molecular probes
life technologies

Quant-iT™ microRNA Reagent

Catalog no. Q32883

Table 1 Contents and storage

Material	Amount	Concentration	Storage	Stability
Quant-iT™ microRNA reagent	1.0 mL	200X in DMSO	• Room temperature • Protect from light • Dessicate	When stored as directed, product is stable for 1 year.
Number of assays: Sufficient material is provided for 1000 assays using 200 µL assay volume in a 96-well microplate format. The Quant-iT™ microRNA reagent can be adapted for use in cuvettes or 384-well microplates.				
Approximate fluorescence excitation/emission maxima: 500/525 nm.				

Introduction

The Quant-iT™ microRNA reagent allows easy and accurate quantification of small RNA (~20 nucleotides or base pairs), even in the presence of common contaminants such as salts, free nucleotides, solvents, detergents, and protein (*Appendix*, Table 2, page 4). The assay is highly selective for small RNA over rRNA or large mRNA (>1000 nt). We have been able to reproducibly quantify small RNA in pure samples at levels as low as 0.5 ng in the assay tube following the supplied protocol below.

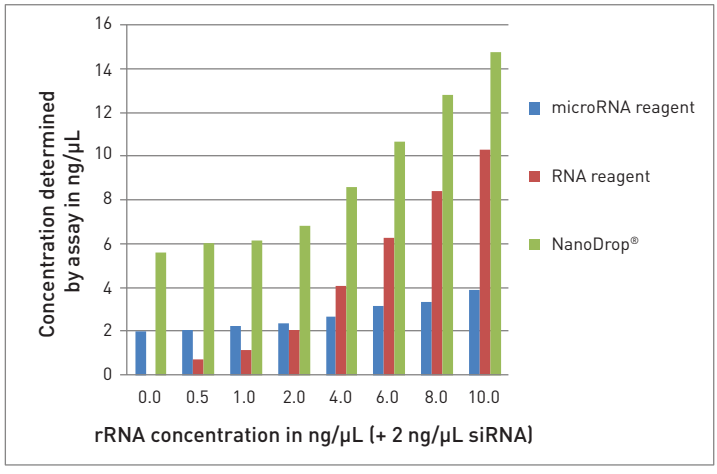

Figure 1 Comparison of detection techniques for accurate quantification of small RNA in the presence of ribosomal RNA. rRNA at the concentrations listed was spiked into solutions containing 2 ng/µL siRNA, then read using the Quant-iT™ microRNA reagent or the Quant-iT™ RNA reagent on the Qubit® fluorometer, or by 260 nm absorbance (A260) on the NanoDrop® spectrophotometer.

The assay accurately detects as little as 0.5 ng small RNA and has a dynamic range of 5 ng/mL to 500 ng/mL (1–100 ng) in the core assay range. The assay is accurate for initial sample concentrations from 0.05 ng/µL to 100 ng/µL. To perform the assay, simply dilute the reagent, add your sample, and read the concentration using a fluorescent microplate reader.

Before you begin

Handling the Quant-iT™ microRNA reagent

There is no data are available addressing the mutagenicity or toxicity of the Quant-iT™ microRNA reagent. This reagent is known to bind nucleic acid and is provided as a solution in DMSO; treat the reagent with the same safety precautions as all other potential mutagens. Dispose of the dye in accordance with local regulations.

Remove the Quant-iT™ microRNA reagent from storage, allow it to equilibrate to room temperature, and mix well. During all steps, protect the Quant-iT™ microRNA reagent concentrate and the working solution from light as much as possible.

Materials required

- 20X TE Buffer*, pH 7.5 (Cat. no. T11493)
- 1X TE Buffer, pH 7.5, with 0.01% CHAPS
- small RNA standard (e.g., *Silencer*® SelectGAPDH Positive Control siRNA, Cat. no. 4390849)
- 96-well microplates

*200 mM Tris, 1 mM EDTA (pH 7.5)

Experimental protocols

Prepare buffers and reagents

1.1 Allow all reagents to come to room temperature.

1.2 Prepare sufficient 1X TE Buffer (10 mM Tris, 1 mM EDTA, pH 7.5) for standard dilutions. This may be prepared by diluting 20X TE Buffer in E-pure™ water. This buffer solution also acts as the "0 small RNA standard" in the microRNA assay.

1.3 Prepare sufficient 1X TE Buffer + 0.01% CHAPS (w/v) assay buffer. All dye working solutions should be prepared in this buffer. For convenience, you may prepare an aqueous stock solution of 1% CHAPS (w/v) and dilute it to 0.01% (w/v) accordingly.

