molecular probes
life technologies

Quant-iT™ microRNA Assay Kit

Catalog no. Q32882

Table 1 Contents and storage

Material	Amount	Concentration	Storage*	Stability
Quant-iT™ microRNA reagent (Component A)	1.0 mL	200X in DMSO	• Room temperature • Dessicate • Protect from light	When stored as directed, kit contents are stable for at least 6 months.
Quant-iT™ microRNA buffer (Component B)	250 mL	NA	• ≤6°C • Protect from light	
microRNA standards (21-mer) (Component C)	set of 8, 500 µL each	0, 0.5, 1, 2, 4, 6, 8, and 10 ng/µL		

*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 at ≤6°C to prevent microbial contamination.
NA = not applicable

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

Introduction

The Quant-iT™ microRNA Assay Kit 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 3, page 7). The assay is highly selective for small RNA over rRNA or large mRNA (>1000 nt) (Figure 1, page 2). 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. The assay detects all types of small RNA, including microRNA and siRNA, both single stranded and double stranded.

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. The kit provides concentrated assay reagent, dilution buffer, and pre-diluted small RNA standards. To perform the assay, simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µL and 20 µL is acceptable), and read the concentration using a fluorescent microplate reader.

If you would like to use this kit with the Qubit® Fluorometer, we have included instructions under *Using the Quant-iT™ microRNA Assay Kit with the Qubit® Fluorometer* on page 4.

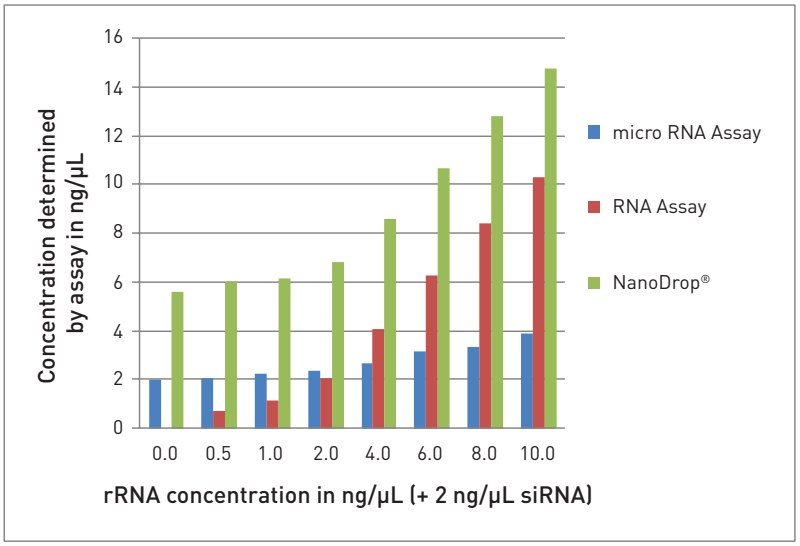

Figure 1 Comparison of detection techniques for accurate quantification of small RNA in the presence of ribosomal RNA. rRNA at the concentrations listed was spiked into solutions containing 2 ng/µL siRNA, then read using the microRNA assay, the RNA assay, or by 260-nm absorbance (A260) on the NanoDrop®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

Before You Begin

Handling the Quant-iT™ reagent

No data are currently 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 Assay Kit from storage, allow the components 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.

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 micro-plate reader equipped with excitation and emission filters appropriate for the Quant-iT™ microRNA reagent (excitation/emission maxima 500/525 nm). Some contaminating substances may interfere with the assay. See Table 3 in the *Appendix* (page 6) for more information. For an overview of the assay procedure, see Figure 2 on page 3.

