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LiQuant™ microRNA Assay Kit

Cat. #: M0133 Size: 200 rxns

Introduction

LiQuant™ 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. 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.

Package Information

Components	M0133
LiQuant™ microRNA Reagent	200 µL
LiQuant™ microRNA Buffer	50 mL
microRNA Standard #1	200 µL
microRNA Standard #2	200 µL

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

Storage

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

Handling and Disposal

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

Protocol

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 µL to 50 µL depending on the concentration of microRNA sample, then adjust the volume of LiQuant™ working solution to 200 µL.

1. Warm up the LiQuant™ microRNA Assay Kit to room temperature. Check the LiQuant™ microRNA reagent for any precipitation. If precipitation is seen, warm up the vial in a water bath and vortex until dissolved.

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

3. Add 190 µL of the LiQuant™ 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 microRNA standard dilutes from microRNA Standard #2 or your known microRNA sample.

5. Add 10 µL 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.

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

7. Measure the fluorescence using a microplate reader with 485 nm excitation and 530 nm emission, with the appropriate cut-off.

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.

Measure microRNA samples using the Qubit® Fluorometer from ThermoFisher

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 µL to 20 µL depending on the concentration of microRNA sample, then adjust the volume of LiQuant™ working solution to 200 µL.

1. Warm up the LiQuant™ microRNA Assay Kit to room temperature. Check the LiQuant™ microRNA reagent for any precipitation. If precipitation is seen, warm up the vial in a water bath and vortex until dissolved.

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

3. Add 190 µL of the LiQuant™ 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 microRNA standard #1, microRNA 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 microRNA Assay program, according to the manufacture's recommendation.

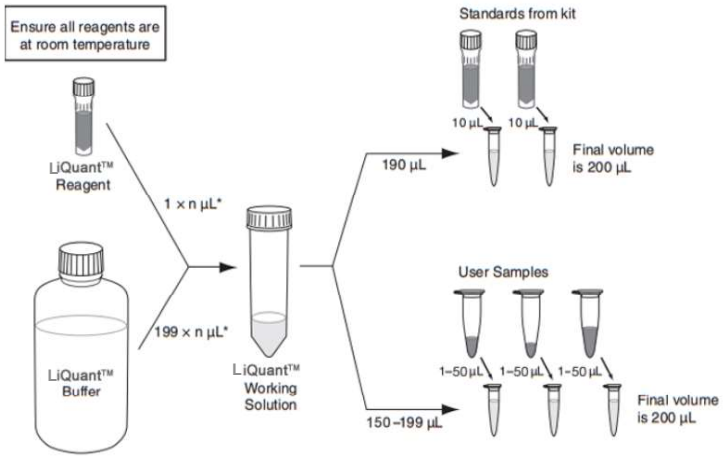
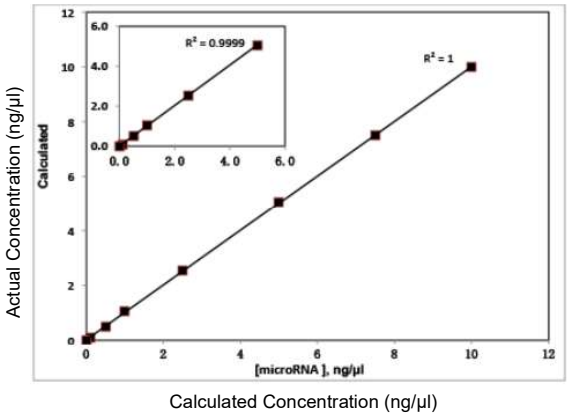


Figure 1. LiQuant™ microRNA Assay workflow



Calculated Concentration (ng/µl)

Figure 2. The 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, further dilute the sample to perform the assay.

Effect of Contaminants in the LiQuant™ microRNA Assay

Contaminant	Final Concentration in Assay	Concentration in 10 µL 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