Qubit™ RNA IQ Assay Kit

Catalog Numbers Q33221 and Q33222

Pub. No. MAN0017405 Rev. C

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

The Qubit™ RNA IQ Assay Kit provides a fast, simple method to check whether an RNA sample has degraded using the Qubit™ 4 or Qubit™ Flex Fluorometer. The assay utilizes two unique dyes: one that binds to large and/or highly structured RNA, and another that selectively binds to small, degraded RNA (Figure 1). Together, the dyes enable you to quickly assess the quality and integrity of an RNA sample. Results are presented as an RNA IQ number (RNA IQ#) that indicates the RNA sample integrity and quality, and also as a calculated percent of large and small RNA in the sample. The RNA IQ# is a value from 1–10, similar to other RNA quality scores, where a small number indicates that the sample is comprised of mainly small RNA fragments and a larger number indicates that the sample consists of mainly large RNA or RNA with tertiary structure.

The Qubit™ RNA IQ Assay Kit includes concentrated assay reagent, dilution buffer, and RNA standards, both intact and degraded. To run the assay, dilute the reagent using the buffer provided, add 0.5–1.5 µg/µL of your sample (any volume from 1–20 µL is acceptable), then read the RNA quality using the Qubit™ Fluorometer. The assay is performed at room temperature, and the results are stable for 1 hour. Common contaminants such as salts, free nucleotides, and RNA stabilization reagents are well tolerated in the assay (“Contaminants tolerated by the Qubit™ RNA IQ Assay” on page 7). In addition to the Qubit™ RNA IQ Assay Kit, we also offer other Qubit™ kits as well as the NanoDrop™ Spectrophotometer for quantification of DNA and RNA.

Note: The Qubit™ RNA IQ Assay Kit only works on Qubit™ 4 or Qubit™ Flex Fluorometer models. The assay is not designed for use with Qubit™, Qubit™ 2, or Qubit™ 3 Fluorometers.

Contents and storage

Material	Amount		Concentration	Storage[1]
	Q33221 (75 assays)	Q33222 (275 assays)		
Qubit™ RNA IQ Reagent (Component A)	2 × 100 µL	10 × 100 µL	200X in DMSO	–5°C to –20°C Desiccated Protect from light
Qubit™ RNA IQ Buffer (Component B)	40 mL	200 mL	—	Room temperature
Qubit™ RNA IQ Standard #1[2] (Component C)	850 µL	2 × 1.5 mL	0 ng/µL RNA in 1 mM citrate buffer	≤–70°C
Qubit™ RNA IQ Standard #2 (Component D)	850 µL	2 × 1.5 mL	100 ng/µL small RNA in 1 mM citrate buffer	
Qubit™ RNA IQ Standard #3 (Component E)	4 × 225 µL	10 × 300 µL	100 ng/µL large RNA in 1 mM citrate buffer	

[1] When stored as directed, the kits are stable for at least 6 months from the date of receipt.
[2] The RNA IQ assay kit standards are stable for shipment and short-term storage at –5°C to –20°C; long-term storage at ≤–70°C is recommended.

Required materials not supplied

- Sterile or nuclease-free plastic container disposable for mixing the Qubit™ RNA IQ working solution
- Nuclease-free pipettors and tips
- Qubit™ Assay Tubes (Cat. No. Q32856) or Qubit™ Flex Assay Tube Strips (Cat. No. Q33252)

Avoid nuclease contamination

- Store the Qubit™ RNA IQ Reagent at ≤–20°C, desiccated and protected from light.
- The Qubit™ RNA IQ Buffer can be stored at ≤–20°C, but it is stable at room temperature.
- Store the Qubit™ RNA IQ Standards at ≤–70°C.

Handling and disposal

No data are currently available that address the mutagenicity or toxicity of the Qubit™ RNA IQ Reagent (Component A). This reagent is an organic dye and is provided as a solution in DMSO. Treat the Qubit™ RNA IQ Reagent with the same safety precautions as other materials with similar properties and dispose of the dye in accordance with local regulations.

