Qubit® dsDNA BR Assay Kits

For use with the Qubit® 2.0 Fluorometer

Catalog nos. Q32850, Q32853

Table 1. Contents and storage information

Material	Amount	Concentration	Storage	Stability
Qubit® dsDNA BR reagent (Component A)	250 µL or 1.25 mL	200X concentrate in DMSO	• Room temperature • Desiccate • Protect from light	When stored as directed, kits are stable for 6 months
Qubit® dsDNA BR buffer (Component B)	50 mL or 250 mL	NA	• Room temperature	
Qubit® dsDNA BR standard #1 (Component C)	1 mL or 5 mL	0 ng/µL in TE buffer	• ≤4°C	
Qubit® dsDNA BR standard #2 (Component D)	1 mL or 5 mL	100 ng/µL in TE buffer		

NA = Not applicable.

Introduction

The Qubit® dsDNA BR Assay Kits for use with the Qubit® 2.0 Fluorometer make DNA quantitation easy and accurate. The kit provides concentrated assay reagent, dilution buffer, and pre-diluted DNA 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® 2.0 Fluorometer. The assay is highly selective for double-stranded DNA (dsDNA) over RNA (*Appendix*, Figure 1) and is accurate for initial sample concentrations from 100 pg/µL to 1000 ng/µL. 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 (*Appendix,* Table 2). In addition to the Qubit® dsDNA BR Assay Kits described here, we offer other kits for assaying RNA, protein, and dsDNA at a lower concentration range (*Appendix,* Table 3).

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

Note: All Qubit® assay kits can also be used with the Qubit® 1.0 Fluorometer.

Before You Begin

Materials required but not provided

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

Storing the Qubit® dsDNA BR Assay Kits

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

Critical assay parameters

Assay temperature

The Qubit® dsDNA BR assay for the Qubit® 2.0 Fluorometer delivers optimal performance when all solutions are at room temperature (22–28˚C). The Qubit® assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay (*Appendix*, Figure 2). To minimize temperature fluctuations, store the Qubit® dsDNA BR reagent and the Qubit® dsDNA BR buffer at room temperature and insert all assay tubes into the Qubit® 2.0 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence; the Qubit® 2.0 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, as this will warm the solution and result in a low reading.

Incubation time

To allow the Qubit® assay to reach maximum 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® reagent

The Qubit® reagents exhibit high photostability in the Qubit® 2.0 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® 2.0 Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (*Appendix*, Figure 2). Note that the temperature inside the Qubit® 2.0 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, you should remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Calibrating the Qubit® 2.0 Fluorometer

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, you should 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 should determine the level of comfort you have using the calibration data stored from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing standards and the samples is stable for no longer than 3 hours. See Figure 3 in the *Appendix* for an example of the calibration curve used to generate the quantitation results.

Handling and disposal

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

Experimental Protocol

Performing the Qubit® dsDNA BR Assay

The protocol below assumes that you will be preparing standards for calibrating the Qubit® 2.0 Fluorometer. If you plan to use the last calibration performed on the instrument, you will need fewer tubes (step 1.1) and less working solution (step 1.3). More detailed instructions on the use of the Qubit® 2.0 Fluorometer (corresponding to steps 1.9–1.12 and 2.1–2.6) can be found in the user manual accompanying the instrument. For sample purity determinations, it is possible to use the Qubit® 2.0 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® dsDNA BR assay requires 2 standards.

Note: Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit® assay tubes (500 tubes, Cat. no. Q32856) or Axygen® PCR-05-C tubes (VWR, part no. 10011-830).

1.2 Label the tube lids.

Note: It is important to label the lid of each standard tube correctly as calibration of the Qubit® 2.0 Fluorometer requires that the standards be introduced to the instrument in the right order.

1.3 Make the Qubit® working solution by diluting the Qubit® dsDNA BR reagent 1:200 in Qubit® dsDNA BR buffer. Use a clean plastic tube each time you make the Qubit® working solution. **Do not mix the working solution in a glass container.**

Note: The final volume in each assay 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 Load 190 µL of Qubit® working solution into 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® dsDNA BR standard is added to 190 µL of Qubit® working solution.

1.6 Load the Qubit® working solution into 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, therefore, load each assay tube with a volume of Qubit® working solution anywhere from 180–199 µL.

1.7 Add each of your samples to assay tubes containing the correct volume of Qubit® working solution (prepared in step 1.6), 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.

1.9 On the Home Screen of the Qubit® 2.0 Fluorometer, press **DNA**, then select **dsDNA Broad Range** as the assay type. The Standards Screen is displayed.

