Quant-iT™ microRNA Assay Kit

Catalog Number Q32882

Pub. No. MAN0009429 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Quant-iT™ microRNA Assay Kit allows for easy and accurate quantification of small RNA (~20 nucleotides or base pairs). The assay is recommended for initial sample concentrations ranging from 50 pg/µL to 100 ng/µL, providing a detection range of 1 ng to 100 ng of RNA. The assay is highly selective for small RNA over rRNA or large mRNA (>1,000 nt) (see Figure 1 on page 2), and tolerant of common contaminants such as salts, free nucleotides, solvents, detergents, or protein ("Effects of contaminating substances" on page 3).

Contents and storage

Component	Amount	Concentration	Storage[1,2]
Quant-iT™ microRNA reagent (Component A)	1.0 mL	200X in DMSO	2°C to 8°C Protect from light
Quant-iT™ microRNA buffer (Component B)	250 mL	Not applicable	2°C to 8°C Avoid freeze/thaw cycles
microRNA standards (21-mer) (Component C)	set of 8, 500 µL each	0, 0.5, 1, 2, 4, 6, 8, and 10 ng/µL	
Number of labelings: 1,000, with a 200 µL assay volume in a 96-well microplate format. The Quant-iT™ microRNA assay can be adapted for use in cuvettes or 384-well microplates.			
Approximate fluorescence excitation/emission maxima: 500/525 nm.			

[1] The Quant-iT™ microRNA buffer (Component B) may be left at room temperature for short-term storage (days); however, for longer periods we recommend storage between 2°C to 8°C to prevent microbial contamination.

[2] When stored as directed, kit contents are stable for at least 6 months.

Required materials not supplied

- Nuclease-free pipettors and tips
- Microplates for Fluorescence-based Assays, 96-well (Cat. No. M33089)

Critical assay parameters

Assay temperature

Quant-iT™ assays deliver optimal performance when all solutions are at room temperature; temperature fluctuations can influence the accuracy of the assay.

Assay selectivity

This assay is highly selective for miRNA (~20 nucleotides) over rRNA or large single- and double-stranded mRNAs (>1,000 bp) (Figure 1).

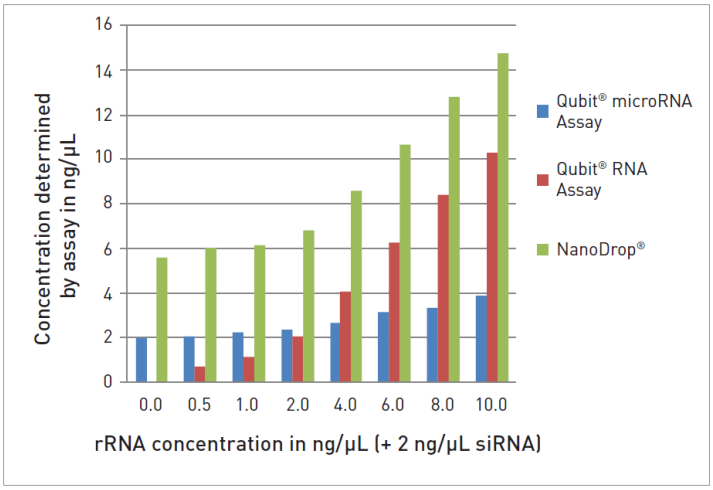

Figure 1 Comparison of detection techniques for accurate quantitation of small RNA in the presence of ribosomal RNA. rRNA at the concentrations listed was spiked into solutions containing 2 ng/µL siRNA, then read using the Qubit™ microRNA assay, the Qubit™ RNA assay, or by 260 nm absorbance (A_{260}) on the NanoDrop™ spectrophotometer.

Incubation time

To allow the Quant-iT™ microRNA assay to reach maximum fluorescence, incubate the assay tubes for 2 minutes after mixing the sample or the standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature when the samples and standards are protected from light.

