Qubit™ microRNA Assay Kits

Catalog Numbers Q32880, Q32881

Pub. No. MAN0009427 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

Qubit™ microRNA Assay Kits, designed for use with Qubit fluorometers, allow for easy and accurate quantification of small RNA (~20 nucleotides or base pairs). Depending on sample volume, the assay is accurate for initial sample concentrations from 25 pg/µL to 150 ng/µL, providing an assay range of 0.5–150 ng. The assay is highly selective for small RNA over rRNA or large mRNA (>1,000 nt) (page 3), and tolerant of common contaminants such as salts, free nucleotides, solvents, detergents, or protein (Table 1 on page 4). We have been able to reproducibly quantify small RNA in pure samples at levels as low as 0.5 ng following the protocol provided in this user guide.

Note: This Qubit assay kit can be used with Qubit 3, Qubit 4, and Qubit Flex Fluorometers.

Contents and storage

Component	Cat. No. Q32880 (100 assays)	Cat. No. Q32881 (500 assays)	Concentration	Storage[1]
Qubit™ microRNA Reagent (Component A)	250 µL	1.25 mL	200X in DMSO	2°C to 8°C Protect from light
Qubit™ microRNA Buffer (Component B)[2]	50 mL	250 mL	Not applicable	2°C to 8°C Avoid freeze/thaw cycles
Qubit™ microRNA Standard #1 (Component C)	1 mL	5 mL	0 ng/µL in TE buffer	2°C to 8°C Avoid freeze/thaw cycles
Qubit™ microRNA Standard #2 (Component D)	4 × 250 µL	10 × 500 µL	10 ng/µL in TE buffer	

[1] When stored as directed, kits are stable for 6 months.

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

Required materials not supplied

- Nuclease-free pipettors and tips
- Qubit™ Assay Tubes (500 tubes, Cat. No. Q32856) or Qubit™ Flex Assay Tube Strips (Cat. No. Q33252)

Guidelines for handling Qubit™ microRNA standards

The calibration standards included in the Qubit™ microRNA Assay Kit are high-quality siRNA 21-mer standards (GAPDH siRNA). The integrity and concentration of these standards is critical to the optimal performance of the Qubit™ microRNA assay. As such, we highly recommend treating the siRNA standards as you would any other RNA, including:

- Use appropriate RNAse-free handling techniques, including RNAse-free gloves, pipette tips, and tubes
- Keep the tube lids closed whenever possible
- Do not touch the pipette to the inside wall of the tube when withdrawing a sample
- Return the siRNA standard to the refrigerator as soon as possible after use

Critical assay parameters

Assay temperature

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

To minimize temperature fluctuations, insert all assay tubes into the Qubit™ Fluorometer only for as much time as it takes for the instrument to measure the fluorescence. Qubit™ Fluorometers 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 because this warms the solution and results in a different reading.

Incubation time

To allow the Qubit™ assay to reach optimal fluorescence, incubate the tubes for 2 minutes after mixing the sample or 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.

Calibrate the Qubit™ Fluorometer

For each assay, you have the option to run a new calibration or use values from the previous calibration. To minimize variables that can affect performance, performing a new calibration for every new assay run is strongly recommended. See Figure 1 for an example of the calibration curve used to generate the quantification results.

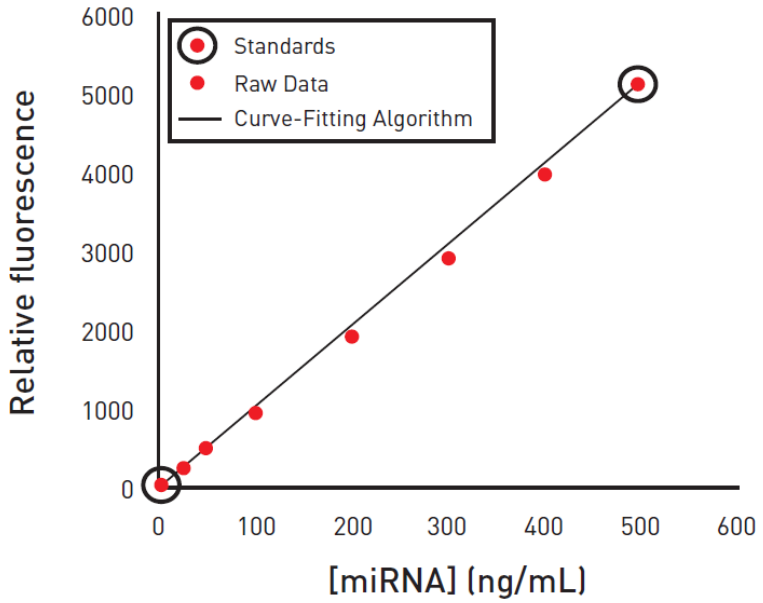

Figure 1 Sample calibration curve.
The plot showing the line corresponding to the curve-fitting algorithm used to calculate concentration in the Qubit™ microRNA assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation.