A solution of 200 mL buffer (20 mg of CHAPS in 200 mL of 1X TE buffer) is sufficient for 1000 assays following the protocol below.

In the presence of CHAPS, dye working solution is stable for at least 3 hours at room temperature.

1.4 Prepare small RNA standards. In the development of the microRNA assay reagent, a range of 0–10 ng/µL was used. A volume of 10 µL will be used for each reaction.

If you are using *Silencer*® Select siRNA, prepare a 100 ng/µL solution by adding 650 µL of 1X TE Buffer only (no CHAPS) to 5 nmoles of *Silencer*® Select siRNA solution. Prepare a 10 ng/µL solution by diluting the 100 ng/µL siRNA solution 10-fold in 1X TE buffer (no CHAPS) (i.e., add 100 µL of 100 ng/µL siRNA solution to 900 µL of 1X TE buffer).

Perform the microRNA assay

The following protocol describes a Quant-iT™ microRNA assay with a total volume of 200 µL per microplate well.

2.1 Prepare the Quant-iT™ microRNA working solution by diluting the Quant-iT™ microRNA reagent 1:200 in 1X TE Buffer + 0.01% CHAPS (w/v). For a 96-well microplate, approximately 20 mL of the Quant-iT™ microRNA working solution will be required for the samples and standard curve.

2.2 Load 200 µL of the Quant-iT™ microRNA working solution (prepared in step 2.1) into the wells containing the standard and samples.

2.3 Pipet 10 µL of the standards prepared in step 1.4 into separate wells of a microplate and mix well.

2.4 Pipet 1–20 µL of each unknown small RNA sample to separate wells of a microplate and mix well. Duplicates or triplicates of the unknown samples are recommended. Some contaminanting substances may interfere with the assay; see Table 2 in the *Appendix,* page 4.

2.5 Incubate the reactions for 2–5 minutes at room temperature.

2.6 Measure the fluorescence using a microplate reader (excitation/emission maxima are 500/525 nm). The fluorescence signal is stable for 3 hours.

2.7 Use a standard curve to determine the small RNA amounts. For the small RNA standards, plot amount vs. fluorescence, and fit a straight line to the data points.

Appendix

Contaminating substances

A number of common contaminants have been tested in the Quant-iT™ microRNA assay, and most are well tolerated (Table 2, below). For untested contaminating substances, and, in general, for highest accuracy, the standards should be assayed under the same conditions as the unknowns. For example, if the experimental samples are in an unusual buffer and if 10 µL volumes of these samples are used, then add 10 µL volumes of the unusual buffer (lacking RNA) to the wells containing the standards.

Table 2 Effects of contaminants in the Quant-iT™ microRNA assay.

Contaminant	Final Concentration in the Assay	Concentration in 20 µL sample	Concentration in 10 µL sample	Result*
Sodium chloride	5 mM	50 mM	100 mM	OK
Magnesium chloride	1 mM	10 mM	20 mM	OK
Sodium acetate	5 mM	50 mM	100 mM	OK
Ammonium acetate	1 mM	10 mM	20 mM	OK
Ethanol	1%	10%	20%	OK
Phenol	0.1%	1%	2%	OK
Chloroform	0.2%	2%	4%	OK‡
SDS	0.01%	0.1%	0.2%	NR
Triton® X-100	0.001%	0.01%	0.02%	OK
NTPs**	1:1 NTP:miRNA	1:1 NTP:miRNA	1:1 NTP:miRNA	OK
dsDNA	10:1 miRNA:dsDNA	10:1 miRNA:dsDNA	10:1 miRNA:dsDNA	OK†
ssDNA	10:1 miRNA:ssDNA	10:1 miRNA:ssDNA	10:1 miRNA:ssDNA	OK†
Oligo DNA	10:1 miRNA:Oligo	10:1 miRNA:Oligo	10:1 miRNA:Oligo	NR

* Results are given either as OK, usually less than 10% perturbation, or as NR (not recommended).

** A mixture of ATP, CTP, GTP, and UTP.

† Some distortion at the high end of the assay; for best results dilute the sample so concentration is ≤ 300 ng/mL.

‡ Immiscible.

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat no.	Product Name	Unit Size
Q32883	Quant-iT™ microRNA reagent	1 mL
Related products		
4390849	*Silencer*® Select GAPDH Positive Control siRNA (Hs, Mm, Rn)	5 nmoles
T11493	20X TE Buffer, RNAse-free	100 mL
ZC10003	Zoom® CHAPS	5 g

Purchaser Notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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