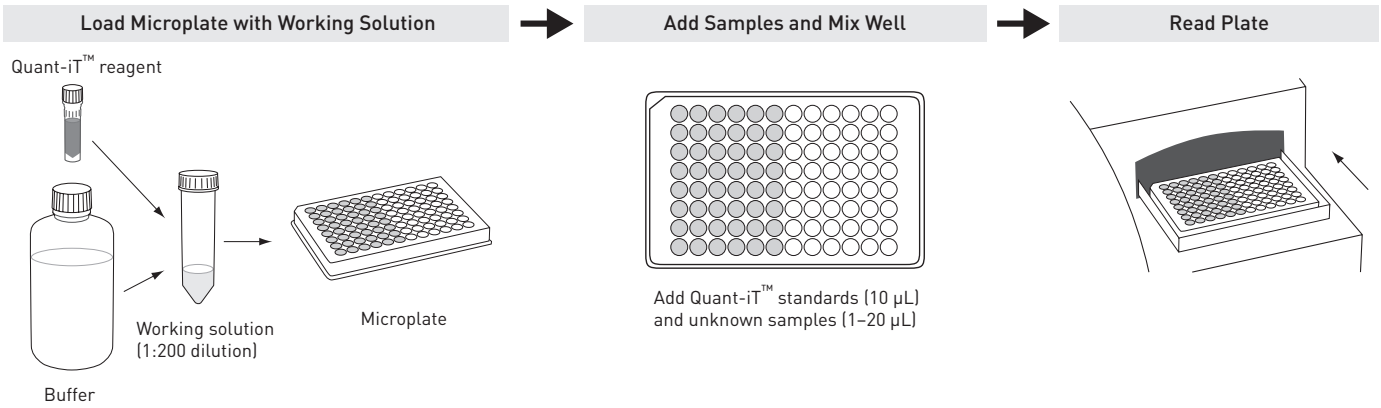

Figure 2 The Quant-iT™ microRNA assay

Assay procedure

1.1 Make a working solution by diluting the Quant-iT™ microRNA reagent 1:200 in Quant-iT™ microRNA buffer. For example, for ~100 assays put 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. Do not use glass containers. Do not use buffers other than the Quant-iT™ microRNA buffer to make the working solution.

1.2 Load 200 µL of the working solution into each microplate well. Diluted Quant-iT™ microRNA reagent is stable for at least 3 hours at room temperature, protected from light.

1.3 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.

1.4 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. Some contaminating substances may interfere with the assay, see the Table 3 in the *Appendix* (page 6).

1.5 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.

1.6 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.

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).

You can easily adapt the Quant-iT™ microRNA Assay Kit for use with the Qubit® Fluorometer. The protocol below is abbreviated from the Qubit® Fluorometer user guide, which is available at **www.lifetechnologies.com**. Although a step-by-step protocol and critical assay parameters are given here, more detail is available in the Qubit® Fluorometer user guide, and you are encouraged to familiarize yourself with this manual before you begin your assay. See Figure 3, below, for an overview of the procedure.

Note: For Qubit® 2.0 Fluorometers purchased before June, 2014, the Quant-iT™ microRNA assay requires the addition of the MyQubit microRNA assay to the Qubit® 2.0 Fluorometer. The assay file can be downloaded from the Qubit® 2.0 Fluorometer web page (**www.lifetechnologies.com/qubit**) and permanently uploaded to your Qubit® 2.0 Fluorometer. The Qubit® 3.0 fluorometer comes pre-loaded with the assay.

