



iQuant™ microRNA Assay Kit

(0.5 – 150 ng)

Catalog Number: N023, N024

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
iQuant™ microRNA Assay Kit (Cat. No. N023)				The product is stable for at least 6 months when stored as directed.
iQuant™ microRNA Reagent (Component A)	200 µL	200X in DMSO	2-8 °C Protect from light	
iQuant™ microRNA Buffer (Component B)	50 mL	1X		
microRNA Standard #1 (Component C)	200 µL	0 ng/µL in TE buffer		
microRNA Standard #2 (Component D)	200 µL	10 ng/µL in TE buffer		
iQuant™ microRNA Assay Kit (Cat. No. N024)				
iQuant™ microRNA Reagent (Component A)	1 mL	200X in DMSO	2-8 °C Protect from light	
iQuant™ microRNA Buffer (Component B)	200 mL	1X		
microRNA Standard #1 (Component C)	1 mL	0 ng/µL in TE buffer		
microRNA Standard #2 (Component D)	1 mL	10 ng/µL in TE buffer		

Approximate fluorescence excitation/emission maxima, in nm: 500/530, bound to microRNA.

Product Description

The iQuant™ microRNA Assay Kit is ideal for quantification of small RNA, including microRNA and siRNA, and both single stranded and double stranded RNA. The assay kit is highly selective for small RNA over rRNA or large mRNA. The assay kit offers advantages in stability, linear dynamic range, and sensitivity over other traditional of RNA quantitation. The assay kit contains concentrated quantitation reagent, dilution buffer, and pre-diluted microRNA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µl and 20 µl is acceptable), and read the fluorescence using fluorescence plate reader or Fluorometer (Figure 2). The assay is well tolerated to common contaminants such as proteins, salts, solvents, detergents, or free nucleotides. The assay can be adapted for use in microplates, tubes or cuvettes.

Handling and Disposal

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

General Protocol

1. Measure microRNA samples using a Fluorescence Microplate Reader

(Note: For simplicity, the following protocol is written using 10 µL of microRNA sample)

volume. In practice, the volume of microRNA sample could be ranging from 1 μ L to 50 μ L depending on the concentration of microRNA sample, then adjust the volume of iQuant™ working solution to 200 μ L.)

- 1.1 Warm up the iQuant™ microRNA Assay Kit to room temperature. Check the iQuant™ microRNA reagent for any precipitation. If precipitation is seen, warm up the vial in a water bath and vortex until dissolved.
- 1.2 Prepare the iQuant™ working solution by diluting the iQuant™ microRNA reagent 1:200 in 1X iQuant™ microRNA 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 μ L of iQuant™ microRNA reagent to 4 mL of 1X iQuant™ microRNA Buffer. Mix well and use immediately.
- 1.3 Add 190 μ L 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 microRNA standard dilutes from microRNA Standard #2 (Component D) or your known microRNA sample.
- 1.5 Add 10 μ L of each microRNA standard dilutes and the unknown microRNA 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 485 nm excitation and 530 nm emission, with the appropriate cut-off.
- 1.8 Generate a linear standard curve by plotting fluorescence versus microRNA concentration of the microRNA standards. Use the standard curve and the fluorescence of the unknown microRNA samples to determine the unknown microRNA concentration.

2. Measure microRNA samples using the Qubit® Fluorometer from Invitrogen

(Note: For simplicity, the following protocol is written using 10 μ L of microRNA sample volume. In practice, the volume of microRNA sample could be ranging from 1 μ L to 20 μ L depending on the concentration of microRNA sample, then adjust the volume of iQuant™ working solution to 200 μ L.)

- 2.1. Warm up the iQuant™ microRNA Assay Kit to room temperature. Check the iQuant™ microRNA reagent for any precipitation. If precipitation is seen, warm up the vial in a water bath and vortex until dissolved.
- 2.2. Prepare the iQuant™ working solution by diluting the iQuant™ microRNA reagent 1:200 in 1X iQuant™ microRNA 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 μ L of iQuant™ microRNA reagent to 2 mL of 1X iQuant™ microRNA Buffer. Mix well and use immediately.
- 2.3. Add 190 μ L 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. 10011-830)).
- 2.4. Add 10 μ L of microRNA standard #1 (Component C), dsDNA microRNA (Component D), and the unknown microRNA samples to the appropriate tubes and mix by vortexing 2-3 seconds, and label the lids of each microRNA 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 **microRNA Assay** program, according to the manufacture's recommendation.

