Qubit[™] RNA IQ Assay Kits

Catalog No. Q33221, Q33222

Pub. No. MAN0017405

Rev. B.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 QubitTM RNA IQ Assay Kits include concentrated assay reagent, dilution buffer, and RNA standards, both intact and degraded. To run the assay, dilute the reagent using the buffer provided, add $0.5-1.5 \,\mu\text{g}/\mu\text{L}$ of your sample (any volume from 1–20 μ L is acceptable), then read the RNA quality using the QubitTM 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 3, page 6). In addition to the QubitTM RNA IQ Assay Kits, we also offer other QubitTM kits (Table 4, page 7) as well as the NanoDropTM Spectrophotometer for quantitation of DNA and RNA.

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

Table 1. Contents and storage

Amount			Storage*	
Q33221 (75 assays)				
2 × 100 µL	10 × 100 μL	200X concentrate in DMSO	 ≤-20°C Desiccate Protect from light 	
40 mL	200 mL	N/A	Room temperature	
850 μL	2 × 1.5 mL	0 ng/µL RNA in 1 mM citrate buffer		
850 μL	2 × 1.5 mL	100 ng/µL small RNA in 1 mM citrate buffer	≤–70°C	
4 × 225 µL	10 × 300 μL	100 ng/µL large RNA in 1 mM citrate buffer		
	Q33221 (75 assays) 2 × 100 μL 40 mL 850 μL 850 μL	Q33221 (75 assays) Q33222 (175 assays) 2 × 100 μL 10 × 100 μL 40 mL 200 mL 850 μL 2 × 1.5 mL 850 μL 2 × 1.5 mL	Q33221 (75 assays)Q33222 (175 assays)Concentration $2 \times 100 \ \mu L$ $10 \times 100 \ \mu L$ $200 \ x \ concentrate \ in \ DMSO$ $40 \ mL$ $200 \ mL$ N/A $40 \ mL$ $200 \ mL$ N/A $850 \ \mu L$ $2 \times 1.5 \ mL$ $0 \ ng/\mu L \ RNA \ in \ 1 \ mM \ citrate \ buffer$ $850 \ \mu L$ $2 \times 1.5 \ mL$ $100 \ ng/\mu L \ small \ RNA \ in \ 1 \ mM \ citrate \ buffer$ $40 \ mL$ $10 \times 300 \ \mu L$ $100 \ ng/\mu L \ large \ RNA \ in \ 1 \ mM \ citrate \ buffer$	

* 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 \leq -20°C; long-term storage at \leq -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 Qubit[™] assay tubes (Cat. No. Q32856)
Storage	 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 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
calibrationFor 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 $^{^{\rm TM}}$ 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 ^{TM} 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 TM working solution by diluting the Qubit TM RNA IQ Reagent 1:200 in Qubit TM RNA IQ Buffer. Use a clean plastic tube each time you prepare the Qubit TM working solution. Do not mix the working solution in a glass container.
	Note: The final volume in each tube must be 200 μ L. Each standard tube requires 190 μ L of Qubit TM working solution, and each sample tube requires anywhere from 180–199 μ L. Prepare sufficient Qubit TM working solution to accommodate all standards and samples.
	For example, for 7 samples, prepare enough working solution for the samples and 3 standards: ~200 μ L per tube for 10 tubes requires 2 mL of working solution (10 μ L of Qubit TM reagent plus 1990 μ L of Qubit TM buffer).
	The Qubit [™] 4 Fluorometer provides a reagent calculator, which quickly computes the necessary volume of working solution needed.
1.4	Add 190 μ L of Qubit TM 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 μ L of each QubitTM standard is added to 190 μ L of QubitTM 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.