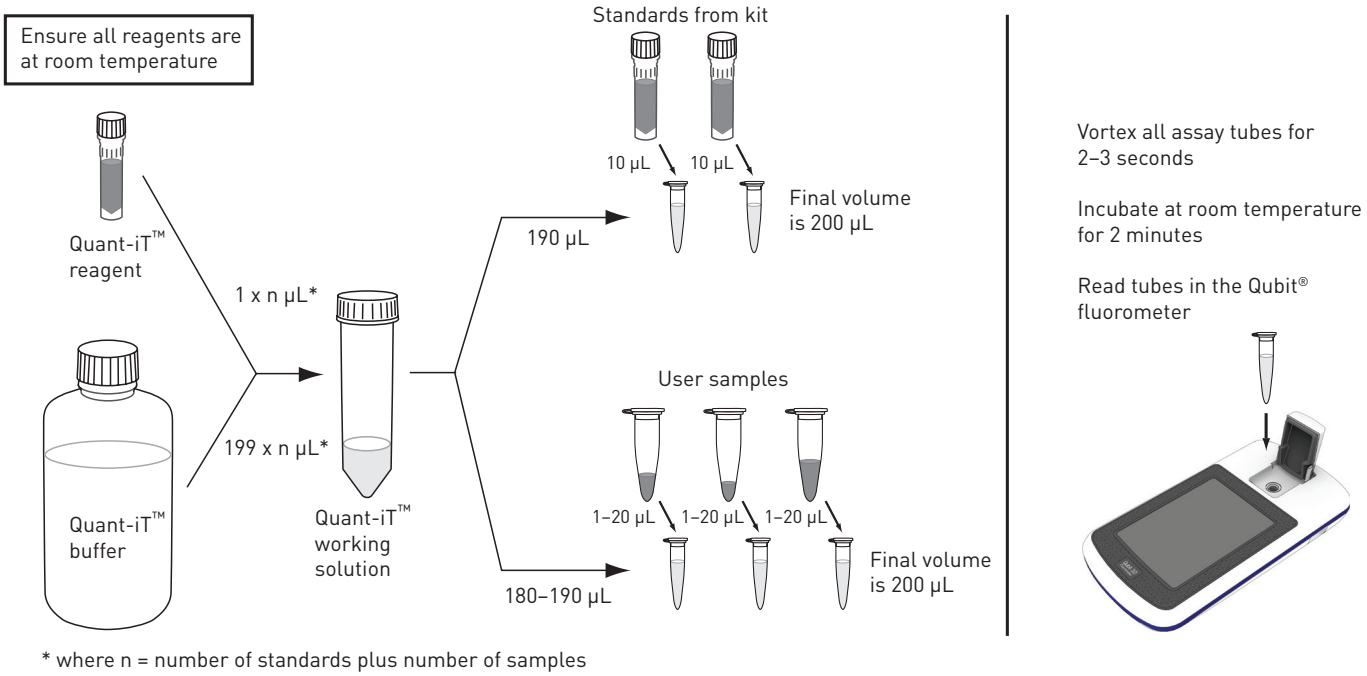

Figure 3 Overview of the Quant-iT™ microRNA assay using the Qubit® Fluorometer

IMPORTANT! Ensure all assay reagents are at room temperature before you begin. Use only thin-wall, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit® assay tubes (500 tubes, Cat. no. Q32856) or Axygen® PCR-05-C tubes (VWR, part no. 10011-830).

Assay procedure

2.1 Label the lids of the assay tubes for the standards and user samples.

Note: The Quant-iT™ microRNA Assay Kit requires two standards for the calibration of the Qubit® Fluorometer. Prepare a dilution of the 0 ng/µL microRNA standard from the Component C set to generate Standard #1, and a dilution of the 10 ng/µL microRNA standard from the Component C set to generate Standard #2 (see step 2.3, page 5).

2.2 Prepare the Quant-iT™ microRNA working solution by diluting the Quant-iT™ microRNA reagent 1:200 in Quant-iT™ buffer.

2.3 Prepare assay tubes according to Table 2, below.

Table 2 Tube setup

	Standard assay tubes	User Sample assay tubes
Volume of working solution (from step 2.2)	190 µL	180–199 µL
Volume of standard (from kit)*	10 µL	—
Volume of user sample	—	1–20 µL
Total volume in each assay tube	200 µL	200 µL

* Prepare Standard #1 by diluting 10 µL of the 0 ng/µL standard, and Standard #2 by diluting 10 µL of the 10 ng/µL standard.

2.4 Vortex all tubes for 2–3 seconds.

2.5 Incubate the tubes for 2 minutes at room temperature.

2.6 Calibrate the Qubit® Fluorometer using Standard #1 and Standard #2.

2.7 Read the user samples in the Qubit® Fluorometer.

2.8 *For Qubit® 2.0 Fluorometer users:* Multiply the readout from the Qubit® 2.0 Fluorometer by the value given by the dilution factor (see the Qubit® 2.0 Fluorometer user guide) to determine the concentration of your original sample. Alternatively, choose **Calculate Sample Concentration** to have the Qubit® 2.0 Fluorometer perform this multiplication for you. For more information, refer to the Qubit® 2.0 Fluorometer user guide.

Note: The Qubit® 3.0 Fluorometer performs this calculation automatically.

Calculate the sample concentration – Qubit® 2.0 Fluorometer

Note: The Qubit® 3.0 Fluorometer performs this calculation automatically.

The Qubit® 2.0 Fluorometer gives values for the Quant-iT™ microRNA assay in ng/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times \frac{200}{x}$$

where QF value is the value given by the Qubit® 2.0 Fluorometer and x is the number of microliters of sample you have added to the assay tube.