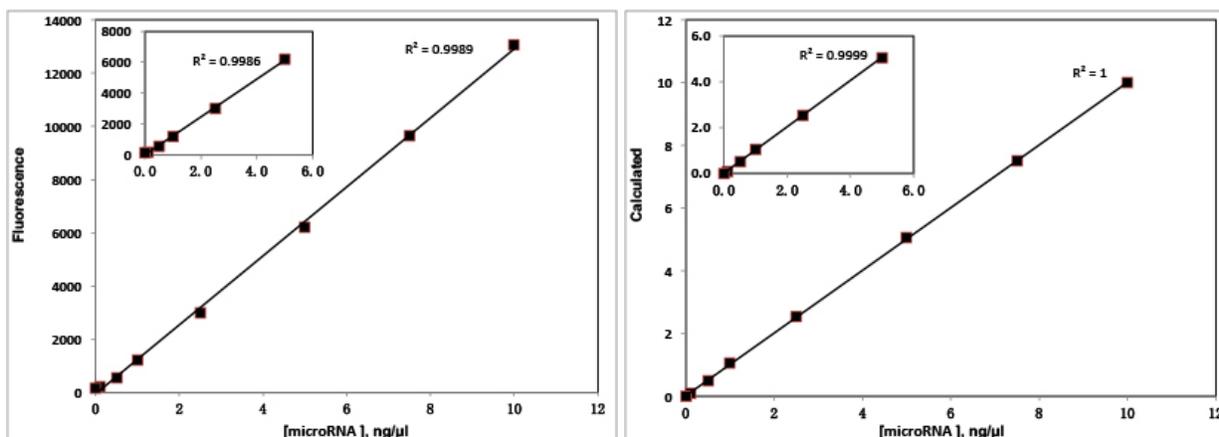


Figure 1. Left: Quantitation of microRNA with iQuant™ microRNA Assay Kit using fluorescence plate reader.
 Right: Quantitation of microRNA with iQuant™ microRNA Assay Kit using Qubit® Fluorometer.

Considerations for Data Analysis

It is more preferring to use a microRNA standard similar to the unknown samples (i.e. similar in size, single stranded vs double stranded). We found using the iQuant™ microRNA reagent most microRNA or siRNA yield similar results. If the fluorescence of an unknown sample is higher than microRNA standard #2 (Component D), further dilute the sample to perform the assay.

Appendix

Table 2. Effect of Contaminants in the iQuant™ dsDNA HS Assay Kit

Contaminant	Final Concentration in Assay	Concentration in 10 μ L Sample	Result
Sodium Chloride	5 mM	100 mM	OK
Magnesium Chloride	1 mM	20 mM	OK
Sodium Acetate	5 mM	100 mM	OK
Ammonium Acetate	1 mM	20 mM	OK
Ethanol	0.5%	10%	OK
Chloroform	0.2%	4%	OK
Phenol	0.1%	2%	OK
Sodium Dodecyl Sulfate	0.01%	0.2%	Not recommended
Triton X-100	0.001%	0.02%	OK
NTPs	1:1 NTP:miRNA	1:1 NTP:miRNA	OK
dsDNA	10:1 miRNA:dsDNA	10:1 miRNA:dsDNA	OK
ssDNA	10:1 miRNA:ssDNA	10:1 miRNA:ssDNA	OK
Oligo DNA	10:1 miRNA:oligo	10:1 miRNA:oligo	Not recommended

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

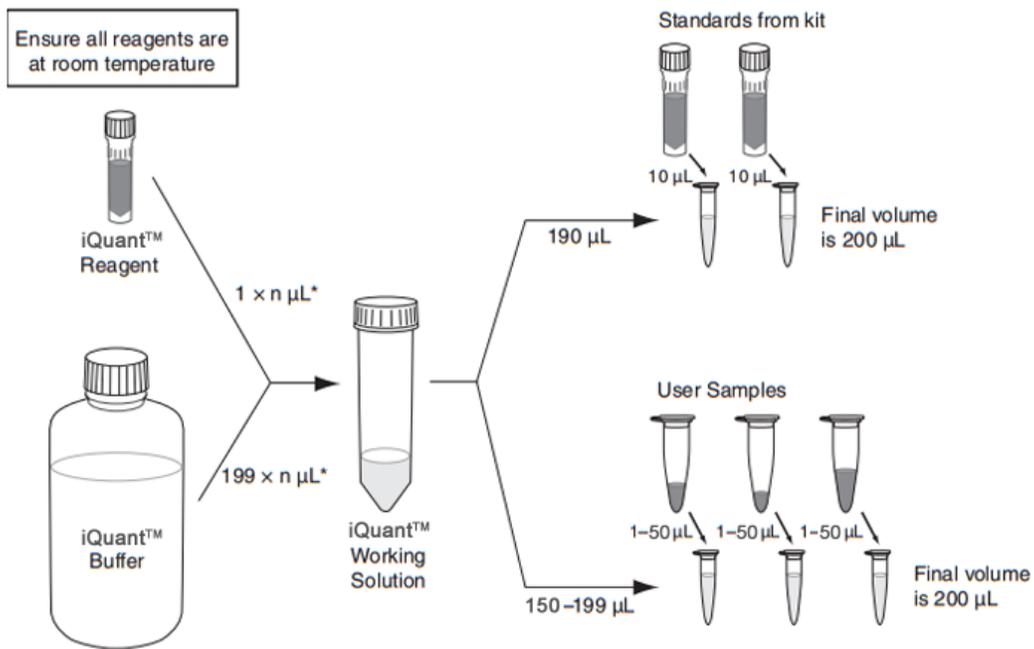


Figure 2. iQuant™ microRNA Assay workflow