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iQuant™ RNA BR Assay Kit
(20 – 1000 ng)
Catalog Number: N018, N019

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
iQuant™ RNA BR Assay Kit (Cat. No. N018)				The product is stable for at least 6 months when stored as directed.
iQuant™ RNA BR Reagent (Component A)	200 µL	200X in DMSO	2-8 °C Protect from light	
iQuant™ RNA BR Buffer (Component B)	50 mL	1X		
RNA Standard #1 (Component C)	200 µL	0 ng/µL in TE buffer		
RNA Standard #2 (Component D)	200 µL	100 ng/µL in TE buffer		
iQuant™ RNA BR Assay Kit (Cat. No. N019)				
iQuant™ RNA BR Reagent (Component A)	1 mL	200X in DMSO	2-8 °C Protect from light	
iQuant™ RNA BR Buffer (Component B)	200 mL	1X		
RNA Standard #1 (Component C)	1 mL	0 ng/µL in TE buffer		
RNA Standard #2 (Component D)	1 mL	100 ng/µL in TE buffer		

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

Product Description

The iQuant™ 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 (Figure 1), 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 (Table 2).

To determine the purity of your sample, use the iQuant™ RNA BR Assay Kit together with the iQuant™ dsDNA BR Assay Kit. These measurements give you a much better indication of sample purity than that produced by measuring the A_{260}/A_{280} ratio.

Handling and Disposal

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

General Protocol

1. 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 iQuant™ working solution to 200 μL.)

1.1 Warm up the iQuant™ RNA BR Assay Kit to room temperature.

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

1.3 Add 190 μL of the iQuant™ 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.

1.4 Prepare a series of RNA standard dilutes from RNA Standard #2 (Component D) or your known RNA sample.

1.5 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.

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

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

1.8 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.

2. Measure RNA samples using the Qubit® Fluorometer from Life Technologies

(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 iQuant™ working solution to 200 μL.)

2.1. Warm up the iQuant™ RNA BR Assay Kit to room temperature.

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

2.3. Add 190 μL of the iQuant™ working solution to each assay tube. (**Note:** Use only thin-wall, clear 0.5 mL PCR tubes. Axygen PCR-05-C tubes (VWR, Cat No. 1011-830)).

2.4. Add 10 μL of RNA standard #1 (Component C), RNA standard #2 (Component D), 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.

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

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

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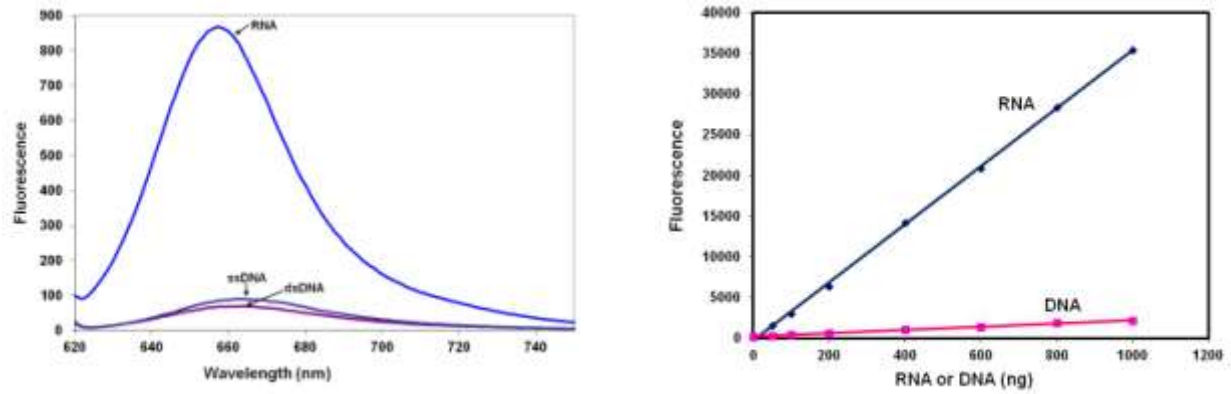

Figure 1. iQuant™ RNA HS Reagent shows great selectivity for RNA over DNA.

