molecular probes
life technologies

Qubit® RNA BR Assay Kits

For use with the Qubit® Fluorometer (all models)

Catalog nos. Q10210, Q10211

Table 1. Contents and storage

Material	Amount		Concentration	Storage	Stability
	Q10210 (100 assays)	Q10211 (500 assays)			
Qubit® RNA BR Reagent (Component A)	250 µL	1.25 mL	200X concentrate in DMSO	• Room temperature • Desiccate • Protect from light	When stored as directed, kits are stable for 6 months.
Qubit® RNA BR Buffer (Component B)	50 mL	250 mL	Not applicable	Room temperature	
Qubit® RNA BR Standard #1 (Component C)	1 mL	5 mL	0 ng/µL in TE buffer	• 2–8°C • Avoid freeze-thaw cycles	
Qubit® RNA BR Standard #2 (Component D)	4 × 250 µL	10 × 500 µL	100 ng/µL in TE buffer		

Introduction

The Qubit® RNA BR (Broad-Range) Assay Kits make RNA quantitation easy and accurate. The kits include concentrated assay reagent, dilution buffer, and prediluted RNA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume from 1–20 µL is acceptable), then read the concentration using the Qubit® Fluorometer. The assay is highly selective for RNA over double-stranded DNA (dsDNA) (Figure 1, page 7) and is accurate for initial sample concentrations from 1 ng/µL to 1 µg/µL, providing an assay range of 20–1000 ng. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (Table 2, page 8). The Qubit® RNA BR assay is intended for total RNA, rRNA, or large mRNA. For small RNA (~20 nt or bp), we recommend the Qubit® microRNA Assay Kit (Cat. nos. Q32880, Q32881). In addition to the Qubit® RNA BR Assay Kits described here, we also offer other kits for assaying DNA and protein (Table 3, page 9).

To determine the purity of your sample, use the Qubit® RNA BR Assay Kit together with the Qubit® dsDNA BR Assay Kit. These measurements give you a much better indication of sample purity than that produced by measuring the A_{260}/A_{280} ratio. To measure protein contamination in nucleic acid samples, simply run 1–20 µL of the sample in the Qubit® Protein Assay.

Note: This Qubit® assay kit can be used with any Qubit® Fluorometer.

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

Before You Begin

Materials required but not provided

- Plastic container (disposable) for mixing the Qubit® working solution (step 1.3, page 3)
- Qubit® assay tubes (500 tubes, Life Technologies, Cat. no. Q32856) or Axygen® PCR-05-C tubes (VWR, part no. 10011-830)

Storing the Qubit® assay kits

The Qubit® RNA BR Reagent and Buffer are designed for room temperature storage. The Qubit® RNA BR Reagent is supplied in DMSO, which freezes at temperatures lower than room temperature. Store the RNA standards at 4°C.

The Qubit® RNA BR Reagent is sensitive to light. Store the vial **in the dark** when not in use.

Critical assay parameters

Assay temperature

The Qubit® RNA BR Assay delivers optimal performance when all solutions are at room temperature (22–28˚C). The Qubit® assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay (Figure 2, page 7). To minimize temperature fluctuations, store the Qubit® RNA BR Reagent and 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 low reading.

Incubation time

To allow the Qubit® assay to reach optimal fluorescence, incubate the tubes for the DNA and RNA assays 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.

Photobleaching of the 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. However, 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 2, page 7). 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.

Calibrating the Qubit® Fluorometer

For each assay, you have the choice to run a new calibration or use the values from the previous calibration. When you first use the instrument, perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you can decide how comfortable you are using the calibration data stored from the last time the instrument was calibrated. Additionally, remember that the fluorescence signal in the tubes containing standards and samples is stable for no longer than 3 hours. See Figure 3 (page 8) for an example of the calibration curve used to generate the quantification results.

RNAse-free handling

The calibration standards included in the Qubit® RNA BR Assay Kit are high-quality rRNA standards. The integrity and concentration of these standards is critical to the optimal performance of the Qubit® RNA BR Assay. As such, we highly recommend treating the rRNA standards as you would any other precious RNA. 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; and return the rRNA standard to the refrigerator as soon as possible after use.

Handling and disposal

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

Preparing samples and standards

This protocol assumes that you are preparing standards for calibrating the Qubit® Fluorometer. If you plan to use the last calibration performed on the instrument (see "Calibrating the Qubit® Fluorometer" on page 2), you need fewer tubes (step 1.1) and less working solution (step 1.3). For sample purity determinations, it is possible to use the Qubit® Fluorometer to calculate the amount of dsDNA and RNA in the same sample — simply perform each assay for your sample.

1.1 Set up the required number of 0.5-mL tubes for standards and samples. The Qubit® RNA BR Assay requires 2 standards.

Note: Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit® assay tubes (Cat. no. Q32856) or Axygen® PCR-05-C tubes (part no. 10011-830).

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® Fluorometer requires the standards to be inserted into the instrument in the right order.

1.3 Prepare the Qubit® working solution by diluting the Qubit® RNA BR Reagent 1:200 in Qubit® RNA BR 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.

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).