Avoid nuclease contamination

To prevent contamination from common sources of RNase, wear gloves at all times and use sterile technique when handling the reagents and samples. The RNaseZap™ Solution (Cat. No. AM9782), which destroys RNases as well as DNases, can be used to clean pipettors and any plasticware.

Critical assay parameters

Assay temperature

The Qubit™ RNA IQ Assay Kit delivers optimal performance when all solutions are at room temperature (18–28°C). Temperature fluctuations can influence the accuracy of the assay (Figure 2).

To minimize temperature fluctuations, store the Qubit™ 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; 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 because this warms the solution and results in a different reading.

Incubation time

To allow the Qubit™ RNA IQ Assay Kit to reach optimal fluorescence, incubate the 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 1 hour at room temperature. For greatest accuracy, the incubation time of the samples should be within 10 minutes of the incubation time of the standards.

Photobleaching of the Qubit™ RNA IQ assay samples

The Qubit™ RNA IQ samples and standards exhibit stable fluorescence for up to 1 hour when kept protected from light. We do not recommend performing multiple readings of a single tube. Perform replicate measurements using separate tubes.

Sample volume

The Qubit™ assays are designed to use 1–20 µL sample. For best results, use the largest volume possible. Larger volumes are easier to pipette accurately than small volumes and will reduce the statistical error in your results.

Qubit™ Fluorometer calibration

For each assay, you have the choice to run a new calibration or use the values from the previous calibration. For the Qubit™ RNA IQ assay, we recommend that the standards are run every time an assay is run to ensure the most accurate IQ measurement. Additionally, remember that the fluorescence signal in the tubes containing standards and samples is stable for no longer than 1 hour.

Prepare standards and samples

1. Set up the required number of assay tubes for standards and samples. The Qubit™ RNA IQ Assay requires 3 standards.

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, though the instrument will perform several checks of the standards during calibration, ensuring proper calibration procedures are followed.

3. Prepare the Qubit™ working solution by diluting the Qubit™ RNA IQ Reagent 1:200 in Qubit™ RNA IQ Buffer. Use a clean plastic tube each time you prepare Qubit™ working solution. Do not mix the working solution in a glass container.

   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. Prepare sufficient Qubit™ working solution to accommodate all standards and samples.

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

4. Add 190 µL of Qubit™ working solution to each of the tubes used for standards.

   Note: As with any solutions containing RNA, ensure that all plasticware coming into contact with the standards and the environment around the preparation area is clean and nuclease-free, due to both RNA sensitivity and the sensitivity of the RNA IQ assay toward RNA degradation.

5. Add 10 µL of each Qubit™ standard to the appropriate tubes, then vortex for 2–3 seconds to mix.

   IMPORTANT! Be careful not to create bubbles.

   Note: Careful pipetting is critical to ensure that exactly 10 µL of each Qubit™ Standard is added to 190 µL of Qubit™ working solution.

6. Add Qubit™ working solution to individual assay tubes so that the final volume in each tube after adding sample is 200 µL.

   Note: Your sample can be anywhere from 1–20 µL. Table 1 lists the appropriate concentration range for different sample volumes.Add a corresponding volume of Qubit™ working solution to each assay tube, anywhere from 180–199 µL.

   Table 1 Recommended sample concentration vs. sample volume

Sample volume	Concentration range
1.0 µL	500–1500 ng/µL
2.0 µL	250–750 ng/µL
5.0 µL	100–300 ng/µL
10.0 µL	50–150 ng/µL
15.0 µL	33–100 ng/µL
20.0 µL	25–75 ng/µL

7. Add each sample to the assay tubes containing the correct volume of Qubit™ working solution, then vortex for 2–3 seconds to mix. The final volume in each tube should be 200 µL.

8. Allow all tubes to incubate at room temperature for 2 minutes, then proceed to “Read standards and samples” on page 3.

