Qubit™ RNA IQ Demonstration Assay Kit, 15 Assays

For use with the Qubit™ Fluorometer

Catalog No. Q33220

Pub. No. MAN0017580 **Rev.** A.0

Product information

The Qubit™ RNA IQ Assay Kit provides a fast, simple method to check whether an RNA sample has degraded using the Qubit™ 4 Fluorometer (required; see **Note** below). 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, page 5). 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 to 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 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 of your sample (any volume from 1–20 µL is acceptable), then read the RNA quality using the Qubit™ 4 Fluorometer (see **Note** below). 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 (Table 2, page 6).

Note: The Qubit™ RNA IQ assay kit can only be used with the Qubit™ 4 Fluorometer. The RNA IQ assay does not work on the original Qubit™, Qubit™ 2.0, or Qubit™ 3 Fluorometers.

Table 1. Contents and storage

Material	Amount	Concentration	Storage*
	Q33220 (15 assays)		
Qubit™ RNA IQ Reagent (Component A)	25 µL	200X concentrate in DMSO	• ≤–20°C • Desiccate • Protect from light
Qubit™ RNA IQ Buffer (Component B)	5 mL	N/A	Room temperature
Qubit™ RNA IQ Standard #1 (Component C)†	250 µL	0 ng/µL RNA in 1 mM citrate buffer	≤–70°C
Qubit™ RNA IQ Standard #2 (Component D)†	250 µL	100 ng/µL small RNA in 1 mM citrate buffer	
Qubit™ RNA IQ Standard #3 (Component E)†	250 µL	100 ng/µL large RNA in 1 mM citrate buffer	
Qubit™ Assay Tubes	15 tubes	N/A	Room temperature

* When stored as directed, the kits are stable for at least 6 months from the date of receipt.

† The RNA IQ assay kit standards are stable for shipment and short-term storage at ≤–20°C; long-term storage at ≤–70°C is recommended.

N/A: Not applicable

Materials required but not provided

- Sterile or nuclease-free plastic container (disposable) for mixing the RNA IQ working solution (step 1.3, page 3)
- Nuclease-free pipettors and tips

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 delivers optimal performance when all solutions are at room temperature (22–28˚C). Temperature fluctuations can influence the accuracy of the assay (Figure 2, page 6). To minimize temperature fluctuations, store the Qubit™ Buffer at room temperature and insert all assay tubes into the Qubit™ 4 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence; the Qubit™ 4 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 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 Qubit™ 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.

Methods

Prepare standards and samples

This protocol assumes that you are preparing standards to calibrate the Qubit™ 4 Fluorometer.

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

Note: Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit™ assay tubes (Cat. No. Q32856)

1.2 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™ 4 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.

1.3 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 the 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.

For example, for 7 samples, prepare enough working solution for the samples and 3 standards: ~200 µL per tube for 10 tubes requires 2 mL of working solution (10 µL of Qubit™ reagent plus 1990 µL of Qubit™ buffer).

The Qubit™ 4 Fluorometer provides a reagent calculator, which quickly computes the necessary volume of working solution needed.

1.4 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.

1.5 Add 10 µL of each Qubit™ standard to the appropriate tube, then mix by vortexing 2–3 seconds. 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.

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

Note: Your RNA sample volume can be anywhere from 1–20 µL. Table 2 lists the appropriate concentration range for different sample volumes. Add a corresponding volume of Qubit™ working solution to each assay tube, anywhere between 180–199 µL.

Table 2. Recommended sample concentration vs. sample volume

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

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

1.8 Allow all tubes to incubate at room temperature for 2 minutes. Proceed to "Read standards and samples".

Read standards and samples

This protocol describes how to run the Qubit™ RNA IQ assay standards and samples on the Qubit™ 4 Fluorometer.

2.1 On the **Home** screen of the Qubit™ 4 Fluorometer, press **RNA**, 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. If you want to use the previous calibration, skip to step 2.5. Otherwise, continue with step 2.2.

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

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.