Note: If you have already performed a calibration for the selected assay, the Qubit® 2.0 Fluorometer prompts you to choose between reading new standards and using the previous calibration. See *Calibrating the Qubit® 2.0 Fluorometer* above for calibration guidelines.

1.10 On the Standards Screen, select to run a new calibration or to use the last calibration:

Press **Yes** to run a new calibration, then:

Insert the tube containing Standard #1 in the Qubit® 2.0 Fluorometer, close the lid, then press **Read**. The reading takes approximately 3 seconds. Remove Standard #1.
Insert the tube containing Standard #2 in the Qubit® 2.0 Fluorometer, close the lid, then press **Read**. Remove Standard #2.

OR

Press **No** to use the last calibration. The Sample Screen is displayed. Insert a sample tube into the Qubit® 2.0 Fluorometer, close the lid, then press **Read**.

After the measurement is completed, the result is displayed on the screen.

Note: The value given by the Qubit® 2.0 Fluorometer at this stage corresponds to the concentration after your sample was diluted into the assay tube. You can record this value and perform the calculation yourself to find out the concentration of your original sample (see *Calculating the concentration of your sample* below) or the Qubit® 2.0 Fluorometer performs this calculation for you (see *Dilution Calculator* on page 5).

1.11 To read the next sample, remove the sample from the Qubit® 2.0 Fluorometer, insert the next sample, then press **Read Next Sample**.

1.12 Repeat sample readings until all samples have been read.

Calculating the concentration of your sample

The Qubit® 2.0 Fluorometer gives values for the Qubit® dsDNA 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:

$$\text{Concentration of your sample} = \text{QF value} \times \left(\frac{200}{x}\right)$$

where:
QF value = the value given by the Qubit® 2.0 Fluorometer
x = the number of microliters of sample you added to the assay tube

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

Dilution Calculator

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.

2.1 After the sample measurement is completed, press **Calculate Stock Conc.** The Dilution Calculator Screen containing the volume roller wheel is displayed.

2.2 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.

2.3 To change the units in which the original sample concentration is displayed, press **µg/mL** A pop-up window opens, showing the current unit selection (indicated by a red dash).

2.4 Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up.

The Qubit® 2.0 Fluorometer automatically converts the units to your selection once the unit selection pop-up window is closed.

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

2.5 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.

2.6 To exit the Dilution Calculator screen, press any navigator button on the bottom of the screen or **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 the units in the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

Appendix

Selectivity of the Qubit® dsDNA BR Assay

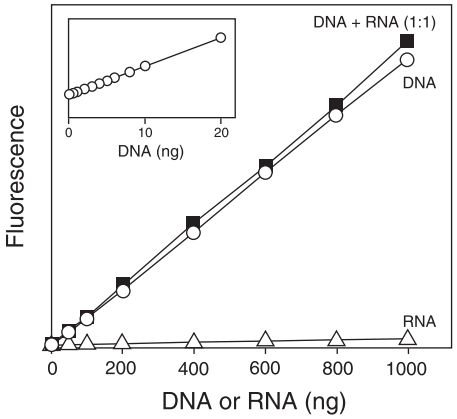

Figure 1. DNA selectivity and sensitivity of the Qubit® dsDNA BR assay (Q32850, Q32853). Triplicate 10-µL samples of λ DNA (○), *E. coli* rRNA (△), or a 1:1 mixture of DNA and RNA (■) were assayed in the Qubit® dsDNA BR assay. Fluorescence was measured at 485/530 nm and plotted versus the mass of nucleic acid for the DNA alone or RNA alone, or versus the mass of the DNA component in the 1:1 mixture. The variation (CV) of replicate DNA determinations was ≤3%. The inset, a separate experiment with octuplicate determinations, shows the sensitivity of the assay for DNA. Background fluorescence has not been subtracted.

Effect of temperature on the Qubit® dsDNA BR Assay

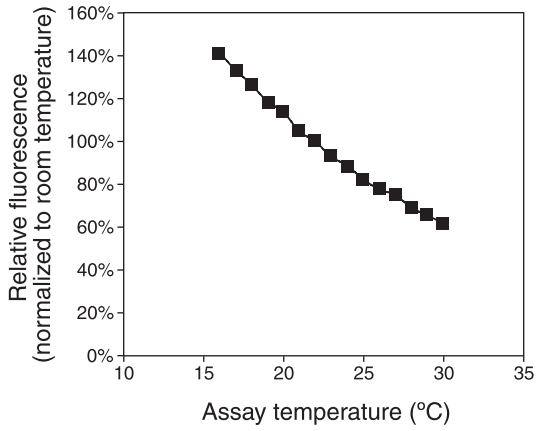

Figure 2. Plot of fluorescence versus temperature for the Qubit® dsDNA BR assay. The Qubit® assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