Read standards and samples

1. On the **Home** screen of the Qubit™ Fluorometer, press the **RNA** icon, then select the **RNA IQ assay** from the list of available RNA assays. The **Read standards** screen is displayed. Press **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. To use the previous calibration, skip step 5. Otherwise, continue with step 2.

2. Insert the assay tube or tube strip containing Standard #1 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete (~7 seconds), remove Standard #1.

   Note: If the reading was successful, a sample tube and a circle will appear indicating success in the first step of calibration. Otherwise, the instrument will alert you to an error.

When calibrating the Qubit™ Flex, all 8 wells will need to be calibrated. The instrument will alert you to any specific wells that have an error.

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

   If the reading was successful, the sample tube will partially fill, as will the circle, indicating success in the second step of calibration. Otherwise, the instrument will alert you to an error.

4. Insert the assay tube or tube strip containing Standard #3 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete, remove Standard #3.

   Note: The instrument displays a notice for a successful calibration on the Read standard screen.

   Note: If you receive the "**Calibration error**" message, you can rerun the standards:

   - In the Error screen, press **OK**.
   - Review the **Read standard** screen.
   - If you wish to rerun the standards, or run new standards, press **Read standard**, then repeat the calibration procedure.

5. Press **Run samples**.

6. On the assay screen, select the sample volume: Press the **+** or **–** buttons on the wheel, or anywhere on the wheel itself, to select the sample volume added to the assay tube or tube strip (from 1–20 µL).

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

   The instrument displays the results on the assay screen. The top value (in large font) in the circular graphical display is the RNA IQ score, or quality score, of the original sample. Within the circle is a graphical display of the results: the entire circle will be blue for an RNA IQ = 10, while the entire circle will be orange for an RNA IQ of 0.The bottom value indicates the percent composition of your RNA sample in % large and/or structured RNA and % small RNA.

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

Ordering information

Product name	Cat. No.	Unit size
Qubit™ RNA IQ Assay Kit, for use with the Qubit™ 4 and Flex Fluorometers	Q33221	75 assays
Qubit™ RNA IQ Assay Kit, for use with the Qubit™ 4 and Flex Fluorometers	Q33222	275 assays
Qubit™ RNA IQ Assay – RNA Standards	Q33235	–

Related products	Cat. No.	Unit size
Qubit™ RNA BR Assay Kit	Q10210	100 assays
Qubit™ RNA BR Assay Kit	Q10211	500 assays
Qubit™ RNA HS Assay Kit	Q32852	100 assays
Qubit™ RNA HS Assay Kit	Q32855	500 assays
Qubit™ RNA XR Assay Kit	Q33223	100 assays
Qubit™ RNA XR Assay Kit	Q33224	500 assays
Qubit™ microRNA Assay Kit	Q32880	100 assays
Qubit™ microRNA Assay Kit	Q32881	500 assays
Qubit™ dsDNA BR Assay Kit	Q32850	100 assays
Qubit™ dsDNA BR Assay Kit	Q32853	500 assays
Qubit™ dsDNA HS Assay Kit	Q32851	100 assays
Qubit™ dsDNA HS Assay Kit	Q32854	500 assays
Qubit™ 1X dsDNA BR Assay Kit	Q33265	100 assays
Qubit™ 1X dsDNA BR Assay Kit	Q33266	500 assays
Qubit™ 1X dsDNA HS Assay Kit	Q33230	100 assays
Qubit™ 1X dsDNA HS Assay Kit	Q33231	500 assays
Qubit™ ssDNA Assay Kit	Q10212	100 assays
Qubit™ Protein Assay Kit	Q33211	100 assays
Qubit™ Protein Assay Kit	Q33212	500 assays
Qubit™ BR Protein Assay Kit, for use with Qubit™ 4 Fluorometers	A50668	100 assays
Qubit™ BR Protein Assay Kit, for use with Qubit™ 4 Fluorometers	A50669	500 assays
Qubit™ Endotoxin Detection Assay Kit, for use with Qubit™ Flex Fluorometers	Q32891	80 assays
Qubit™ 4 System Verification Assay Kit	Q33237	–
Qubit™ Flex System Verification Assay Kit	Q33254	–
Qubit™ Assay Tubes	Q32856	500 tubes
Qubit™ Flex Assay Tube Strips	Q33252	125 count
Qubit™ Flex Pyrogen-Free Tube Strips	Q32893	120 count