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

Table 2. Recommended sampe concentration vs. sample volume

- **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^{TM} RNA IQ assay standards and samples on the Qubit^{TM} 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^M 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 $1-20 \,\mu L$)
- 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

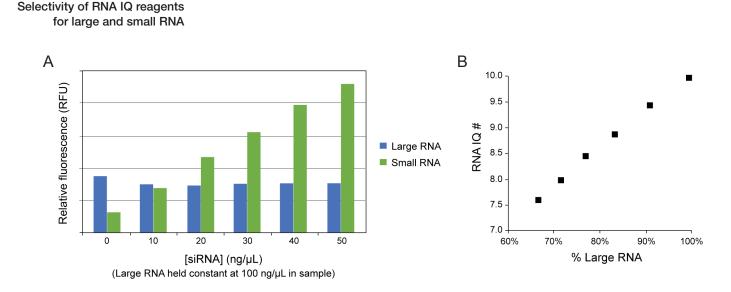


Figure 1. Selectivity of the Qubit[™] RNA IQ reagents for large and small RNA. Triplicate samples containing 100 ng/µL of rRNA (*E. coli*) and varying amounts of siRNA (0 to 50 ng/µL) were assayed with the Qubit[™] RNA IQ Assay (Cat. Nos. Q33221, Q33222) on the Qubit[™] 4 Fluorometer (Cat. No. Q33226). Relative fluorescent units (RFUs) (A) and IQ#'s (B) were plotted for these samples.

Effect of temperature on the Qubit[™] RNA IQ Assay

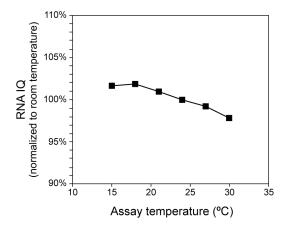


Figure 2. Plot of RNA IQ vs. temperature for the Qubit[™] RNA IQ Assay. The Qubit[™] assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

Contaminants tolerated by the Qubit[™] RNA IQ Assay

Table 3. Effect of contaminants in the Qubit [™] RNA IQ Assay ³

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	ОК
EDTA	1 mM	10 mM	20 mM	ОК
Ethanol	0.5%	5%	10%	ОК
SDS	0.005%	0.05%	0.1%	ОК
Phenol	0.05%	0.5%	1%	ОК
BSA	20 µg/mL	200 µg/mL	400 µg/mL	ОК
RNA Later	Up to 0.025%	Up to 0.25%	Up to 0.5%	OK [†]
Guanidine•HCI	Up to 1 mg/mL	Up to 10 mg/mL	Up to 20 mg/mL	OK [†]
THE RNA Storage solution	10%	Neat	Neat	ОК
Sodium Citrate	10 mM	100 mM	200 mM	ОК
RNaseOUT	5 Units	50 Units	100 Units	ОК
DNA	NR	NR	NR	tt

* 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.

Qubit[™] assay kits compatible with the Qubit[™] Fluorometer

A number of fluorescence-based quantification kits are available for use with the Qubit[™] Fluorometer. Use Table 4 to choose a kit based on the target molecule being measured and the number of assays you require.