Assay selectivity

This assay is highly selective for miRNA (~20 nucleotides) over rRNA or large single- and double-stranded mRNAs (>1,000 bp) (Figure 2).

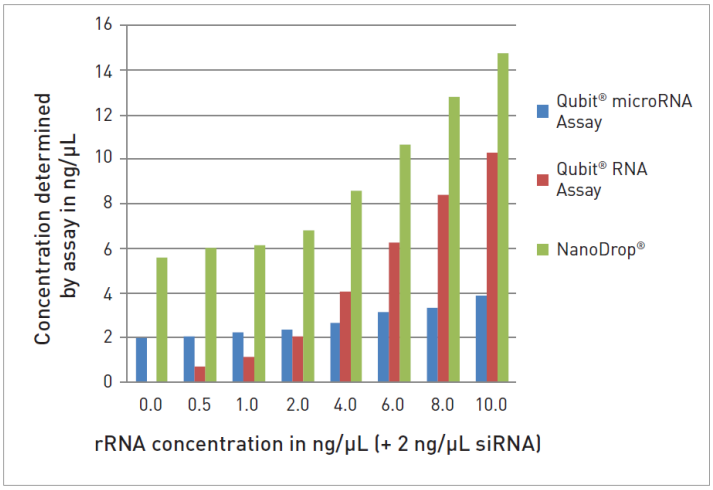

Figure 2 Comparison of detection techniques for accurate quantitation of small RNA in the presence of ribosomal RNA.
rRNA at the concentrations listed was spiked into solutions containing 2 ng/µL siRNA, then read using the Qubit™ microRNA assay, the Qubit™ RNA assay, or by 260 nm absorbance (A_{260})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

Photostability of Qubit™ reagents

The Qubit™ reagents exhibit 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 (Figure 3). 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, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

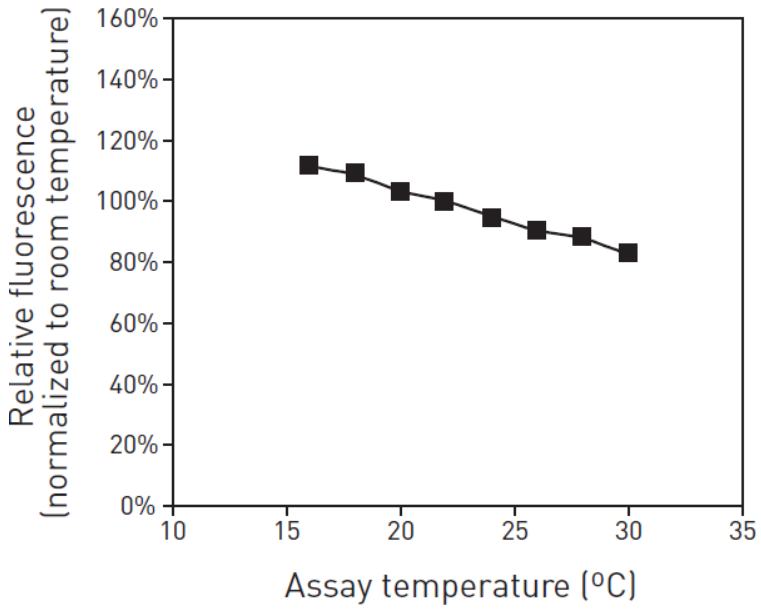

Figure 3 Plot of fluorescence vs. temperature for the Qubit™ microRNA assay.
The Qubit™ assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

Handling and disposal

No data are currently available addressing the mutagenicity or toxicity of the Qubit™ microRNA reagent (Component A). This reagent is known to bind nucleic acid and is provided as a solution in DMSO. Treat the Qubit™ microRNA reagent with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

Effects of contaminating substances

A number of common contaminants have been tested with the Qubit™ microRNA Assay Kit, 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 10 µL of each sample is used, add 10 µL of the unusual buffer (lacking microRNA) to each standard.

Table 1 Effects of contaminants in the Qubit™ 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 samples and standards

This protocol provides instructions to prepare standards for calibrating the Qubit™ Fluorometer. If you plan to use the values of a previous calibration, fewer tubes (step 1) and less working solution (step 4) will be needed (see “Calibrate the Qubit™ Fluorometer” on page 2).