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer (i.e., if the Qubit® 2.0 Fluorometer gave a concentration in ng/mL, the result of the equation will be in ng/mL).

Alternatively, you may choose **Calculate Sample Concentration** to have the Qubit® 2.0 fluorometer perform this multiplication for you (available on Qubit® fluorometers with firmware version 1.22 or later).

Appendix: Critical Assay Parameters

Assay temperature

The Quant-iT™ microRNA assay for the Qubit® Fluorometer delivers optimal performance when all solutions are at room temperature. The Quant-iT™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay (see Figure 4, below). To minimize temperature fluctuations, store the Quant-iT™ microRNA reagent and the Quant-iT™ microRNA buffer at room temperature and insert all assay tubes into the Qubit® Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, as the Qubit® Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. **Do not hold the assay tubes in your hand before reading, as this will warm the solution and result in a low reading.**

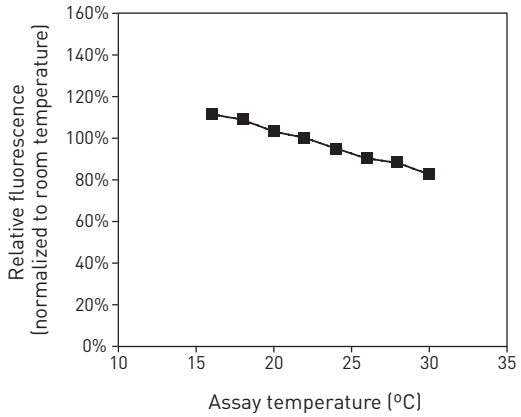

Figure 4 Plot of fluorescence vs. temperature for the Quant-iT™ microRNA assay. The Quant-iT™ assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

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.

Photobleaching of the Quant-iT™ reagent

The Quant-iT™ microRNA reagent exhibits high photostability in the Qubit® Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit® Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (see Figure 4, above). Note that the temperature inside the Qubit® Fluorometer may be as much as 3°C above room temperature after 1 hour. For this reason, if you want to perform multiple readings of a single tube, you should remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Assay tubes to use with the Qubit® Fluorometer

Use only thin-wall, clear 0.5 mL PCR tubes with the Qubit® Fluorometer. Acceptable tubes include Qubit® assay tubes (Cat. no. Q32856, 500 tubes) or Axygen® PCR-05-C tubes (VWR, part number 10011-830). The assay volume must be 200 µL for an accurate read.

Calibrating the Qubit® Fluorometer

When quantifying your samples using the Qubit® Fluorometer, you have the choice to calibrate the instrument using freshly prepared calibration solutions or to apply the values from a previously run calibration. *Using the Quant-iT™ microRNA Assay Kit with the Qubit® Fluorometer*, page 4, describes the preparation of fresh calibration standards. Consult the user guide for the Qubit® Fluorometer for guidance on choosing a calibration mode.

Contaminating substances

A number of common contaminants have been tested in the Quant-iT™ microRNA assay, and most are well tolerated (Table 3, below). For untested contaminating substances and in general, the standards should be assayed under the same conditions as the unknowns for highest accuracy. 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 microRNA) to the tubes or wells containing the standards.

Table 3 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
Q32882	Quant-iT™ microRNA Assay Kit, 1000 assays *5–500 ng*	1 kit
Related products		
Q33120	Quant-iT™ dsDNA Assay Kit, High Sensitivity, 1000 assays *0.2–100 ng*	1 kit
Q33130	Quant-iT™ dsDNA Assay Kit, Broad Range, 1000 assays *2–1000 ng*	1 kit
Q10213	Quant-iT™ RNA Assay Kit, Broad Range, 1000 assays *20–1000 ng*	1 kit
Q33140	Quant-iT™ RNA Assay Kit, 1000 assays *5–100 ng*	1 kit
Q33210	Quant-iT™ Protein Assay Kit, 1000 assays *0.25–5 µg*	1 kit

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.

Obtaining Support
For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:
- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

SDS
Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/sds**.

Certificate of Analysis
The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the product packaging (tube, pouch, or box).

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 found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Disclaimer
LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Important Licensing Information
This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

16 February 2015