How the Qubit® 2.0 Fluorometer calculates concentration

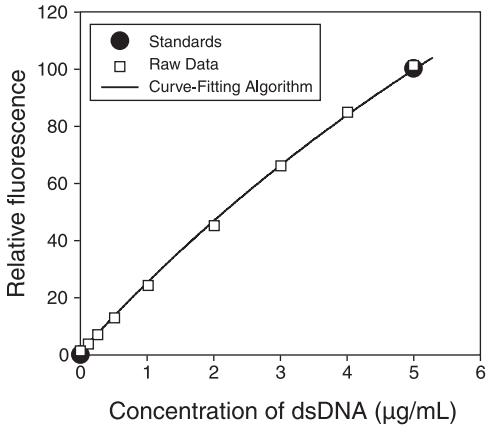

Figure 3. The curve-fitting algorithm used to determine concentration in the Qubit® dsDNA BR assay. The Qubit® 2.0 Fluorometer generates concentration data based on the relationship between the two standards used in the calibration. This plot shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit® dsDNA 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® dsDNA BR Assay

Table 2. Effect of contaminants in the Qubit® dsDNA BR assay, tested over the range of 0.01 to 5 µg/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	1%	10%	20%	OK
Phenol	0.1%	1%	2%	OK
Chloroform‡	0.2%	2%	4%	OK
SDS	0.01%	0.1%	0.2%	OK
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
RNA	6X	6X	6X	OK
ssDNA	1X	1X	1X	OK
Oligos	3X	3X	3X	OK

* DNA 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. In all cases, results are given as OK (usually <10% perturbation). † 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.

Qubit® Assay Kits compatible with the Qubit® 2.0 Fluorometer

A number of fluorescence-based quantitation kits are available for use with the Qubit® 2.0 Fluorometer. Table 3 can help you 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® 2.0 Fluorometer

Product	Catalog 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
Qubit® dsDNA BR Assay Kit	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
Qubit® dsDNA HS Assay Kit	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 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
Qubit® RNA Assay Kit	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 5–6 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
Qubit® RNA BR Assay Kit	Q10211	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, β-mercaptoethanol, amino acids, and DNA • Signal is stable for 3 hours
Qubit® Protein Assay Kit	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
Q32850	Qubit® dsDNA BR Assay Kit, 100 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32853	Qubit® dsDNA BR Assay Kit 500 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Related products		
Q32851	Qubit® dsDNA HS Assay Kit, 100 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer	1 kit
Q32854	Qubit® dsDNA HS Assay Kit, 500 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10212	Qubit® ssDNA Assay Kit, 100 assays *1–200 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10210	Qubit® RNA BR Assay Kit, 100 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10211	Qubit® RNA BR Assay Kit, 500 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32852	Qubit® RNA Assay Kit, 100 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32855	Qubit® RNA Assay Kit, 500 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33211	Qubit® Protein Assay Kit, 100 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33212	Qubit® Protein Assay Kit, 500 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32856	Qubit® assay tubes *set of 500*	1 set
Q32866	Qubit® 2.0 Fluorometer	each
Q32867	Qubit® 2.0 Fluorometer USB	each
Q32868	Qubit® 2.0 Fluorometer International Power Cord (replacement)	each

Contact Information

Corporate Headquarters
5791 Van Allen Way
Carlsbad, CA 92008
USA
Phone: +1 760 603 7200
Fax: +1 760 602 6500
Email: techsupport@lifetech.com

European Headquarters
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF
UK
Phone: +44 141 814 6100
Toll-Free Phone: 0800 269 210
Toll-Free Tech: 0800 838 380
Fax: +44 141 814 6260
Tech Fax: +44 141 814 6117
Email: euroinfo@invitrogen.com
Email Tech: eurotech@invitrogen.com

Japanese Headquarters
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Japan
Phone: +81 3 5730 6509
Fax: +81 3 5730 6519
Email: jpinfo@invitrogen.com

Additional international offices are listed at **www.lifetechnologies.com**

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**. At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- 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
The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the product packaging (tube, pouch, or box).

Limited Warranty
Invitrogen (a part of Life Technologies Corporation) is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Support Representatives.
All Invitrogen products are warranted to perform according to specifications stated on the certificate of analysis. The Company will replace, free of charge, any product that does not meet those specifications. This warranty limits the Company's liability to only the price of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. The Company reserves the right to select the method(s) used to analyze a product unless the Company agrees to a specified method in writing prior to acceptance of the order.
Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore the Company makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives.
Life Technologies Corporation shall have no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

Limited Use Label License: Research Use Only
The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial services of any kind, including, without limitation, reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.