Photostability of the Quant-iT™ reagent

Avoid multiple sequential fluorescence reads to minimize reagent photobleaching.

Handling the Quant-iT™ reagent

No data are currently available addressing the mutagenicity or toxicity of the Quant-iT™ microRNA reagent (Component A). 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.

Effects of contaminating substances

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

Table 1 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[1]
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[2]
SDS	0.01%	0.1%	0.2%	NR
Triton™ X-100	0.001%	0.01%	0.02%	OK
NTPs[3]	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[4]
ssDNA	10:1 miRNA:ssDNA	10:1 miRNA:ssDNA	10:1 miRNA:ssDNA	OK[4]
Oligo DNA	10:1 miRNA:oligo	10:1 miRNA:oligo	10:1 miRNA:oligo	NR

[1] Results are given either as OK, usually less than 10% perturbation, or as NR (not recommended).
[2] Immiscible.
[3] A mixture of ATP, CTP, GTP, and UTP.
[4] Some distortion at the high end of the assay; for best results dilute the sample so concentration is ≤ 300 ng/mL.

Prepare and read samples and standards using the Quant-iT™ microRNA Assay Kit with a fluorescence microplate reader

This protocol describes the use of the Quant-iT™ microRNA Assay Kit with a fluorescence microplate reader equipped with either a monochrometer or excitation and emission filters appropriate for the Quant-iT™ microRNA reagent (excitation/emission maxima 500/525 nm). For an overview of the assay procedure, see Figure 2.

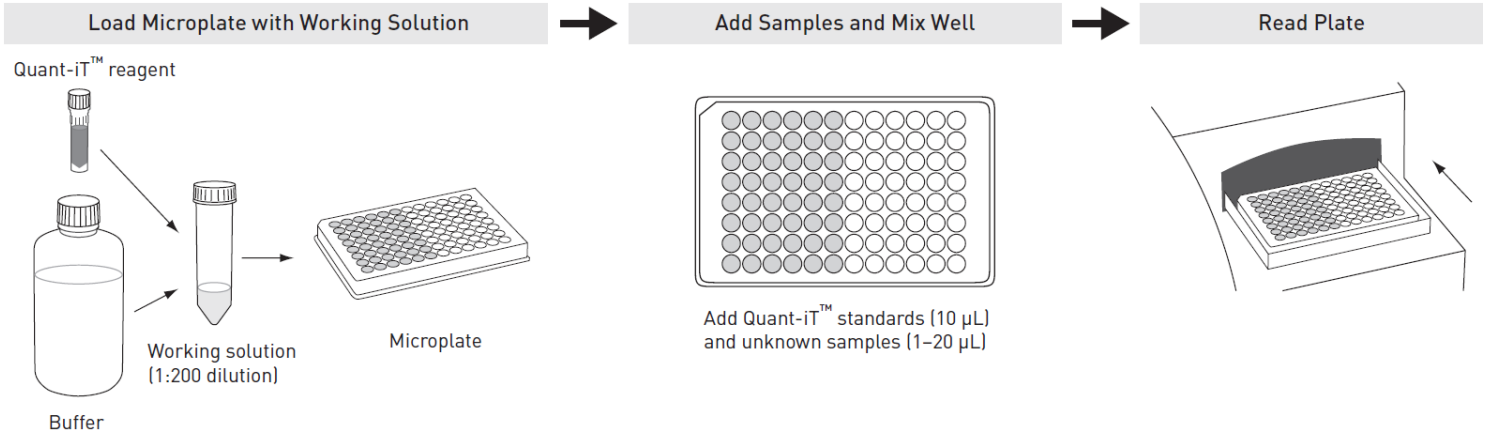

Figure 2 Overview of Quant-iT™ microRNA assay.

IMPORTANT! For best results, ensure that all materials and reagents are at room temperature.