Selectivity of the Qubit™ RNA IQ Assay

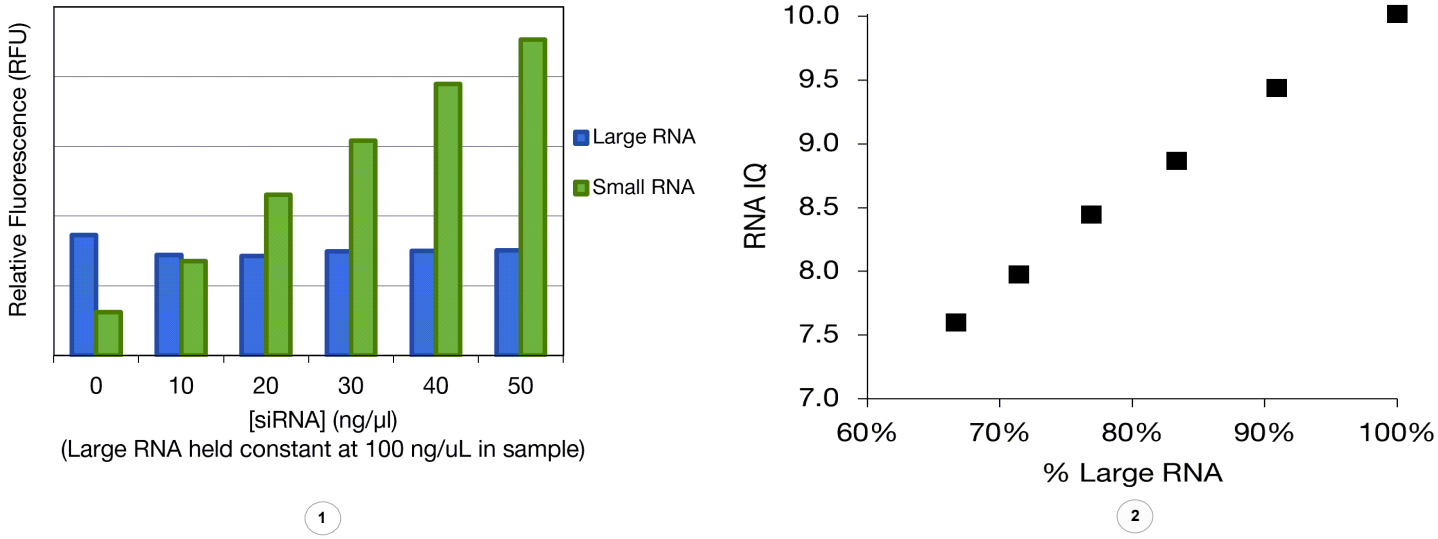

Figure 1 Selectivity of the Qubit™ RNA IQ reagents for large and small RNA

Triplicate samples containing 100 ng/µL of rRNA (*E. coli*) and varying amounts of siRNA (0–50 ng/µL) were assayed with the Qubit™ RNA IQ Assay (Cat. Nos. Q33221 and Q33222) on the Qubit™ 4 Fluorometer (Cat. No. Q33226). Relative fluorescent units (RFUs) (**1**) and IQ#'s (**2**) were plotted for these samples.

