molecular probes
life technologies

Quant-iT™ dsDNA High-Sensitivity Assay Kit

Catalog no. Q33120

Table 1. Contents and storage

Material	Amount	Concentration	Storage	Stability
Quant-iT™ dsDNA HS reagent (Component A)	1.0 mL	200X in DMSO	• Room temperature • Protect from light • Desiccate	When stored as directed, kit contents are stable for at least 6 months.
λ dsDNA HS standards (Component C)	set of 8 (500 µL each)	0, 0.5, 1, 2, 4, 6, 8, and 10 ng/µL	≤6°C	
Quant-iT™ dsDNA HS buffer (Component B)	250 mL	NA	• ≤6°C * • Protect from light	
* For short-term storage (days), the buffer may be left at room temperature; however, for longer periods we recommend storage at ≤6°C to prevent microbial contamination.				
Number of labelings: 1,000, with a 200 µL assay volume in a 96-well microplate format. The Quant-iT™ dsDNA HS assay can be adapted for use in cuvettes or 384-well microplates.				
Approximate fluorescence excitation/emission maxima: 502/523 nm (see Figure 1, page 2)				

Introduction

The Quant-iT™ dsDNA High-Sensitivity Assay Kit makes DNA quantification easy and accurate. The kit provides concentrated assay reagent, dilution buffer, and pre-diluted DNA standards. Simply dilute the reagent 1:200, load 200 µL into the wells of a microplate, add 1–20 µL sample volumes, mix, then read the fluorescence. The assay is highly selective for double-stranded DNA over RNA, and in the range of 0.2–100 ng, the fluorescence signal is linear with DNA (Figure 2, page 2). The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants, such as salts, solvents, detergents, or protein are well tolerated in the assay.

In addition to the Quant-iT™ dsDNA High-Sensitivity Assay Kit described here, we also offer the Quant-iT™ dsDNA Broad-Range Assay Kit (Cat. no. Q33130). The Quant-iT™ dsDNA Broad-Range Kit is designed for assaying samples containing 2–1000 ng of DNA.

If you would like to use this kit with the Qubit® fluorometer, we have included instructions under *Using the Quant-iT™ dsDNA High-Sensitivity Assay Kit with the Qubit® Fluorometer* (page 5).

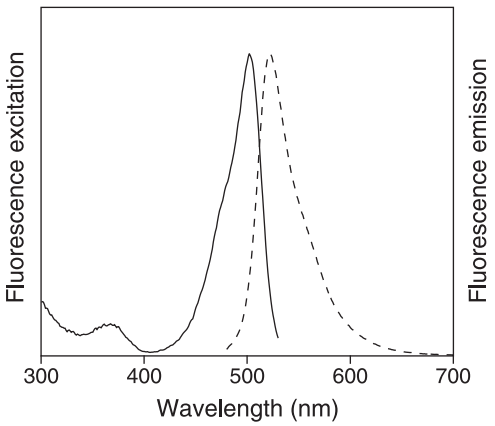

Figure 1. Excitation and emission maxima for the Quant-iT™ dsDNA HS reagent bound to DNA.

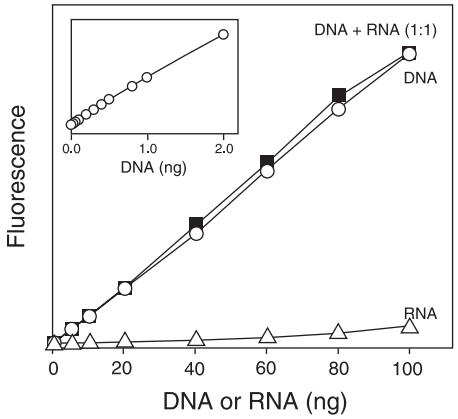

Figure 2. DNA selectivity and sensitivity of the Quant-iT™ dsDNA HS assay. Triplicate 10 µL samples of λ DNA (○), *E. coli* rRNA (△), or a 1:1 mixture of DNA and RNA (■) were assayed in the Quant-iT™ dsDNA HS assay. Fluorescence was measured at 485/530 nm and plotted versus the mass of nucleic acid for 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 ≤2%. The inset, a separate experiment with octuplicate determinations, shows the extreme sensitivity of the assay for DNA. Background fluorescence has not been subtracted.