2.3 Insert the tube 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.

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

The instrument displays a notice for a successful calibration on the Read standard 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**.

2.5 Press **Run samples**.

2.6 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 (from 1–20 µL)

2.7 Insert a sample tube into the sample chamber, close the lid, then press **Read tube**. When the reading is complete (~4 seconds), remove the sample tube.

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. For information on interpreting the sample results, refer to the *Qubit™ 4 Fluorometer User Guide*.

2.8 Repeat step 2.7 until all samples have been read.

Appendix

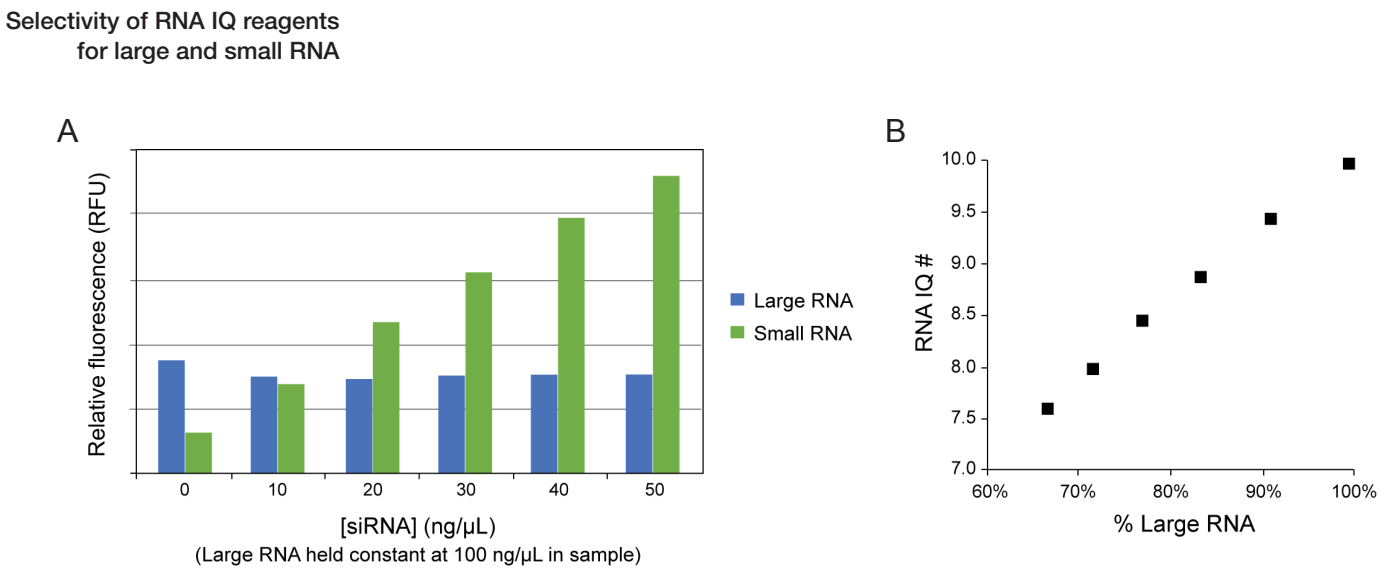

Figure 1. Selectivity of the Qubit™ RNA IQ reagents for large and small RNA. Triplicate samples containing 100 ng/mL of rRNA (*E. coli*) and varying amounts of siRNA (0 to 50 ng/µL) were assayed with the Qubit™ RNA IQ Assay on the Qubit™ 4 Fluorometer (Cat. No. Q33226). Relative fluorescent units (RFUs) **(A)** and IQ#'s **(B)** were plotted for these samples.