1.4 Add 190 µL of Qubit® working solution to each of the tubes used for standards.

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 sample can be anywhere from 1–20 µL. Add a corresponding volume of Qubit® working solution to each assay tube: anywhere from 180–199 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 "Reading standards and samples"; follow the procedure appropriate for your instrument:

- "Qubit® 3.0 Fluorometer", below
- "Qubit® 2.0 Fluorometer" on page 5

Reading standards and samples

Qubit® 3.0 Fluorometer

2.1 On the Home screen of the Qubit® 3.0 Fluorometer, press **RNA**, then select **RNA Broad Range** as the assay type. 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 2.4. 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 (~3 seconds), remove Standard #1.

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.

The instrument displays the results on the Read standard screen. For information on interpreting the calibration results, refer to the *Qubit® 3.0 Fluorometer User Guide*.

2.4 Press **Run samples**.

2.5 On the assay screen, select the sample volume and units:

a. Press the **+** or **–** buttons on the wheel to select the sample volume added to the assay tube (from 1–20 µL).

b. From the dropdown menu, select the units for the output sample concentration.

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

The instrument displays the results on the assay screen. The top value (in large font) is the concentration of the original sample. The bottom value is the dilution concentration. For information on interpreting the sample results, refer to the *Qubit® 3.0 Fluorometer User Guide*.

2.7 Repeat step 2.6 until all samples have been read.

Qubit® 2.0 Fluorometer

3.1 On the Home screen of the Qubit® 2.0 Fluorometer, press **RNA**, then select **RNA Broad Range** as the assay type. The Standards screen is displayed.

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 **No** and skip to step 3.5. Otherwise, continue with step 3.2**.**

3.2 On the Standards screen, press **Yes** to read the standards.

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

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

When the calibration is complete, the instrument displays the Sample screen.

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

The instrument displays the results on the Sample screen. The value displayed corresponds to the concentration after your sample was diluted into the assay tube. To find the concentration of your original sample, you can record this value and perform the calculation yourself (see "Calculating the sample concentration", below) or the instrument can perform this calculation for you (see "Dilution Calculator", page 6).

3.6 Repeat step 3.5 until all samples have been read.

Calculating 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 Qubit® RNA BR Assay in µg/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 = the value given by the Qubit® 2.0 Fluorometer
x = the number of microliters of sample added to the assay tube

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer. For example, if the Qubit® 2.0 Fluorometer gave a concentration in µg/mL, the result of the equation is in µg/mL.

Dilution Calculator– Qubit® 2.0 Fluorometer

The Dilution Calculator feature of the Qubit® 2.0 Fluorometer calculates the concentration of your original sample based on the volume of sample you added to the assay tube. To have the Qubit® 2.0 Fluorometer perform this calculation for you, follow the instructions below.

4.1 After the sample measurement is complete, press **Calculate Stock Conc.** The Dilution Calculator screen is displayed.

4.2 Using the volume roller wheel, select the volume of your original sample that you added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.

4.3 To change the units in which the original sample concentration is displayed:

a. Press **ng/mL**.

b. On the unit selection pop-up window, select a unit for your original sample concentration.

c. Touch anywhere on the screen to close the pop-up window. The Qubit® 2.0 Fluorometer automatically converts the units to your selection.

Note: The unit button next to your sample concentration reflects the change in units. For example, if you changed the unit to pg/µL, the button displays pg/µL.

4.4 To save the data from your calculation to the Qubit® 2.0 Fluorometer, press **Save** on the Dilution Calculator screen. The last calculated value of your measurement is saved in the *.csv file and tagged with a time and date stamp.

4.5 To exit the Dilution Calculator screen, press any navigator button on the bottom of the screen or press **Read Next Sample**.

Note: When you navigate away from the Dilution Calculator screen, the Qubit® 2.0 Fluorometer saves the last values for the sample volume and units on the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

Appendix

Selectivity of the Qubit® RNA BR Assay

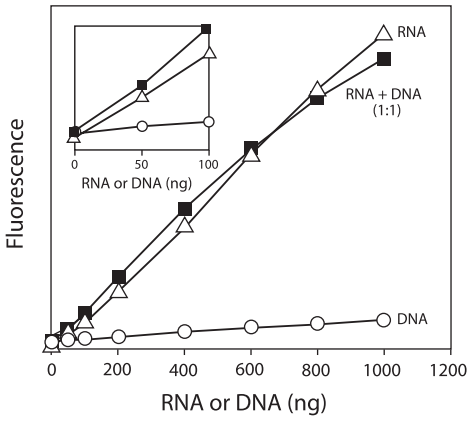

Figure 1. RNA selectivity and sensitivity of the Qubit® RNA BR Assay (Q10210, Q10211). Triplicate 10-µL samples of *E. coli* rRNA (△), λ DNA (○), or a 1:1 mixture of RNA and DNA (■) were assayed in the Qubit® RNA BR Assay. Fluorescence was measured at 630/660 nm and plotted versus the mass of nucleic acid for the RNA alone or DNA alone, or versus the mass of the RNA component in the 1:1 mixture. The variation (CV) of replicate RNA determinations was ≤10%. The inset is an enlargement of the graph to show the sensitivity of the assay for RNA. Background fluorescence has not been subtracted.