1. Make a working solution by diluting the Quant-iT™ microRNA reagent 1:200 in Quant-iT™ microRNA buffer. For example, for ~100 assays combine 100 µL of Quant-iT™ microRNA reagent (Component A) and 20 mL of Quant-iT™ microRNA buffer (Component B) in a disposable plastic container and mix well. The Quant-iT™ working solution is stable for at least 3 hours at room temperature, protected from light.

   IMPORTANT! Do not use glass containers. Do not use buffers other than the Quant-iT™ microRNA buffer to make the working solution.

2. Add 10 µL of each microRNA standard (Component C) to separate wells and mix well. Take care not to introduce nucleases into the tubes of RNA standard as you remove aliquots for the assay. Duplicates or triplicates of the standards are recommended.

3. Add 1–20 µL of each unknown small RNA sample to separate wells and mix well. Duplicates or triplicates of the unknown samples are recommended.

4. Load 200 µL of the working solution into each microplate well. This can be done readily using a multichannel pipettor.

5. If possible, mix your 96-well plate using a plate mixer or using the plate reader for about 3–10 seconds. After mixing, allow the plate to incubate at room temperature for 2 minutes.

6. Measure the fluorescence using a microplate reader (excitation/emission maxima are 500/525 nm). The fluorescence signal is stable for 3 hours at room temperature, when protected from light.

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

Note: Many curve-fitting programs will calculate the y-intercept. However, for best results, manually set the y-intercept as the RFU value (relative fluorescence unit) obtained from the 0 ng/µL RNA standard.

Data analysis considerations–standard curves and extended ranges

The fluorescence of the Quant-iT™ microRNA reagent bound to small RNA is extremely linear between 0.5–100 ng. For best results at the low end of the standard curve, the line should be forced through the background point (or through zero, if background has been subtracted).

Related products

Table 2 Bulk Reagents and Kits

Product	Quantity	Cat. No.
Quant-iT™ PicoGreen™ dsDNA Assay Kit	1 mL assay kit 10 x 100 µL	P7589 P11496
Quant-iT™ PicoGreen™ dsDNA Reagent	1 mL reagent 10 x 100 µL	P7581 P11495
TE Buffer (20X), RNase-free	100 mL	T11493
Quant-iT™ RiboGreen™ RNA Assay Kit	1 mL assay kit	R11490
Quant-iT™ RiboGreen™ RNA Reagent	1 mL reagent	R11491
Quant-iT™ RediPlate™ 96 RiboGreen™ RNA Quantitation Kit	1 plate	R32700
Quant-iT™ OliGreen™ ssDNA Assay Kit	1 mL assay kit	O11492
Quant-iT™ OliGreen™ ssDNA Assay Reagent	1 mL reagent	O7582

Table 3 Microplate Reader Assays

Product	Dynamic Range	Quantity	Cat. No.
Quant-iT™ 1X dsDNA Assay Kit, High Sensitivity	200 pg–100 ng	1,000 reactions	Q33232
Quant-iT™ 1X dsDNA Assay Kit, Broad-Range	4 ng–2 µg	1,000 reactions	Q33267
Quant-iT™ DNA Assay Kit, High Sensitivity	200 pg–100 ng	1,000 reactions	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	4 ng–1 µg	1,000 reactions	Q33130
Quant-iT™ RNA Assay Kit	5–100 ng	1,000 reactions	Q33140
Quant-iT™ RNA Reagent	5–100 ng	1,000 reactions	Q32884
Quant-iT™ RNA Assay Kit, Broad Range	20 ng–1 µg	1,000 reactions	Q10213
Quant-iT™ RNA XR Assay Kit	200 ng–10 µg	1,000 reactions	Q33225
Quant-iT™ microRNA Assay Kit	1–100 ng	1,000 reactions	Q32882
Quant-iT™ Protein Assay Kit	250 ng–5 µg	1,000 reactions	Q33210
Microplates for Fluorescence-based Assays, 96-well	—	10 plates	M33089

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No.MAN0009429

Revision	Date	Description
B.0	February 3, 2022	Updated format and content
A.0	February 2015	New doc