Effect of temperature on the Qubit™ RNA IQ Assay

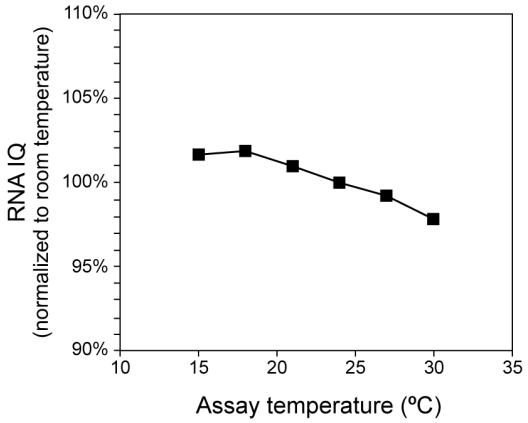

Figure 2. Plot of RNA IQ vs. temperature for the Qubit™ RNA IQ Assay. The Qubit™ assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

Contaminants tolerated by the Qubit™ RNA IQ 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	50 mM	250 mM	NR
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	Up to 0.025%	Up to 0.25%	Up to 0.5%	OK†
Guanidine•HCl	Up to 1 mg/mL	Up to 10 mg/mL	Up to 20 mg/mL	OK†
THE RNA Storage solution	10%	Neat	Neat	OK
Sodium Citrate	10 mM	100 mM	200 mM	OK
RNaseOUT	5 Units	50 Units	100 Units	OK
DNA	NR	NR	NR	††

* RNA IQ standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 20-µL or 10-µL sample volumes are also listed. Results are given as OK, usually less than 10% perturbation, or as NR (not recommended).

† An acceptable result, but concentrations above those listed result in unacceptable signal deviations.

†† 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.

Ordering information

Cat. No.	Product name	Unit size
Q33220	Qubit™ RNA IQ Demonstration Assay Kit, 15 assays *for use with the Qubit™ 4 Fluorometer*	1 kit
Related products		
Q33221	Qubit™ RNA IQ Assay Kit, 75 assays *for use with the Qubit™ 4 Fluorometer*	1 kit
Q33222	Qubit™ RNA IQ Assay Kit, 275 assays *for use with the Qubit™ 4 Fluorometer*	1 kit
Q32850	Qubit™ dsDNA BR Assay Kit, 100 assays *2–1000 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q32853	Qubit™ dsDNA BR Assay Kit 500 assays *2–1000 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q32851	Qubit™ dsDNA HS Assay Kit, 100 assays *0.2–100 ng* *for use with the Qubit™ Fluorometer	1 kit
Q32854	Qubit™ dsDNA HS Assay Kit, 500 assays *0.2–100 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q10210	Qubit™ RNA BR Assay Kit, 100 assays *20–1000 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q10211	Qubit™ RNA BR Assay Kit, 500 assays *20–1000 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q32852	Qubit™ RNA HS Assay Kit, 100 assays *5–100 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q32855	Qubit™ RNA HS Assay Kit, 500 assays *5–100 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q10212	Qubit™ ssDNA Assay Kit, 100 assays *1–200 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q32880	Qubit™ microRNA Assay Kit, 100 assays *1–100 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q32881	Qubit™ microRNA Assay Kit, 500 assays *1–100 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q33211	Qubit™ Protein Assay Kit, 100 assays *0.25–5 µg* *for use with the Qubit™ Fluorometer*	1 kit
Q33212	Qubit™ Protein Assay Kit, 500 assays *0.25–5 µg* *for use with the Qubit™ Fluorometer*	1 kit
Q32856	Qubit™ assay tubes *set of 500*	1 set
Q33226	Qubit™ 4 Fluorometer	1 fluorometer
Q33227	Qubit™ 4 Quantitation Starter Kit	1 kit
Q33228	Qubit™ 4 NGS Starter Kit	1 kit
Q33229	Qubit™ 4 RNA IQ Starter Kit	1 set

Documentation and support

Customer and Technical Support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

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.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

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

For Research Use Only. Not for use in diagnostic procedures.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0017580

Revision	Date	Description
A.0	24 January 2018	New user guide

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Manufacturer: Life Technologies Corporation | 29851 Willow Creek Road | Eugene, OR 97402

Trademarks: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

ThermoFisher SCIENTIFIC