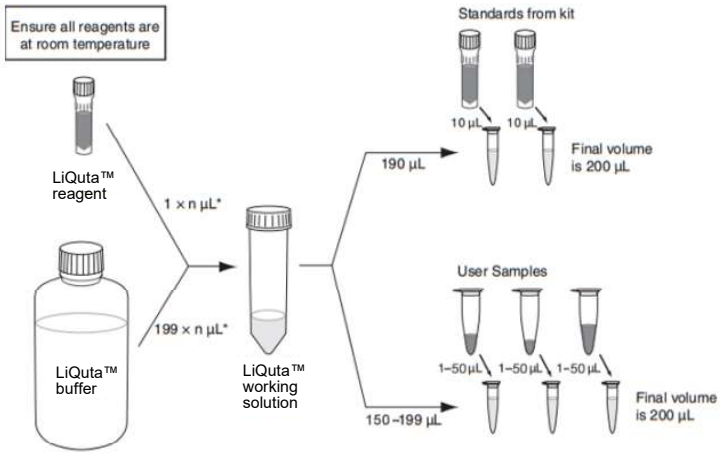
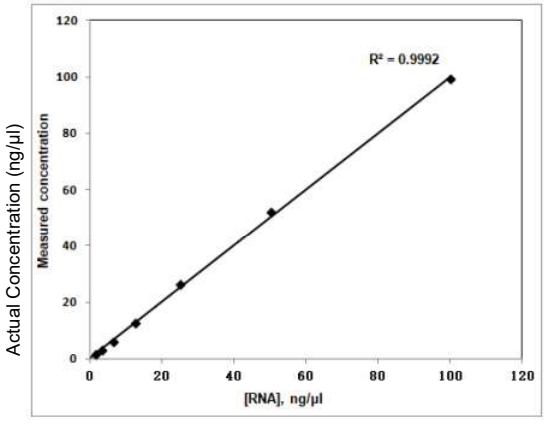


Figure 1. LiQuta™ RNA BR Assay workflow



Calculated Concentration (ng/µl)

Figure 2. The quantitation of rRNA with LiQuta™ RNA BR Assay Kit using Qubit® Fluorometer.

Considerations for Data Analysis

It is more prefer to use a RNA standard similar to the unknown samples (i.e. similar in size, linear vs circular). We found using the LiQuta™ RNA BR reagent most RNA yield similar results. If the fluorescence of an unknown sample is higher than RNA standard #2, further dilute the sample and add 10 µL of diluted sample to perform the assay.

Effect of Contaminants in the LiQuta™ RNA BR Assay

Contaminant	Final Concentration in Assay	Concentration in 10 µL Sample	Result
Proteins			
Bovine Serum Albumin	20 µg/mL	400 µg/mL	OK
Salts			
Sodium Chloride	10 mM	200 mM	OK
Magnesium Chloride	2 mM	40 mM	OK
Sodium Acetate	10 mM	200 mM	OK
Ammonium Acetate	10 mM	200 mM	OK
Organic Solvents			
Ethanol	1%	20%	OK
Chloroform	0.1%	2%	OK
Phenol	0.1%	2%	OK
Detergents			
Sodium Dodecyl Sulfate	0.01%	0.2%	OK
Triton X-100	0.001%	0.02%	OK
Other Compounds			
dNTPs	100 µM	2 mM	OK
DNA	1X	1X	OK
NTPs	1X	1X	OK
Oligos	1X	1X	OK