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

1. Set up the required number of Qubit™ tubes for standards and samples. The Qubit™ microRNA assay requires 2 standards.

   Note: Use only thin-wall, clear, 0.5-mL PCR tubes (Cat. No. Q32856) for the Qubit™ 4 Fluorometer and 8 × 200-µL tube strips (Cat. No. Q33252) for the Qubit™ Flex Fluorometer.

2. Label the tube lids.

   Note: Do not label the side of the tube as this could interfere with the sample read. Label the lid of each standard tube correctly. Calibration of the Qubit™ Fluorometer requires the standards to be inserted into the instrument in the right order.

3. Prepare the Qubit™ working solution by diluting the Qubit™ microRNA reagent 1:200 in the Qubit™ microRNA buffer. Use a clean plastic tube each time you prepare a new Qubit™ working solution.

   IMPORTANT! Do not mix the working solution in a glass container.

4. Add the Qubit™ microRNA working solution to each tube such that the final volume is 200 µL.

	Standard assay tubes	User sample assay tubes
Volume of working solution	190 µL	180–199 µL
Volume of standard	10 µL	—
Volume of user sample	—	1–20 µL
Total volume in each assay tube	200 µL	200 µL

Note: The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit™ working solution, and each sample tube requires anywhere from 180–199 µL. Ensure that you have sufficient Qubit™ working solution to accommodate all standards and samples. For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit™ reagent plus 1990 µL of Qubit™ buffer).

Qubit™ Fluorometers provide a reagent calculator, which quickly computes the necessary volume of working solution needed.

5. Add 10 µL of each Qubit™ standard to the appropriate tube.

6. Add 1–20 µL of each user sample to the appropriate tube.

   Note: If you are adding 1–2 µL of sample, use a 2-µL pipette for best results.

7. Vigorously vortex for 3–5 seconds.

8. Allow all tubes to incubate at room temperature for 2 minutes, then proceed to read standards and samples (next section).

Read standards and samples

Follow the procedure appropriate for your instrument.

Read samples and standards with the Qubit™ 4 Fluorometer

For a more complete overview on using the Qubit™ 4 Fluorometer, please refer to *Qubit™ 4 Fluorometer User Guide* (Pub. No. MAN0017209), available for download at **thermofisher.com/qubit**.

1. On the **Home** screen, touch **RNA**, then select **microRNA** as the assay type. Touch **Read standards** to proceed.

   Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, skip to step 4. Otherwise, continue with step 2.

2. Insert the tube containing Standard #1 into the sample chamber, close the lid, then touch **Read standard**. When the reading is complete (~3 seconds), remove Standard #1.

3. Insert the tube containing Standard #2 into the sample chamber, close the lid, then touch **Read standard**. When the reading is complete, remove Standard #2.

   Note: The instrument displays the results on the Read Standards screen. For information on interpreting the calibration results, refer to the *Qubit™ 4 Fluorometer User Guide* (Pub. No. MAN0017209), available for download at **thermofisher.com/qubit**.

4. Touch **Run samples**.

5. On the assay screen, select the **Sample volume** and units.
   a. Touch the + or – buttons on the wheel, or anywhere on the wheel itself, to select the sample volume added to the assay tube (1–20 µL).
   b. From the **Unit** dropdown menu, select the units for the output sample concentration.

6. Insert a sample tube into the sample chamber, close the lid, then touch **Read tube**. When the reading is complete (~3 seconds), remove the sample tube. The top value (in large font) is the concentration of the original sample and the bottom value is the dilution concentration. For information on interpreting the sample results, refer to the *Qubit™ 4 Fluorometer User Guide* (Pub. No. MAN0017209).

7. Repeat step 6 until all samples have been read.

Read standards and samples with the Qubit™ Flex Fluorometer

For a more complete overview on using the Qubit™ Flex Fluorometer, please refer to *Qubit™ Flex Fluorometer User Guide* (Pub. No. MAN0018186), available for download at **thermofisher.com/qubit**.

1. On the **Home** screen, select **microRNA** as the assay type. Touch **Read standards & run samples** to proceed.

   Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, press **Run samples** and skip to step 4. Otherwise, continue with step 2.

2. Insert the tube strip containing Standard #1 into the sample chamber, close the lid, then touch **Run standards**. When the reading is complete (~3 seconds), remove Standard #1.

3. Insert the tube strip containing Standard #2 into the sample chamber, close the lid, then touch **Run standards**. When the reading is complete, remove Standard #2.