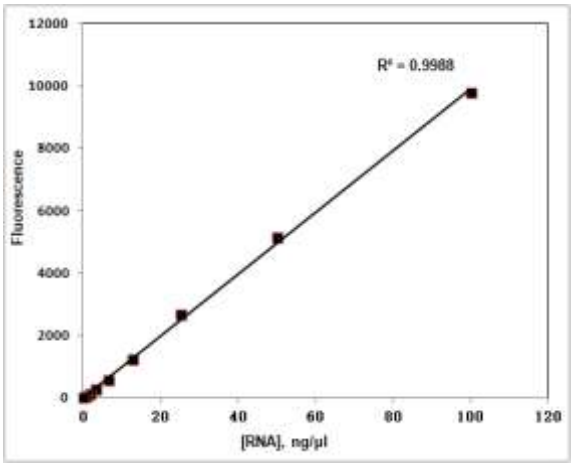

Figure 2. The quantitation of rRNA with iQuant™ RNA BR Assay Kit using fluorescence plate reader.

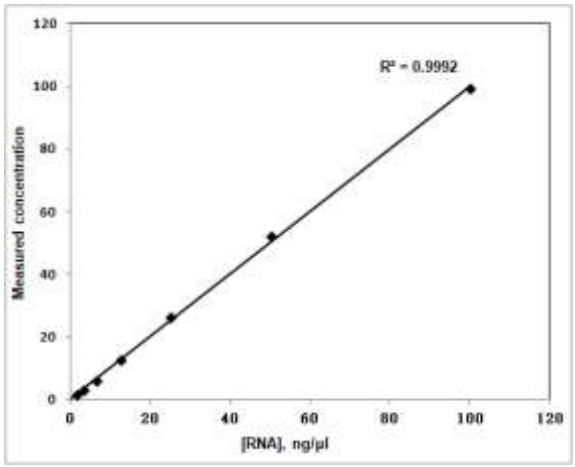

Figure 3. The quantitation of rRNA with iQuant™ RNA BR Assay Kit using Qubit® Fluorometer.

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 iQuant™ BR RNA reagent most RNA yield similar results. If the fluorescence of an unknown sample is higher than RNA standard #2 (Component D), further dilute the sample and add 10 µL of diluted sample to perform the assay.

Appendix

Table 2. Effect of Contaminants in the iQuant™ 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

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

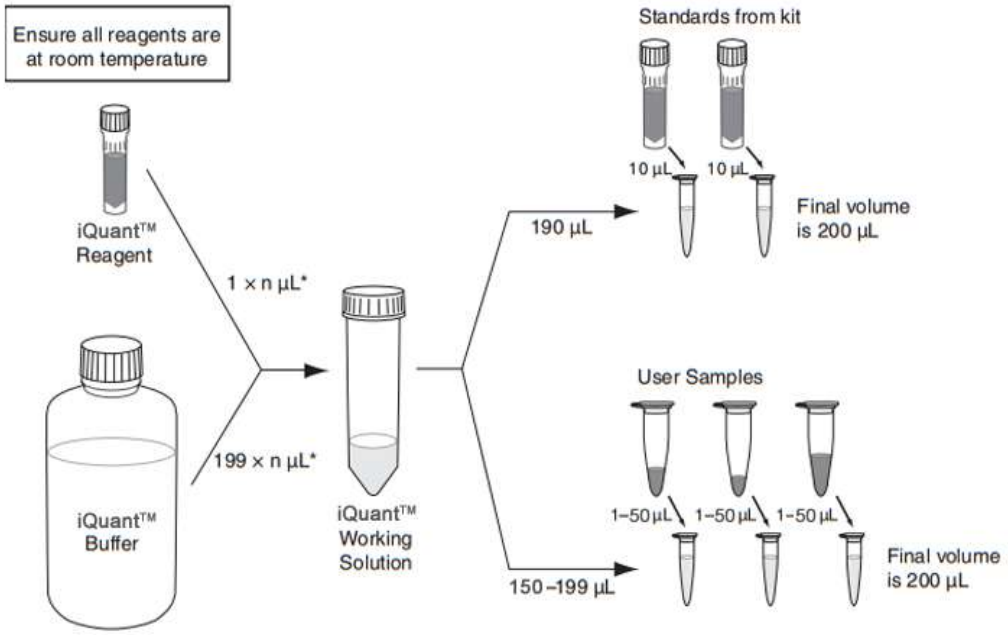

Figure 4. iQuant™ RNA BR Assay workflow