Effect of temperature on the Qubit® RNA BR Assay

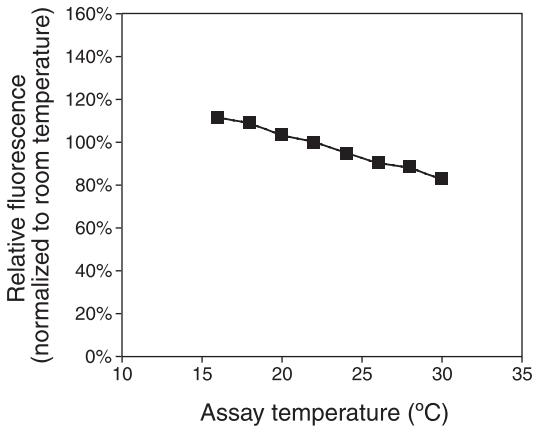

Figure 2. Plot of fluorescence versus temperature for the Qubit® RNA BR Assay. The Qubit® assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

How the Qubit® Fluorometer calculates concentration

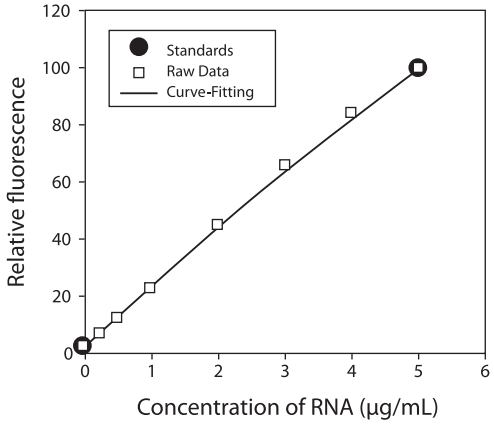

Figure 3. The curve-fitting algorithm used to determine concentration in the Qubit® RNA BR Assay. The Qubit® Fluorometer generates concentration data based on the relationship between the two standards used in calibration. This plot shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit® RNA BR 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.

Contaminants tolerated by the Qubit® RNA BR Assay

Table 2. Effect of contaminants in the Qubit® RNA BR Assay, tested over a range of 500–5000 ng/mL*

Contaminant	Final concentration in the assay	Concentration in 20-µL sample	Concentration in 10-µL sample	Result
Sodium chloride	10 mM	100 mM	200 mM	OK
Magnesium chloride	2 mM	20 mM	40 mM	OK†
Sodium acetate	10 mM	100 mM	200 mM	OK†
Ammonium acetate	10 mM	100 mM	200 mM	OK
Potassium phosphate, pH 7.4	5 mM	50 mM	100 mM	OK
Ethanol	0.1%	1%	2%	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
dNTPs§	100 µM	1 mM	2 mM	OK
BSA	20 µg/mL	200 µg/mL	400 µg/mL	OK
IgG	10 µg/mL	100 µg/mL	200 µg/mL	OK
NTPs	1X◊	1X◊	1X◊	OK
ssDNA	1X◊	1X◊	1X◊	OK
Oligos	1X◊	1X◊	1X◊	OK
dsDNA	1X◊	1X◊	1X◊	OK

**E.coli* rRNA 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 either as OK, usually less than 10% perturbation, or as NR, not recommended.

†An acceptable result, but with some distortion of the standard curve; for best results, add the same amount of contaminant to the standard samples.

‡Immiscible.

§A mixture of dATP, dCTP, dGTP, and dTTP.

◊1X indicates a concentration equal to the concentration of rRNA.

Qubit® assay kits compatible with the Qubit® Fluorometer

A number of fluorescence-based quantitation kits are available for use with the Qubit® Fluorometer. Use Table 3 to choose a kit based on the target molecule being measured and the number of assays you require.

Table 3. Qubit® assay kits for use with the Qubit® Fluorometer

Product	Cat. no.	Number of assays*	Target	Notes
Qubit® dsDNA BR Assay Kit	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† • Useful for quantitation of genomic and miniprep DNA samples • Accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
	Q32853	500		
Qubit® dsDNA HS Assay Kit	Q32851	100	dsDNA	• 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† • Useful for quantitation of PCR products, viral DNA, and samples for subcloning • Accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
	Q32854	500		
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 Assay Kit	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† • 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
	Q32855	500		
Qubit® RNA BR Assay Kit	Q10210	100	RNA	• 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† • 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
	Q10211	500		
Qubit® microRNA Assay Kit	Q32880	100	RNA	• 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† • 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
	Q32881	500		
Qubit® Protein Assay Kit	Q33211	100	Protein	• 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† • Little protein-to-protein difference in signal • Accurate in the presence of DTT, ß-mercaptoethanol, amino acids, and DNA • Signal is stable for 3 hours
	Q33212	500		

*Based on an assay volume of 200 µL.
†Concentration ranges refer to the concentration of sample after dilution in the assay tube.

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product name	Unit size
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
Related products		
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
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
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

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**.

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- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
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- 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
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