Table 4. Qubit[™] assay kits for use with the Qubit[™] Fluorometer

	Cat. No.	No. of assays*	Target	Notes
Qubit [™] dsDNA	Q32850	100	dsDNA	 Core range (high confidence): 0.01 μg/mL to 5 μg/mL[†] Extended range (moderate confidence): 5 μg/mL to 10 μg/mL[†]
BR Assay Kit	Q32853	500	USDNA	 Useful for quantitation of genomic and miniprep DNA samples Accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit [™] dsDNA	Q32851 Q33230	100		 Core range (high confidence): 1 ng/mL to 500 ng/mL[†] Extended ranges (moderate confidence): 0.5 ng/mL to 1 ng/mL and 500 ng/mL to 600 ng/mL[†]
HS Assay Kit	Q32854 Q33231	500	dsDNA	 Useful for quantitation of PCR products, viral DNA, and samples for subcloning Accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ ssDNA Assay Kit	Q10212	100	ssDNA	 Core range (high confidence): 5 ng/mL to 1000 ng/mL[†] Extended ranges (moderate confidence): 1 ng/mL to 5 ng/mL and 1000 ng/mL to 1200 ng/mL[†] Useful for quantitation of oligos, primers, denatured DNA, PCR products Accurate in the presence of salts, urea, solvents, proteins, ATP, and agarose
Qubit [™] RNA HS	Q32852	100	RNA	 Core range (high confidence): 25 ng/mL to 500 ng/mL[†] Extended ranges (moderate confidence): 20 ng/mL to 25 ng/mL and 500 ng/mL to 1000 ng/mL[†]
Assay Kit	Q32855	500		 Useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures Accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit [™] RNA BR	Q10210	100		 Core range (high confidence): 0.1 μg/mL to 5 μg/mL[†] Extended ranges (moderate confidence): 0.05 μg/mL to 0.1 μg/mL and 5 μg/mL to 6 μg/mL[†]
Assay Kit	Q10211	500	RNA	 Useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures Accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit [™] RNA XR	Q33233	100	RNA	• Core range: 1 ng/mL to 8 μg/mL [†]
Assay Kit	Q33234	500		 Useful for quantitation of samples for RT-PCR, qRT-PCR or RNA-SEQ Accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit [™] microRNA	Q32880	100		 Core range (high confidence): 5 ng/mL to 500 ng/mL[†] Extended ranges (moderate confidence): 2.5 ng/mL to 5 ng/mL and 500 ng/mL to 750 ng/mL[†]
Assay Kit	Q32881	500	RNA	 Useful for quantification of samples for qRT-PCR and sequencing applications Accurate in the presence of rRNA, large mRNA (>1000 bp), salts, solvents, proteins, and free nucleotides

[†]Concentration ranges refer to the concentration of the sample after dilution in the assay tube.

Table 4 (continued). Qubit ${}^{\scriptscriptstyle {\rm M}}$ assay kits for use with the Qubit ${}^{\scriptscriptstyle {\rm M}}$ Fluorometer

Product	Cat. No.	No. of assays*	Target	Notes
Qubit [™] Protein	Q33211	100	 Protein Extended ranges (moderate confidence): 1 µg/mL to to 26 µg/mL[†] Little protein-to-protein difference in signal Accurate in the processor of DTT 0, monochapterate 	 Core range (high confidence): 1.25 μg/mL to 25 μg/mL[†] Extended ranges (moderate confidence): 1 μg/mL to 1.25 μg/mL and 25 μg/mL to 26 μg/mL[†]
Assay Kit	Q33212	500		• Accurate in the presence of DTT, β -mercaptoethanol, amino acids, and DNA
Qubit [™] RNA IQ	Q33221 75	RNA dye	Accurate in the presence of salts, protein, solvents and RNA stabilization	
Assay Kit	Q33222	275	and quality	 Signal is stable for 1 hour For use with the Qubit[™] 4 Fluorometer; the assay does not work on the original Qubit[™], Qubit[™] 2.0, or Qubit[™] 3 Fluorometers
*Based on an assay volume of 200 μL. [†] Concentration ranges refer to the concentration of the sample after dilution in the assay tube.				

Ordering information

Cat. No.	Product name	Unit size
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
Related pro	oducts	
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
Q33223	Qubit [™] RNA XR Assay Kit, 100 assays	1 kit
Q33224	Qubit [™] RNA XR Assay Kit, 500 assays	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
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
Q33230	Qubit [™] 1X dsDNA HS Assay Kit, 100 assays	1 kit
Q33231	Qubit [™] 1X dsDNA HS Assay Kit, 500 assays	1 kit
Q10212	Qubit [™] ssDNA Assay Kit, 100 assays *1–200 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
Q33233	Qubit [™] 1X dsDNA HS Assay - Lambda DNA Standard	5 mL
Q33234	Qubit [™] 1X dsDNA HS Assay - Calf Thymus DNA Standard	5 mL
Q33235	Qubit [™] RNA IQ Assay - RNA Standards	1 set
Q33236	Qubit [™] XR Assay - RNA Standard	1 set
Q32856	Qubit [™] assay tubes *set of 500*	1 set

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Revision history: Pub. No. MAN0017405

Revision	Date	Description
B.0	05 December 2017	Correct sample concentration in Product information, add sample volume vs. sample concentration table for step 1.6, expand Table 4 to include additional Qubit [™] assay kits.
A.0	12 October 2017	New user guide

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