Effect of temperature on the Qubit™ RNA IQ Assay

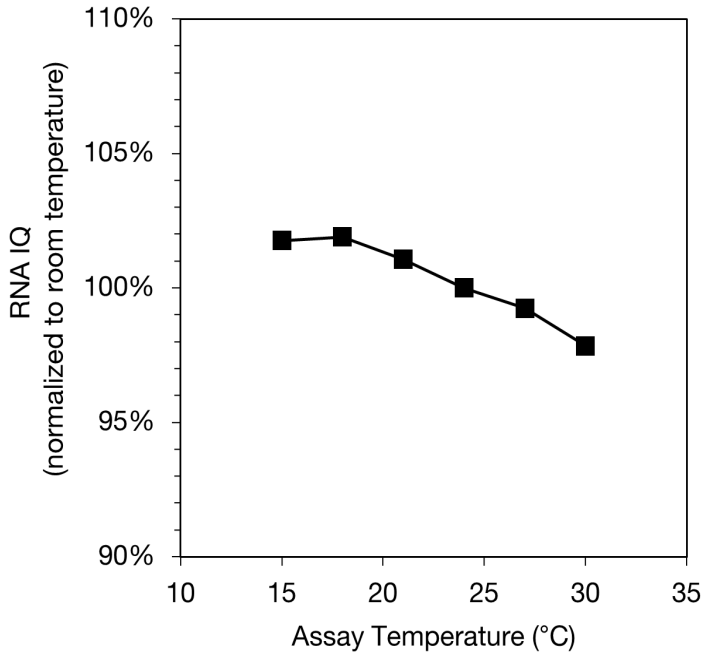

Figure 2 Plot of fluorescence vs. temperature for the Qubit™ RNA IQ Assay Qubit™ assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

Table 2 Effect of contaminants in the Qubit™ RNA IQ Assay

Contaminant	Final concentration in the assay	Concentration in 20 µL sample	Concentration in 10 µL sample	Result
Sodium chloride	25 mM	250 mM	500 M	NR[1]
Magnesium chloride	2.5 mM	25 mM	50 mM	NR
Ammonium acetate	2.5 mM	25 mM	50 mM	NR
Sodium acetate	2.5 mM	25 mM	50 mM	NR
Sodium azide	0.5 mM	5 mM	10 mM	OK
EDTA	1 mM	10 mM	20 mM	OK
Ethanol	0.5%	5%	10%	OK
SDS	0.005%	0.05%	0.1%	OK
Phenol	0.05%	0.5%	1%	OK
BSA	20 µg/mL	200 µg/mL	400 µg/mL	OK
RNA later	upto 0.025%	upto 0.25%	upto 0.5%	OK[2]
Guanidine•HCl	upto 0.05 µg/mL	upto 0.5 µg/mL	upto 1 µg/mL	OK
RNA storage solution	10%	Neat	Neat	OK
Sodium citrate	0.5 mM	5 mM	10 mM	OK
RNaseOUT	5 units	50 units	100 units	OK
DNA	NR	NR	NR	[3]

[1] Not recommended
[2] An acceptable result, but concentrations above those listed result in unacceptable signal deviations.
[3] Presence of DNA results in a decrease of the RNA quality score. As a result, samples with significant DNA content should be treated with DNase to remove residual DNA contamination before assaying.

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  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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

Revision history: Pub. No. MAN0017405 C

Revision	Date	Description
C	30 January 2025	Updated “Product description” on page 1, “Required materials not supplied” on page 2,“Critical assay parameters” on page 2, “Prepare standards and samples” on page 3, “Read standards and samples” on page 3, and “Contaminants tolerated by the Qubit™ RNA IQ Assay” on page 7. Removed Qubit™ assay kits compatible with the Qubit™ Fluorometer table.
B	5 December 2017	Corrected sample concentration in Product description, added sample volume vs. sample concentration table for step 1.6, expand Qubit™ assay kits compatible with the Qubit™ Fluorometer to include additional Qubit™ assay kits.
A	12 October 2017	New document for Qubit™ RNA IQ Assay Kit.

The information in this guide is subject to change without notice.