   Note: The instrument displays graphical results on the Standards complete screen. For information on interpreting the calibration results, refer to the *Qubit™ Flex Fluorometer User Guide* (Pub. No. MAN0018186), available for download at **thermofisher.com/qubit**.

4. Press **Next** from the Standards complete screen. When prompted, load the tube strips with your samples as shown in the Insert samples screen. If you have fewer than 8 samples, touch to deselect the tube positions that do not contain a sample.

5. Select the units for the output sample concentration, then touch **Next**.

6. (Optional) Select **More options** to add the assay kit lot #, tags, or sample IDs. For information on using these options, refer to the *Qubit™ Flex Fluorometer User Guide*.

7. In the **Sample volume** screen, enter the sample volume added to the assay tube (1–20 µL). Enter the volume directly in the **Sample volume** text box, use the + and – buttons, or adjust the sample volume wheel to select the **Sample volume** added to the assay tube.

   Note: The sample volume used (1–20 µL) changes the assay accuracy range. A different sample volume or assay may be required if the sample concentration is outside of what the assay can accurately quantify.

8. Insert a sample tube strip into the sample chamber, close the lid, then touch **Run samples**. When the reading is complete (~3 seconds), remove the sample tube strip.

   Standards and sample measurements are displayed on a graph with the results in a list below it.

   Touch the graph icon to switch to the results list-only view. The values listed are the concentrations of the original samples. For information on interpreting the sample results, refer to the *Qubit™ Flex Fluorometer User Guide* (Pub. No. MAN0018186).

9. Select **Add samples** and repeat step 8 to read more samples.

Related products

Table 2 Assays

Product	Quantitation range	Quantity	Cat. No.
Qubit™ Protein BR Assay Kit[1]	0.1–20 mg	100 reactions 500 reactions	A50668 A50669
Qubit™ Protein Assay Kit	12.5–5,000 µg	100 reactions 500 reactions	Q33211 Q33212
Qubit™ 1X dsDNA HS Assay Kit	0.1–120 ng	100 reactions 500 reactions	Q33230 Q33231
Qubit™ 1X dsDNA BR Assay Kit	4–4,000 ng	100 reactions 500 reactions	Q33265 Q33266
Qubit™ dsDNA HS Assay Kit	0.1–120 ng	100 reactions 500 reactions	Q32851 Q32854
Qubit™ dsDNA BR Assay Kit	4–2,000 ng	100 reactions 500 reactions	Q32850 Q32853
Qubit™ ssDNA Assay Kit	0.2–240 ng	100 reactions	Q10212
Qubit™ RNA IQ Assay Kit	N/A	75 reactions 275 reactions	Q33221 Q33222
Qubit™ RNA HS Assay Kit	4–200 ng	100 reactions 500 reactions	Q32852 Q32855
Qubit™ RNA BR Assay Kit	10–1,200 ng	100 reactions 500 reactions	Q10210 Q10211
Qubit™ RNA XR Assay Kit	100–20,000 ng	100 reactions 500 reactions	Q33223 Q33224
Qubit™ microRNA Assay Kit	0.5–150 ng	100 reactions 500 reactions	Q32880 Q32881
Qubit™ 4 System Verification Assay Kit	N/A	50 reactions	Q33237
Qubit™ Flex System Verification Assay Kit	N/A	50 reactions	Q33254

[1] Qubit™ Protein BR Assay Kit is designed for use with Qubit™ 4 only.

Table 3 Instruments

Product	Cat. No.
Qubit™ Flex Fluorometer	Q33327
Qubit™ Flex Fluorometer NGS Starter Kit	Q45893
Qubit™ Flex Fluorometer Quantitation Starter Kit	Q45894
Qubit™ 4 Fluorometer	Q33238
Qubit™ 4 NGS Starter Kit	Q33240
Qubit™ 4 Quantitation Starter Kit	Q33239
Qubit™ 4 RNA IQ Starter Kit	Q33241
Qubit™ 4 Protein BR Starter Kit	A51292

Table 4 Consumables/Accessories

Product	Quantity	Cat. No.
Qubit™ Flex Assay Tube Strips	125 tube strips	Q33252
Qubit™ Assay Tubes	500 tubes	Q32856
Qubit™ 4 Fluorometer International Power Supply (replacement)	1 each	A36204
Qubit™ 4 USB Flash Drive	1 each	Q46009

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**.

Life Technologies Corporation | 29851 Willow Creek | Eugene, Oregon 97402 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No.MAN0009427

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