Before You Begin

Handling the Quant-iT™ reagent

No data are currently available addressing the mutagenicity or toxicity of the Quant-iT™ dsDNA HS reagent. This reagent is known to bind nucleic acid and is provided as a solution in DMSO; treat the reagent with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

Remove the Quant-iT™ dsDNA High-Sensitivity Assay Kit from storage and allow the components to equilibrate to room temperature. During all steps, protect the Quant-iT™ dsDNA HS reagent concentrate and the working solution from light as much as possible.

This protocol describes the use of the Quant-iT™ dsDNA High-Sensitivity Assay Kit with a fluorescence microplate reader that is equipped with excitation and emission filters appropriate for fluorescein or Alexa Fluor® 488 dye. Some contaminating substances may interfere with the assay. See *Contaminating substances*, page 7, for more information. For an overview of this procedure, see Figure 3, below.

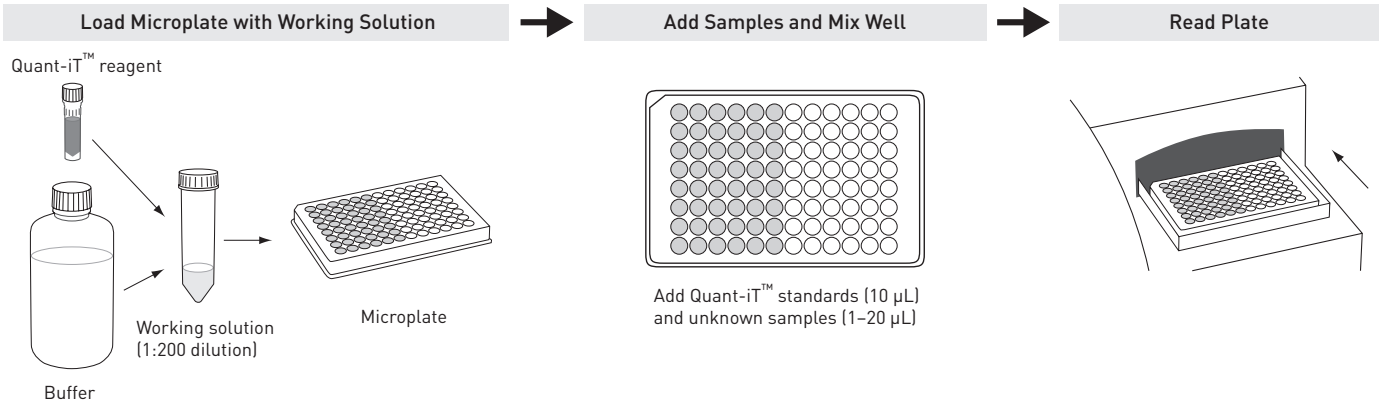

Figure 3. The Quant-iT™ dsDNA High-Sensitivity assay.

Assay procedure

1.1 Make a working solution by diluting Quant-iT™ dsDNA HS reagent 1:200 in Quant-iT™ dsDNA HS buffer. For example, for ~100 assays put 100 µL of Quant-iT™ dsDNA HS reagent (Component A) and 20 mL of Quant-iT™ dsDNA HS buffer (Component B) in a disposable plastic container and mix well. Do not use glass containers. Do not use buffers other than the Quant-iT™ dsDNA HS buffer to make the working solution.

1.2 Load 200 µL of the working solution into each microplate well. Diluted Quant-iT™ dsDNA HS reagent is stable for at least 3 hours at room temperature, protected from light.

1.3 Add 10 µL of each λ DNA standard (Component C) to separate wells and mix well. Take care not to introduce nucleases into the tubes of DNA standard as you remove aliquots for the assay. Duplicates or triplicates of the standards are recommended.

1.4 Add 1–20 µL of each unknown DNA sample to separate wells and mix well. Duplicates or triplicates of the unknown samples are recommended. Some contaminating substances may interfere with the assay, see *Contaminating substances* on page 7.

1.5 Measure the fluorescence using a microplate reader (excitation/emission maxima are 502/523 nm; see Figure 1, page 2). Standard fluorescein wavelengths (excitation/emission at ~480/530 nm) are appropriate for this dye. The fluorescence signal is stable for 3 hours at room temperature.

1.6 Use a standard curve to determine the DNA amounts. For the λ DNA standards, plot amount vs. fluorescence, and fit a straight line to the data points.

Data analysis considerations – standard curves and extended ranges

The fluorescence of the Quant-iT™ dsDNA HS reagent bound to dsDNA is extremely linear from 0–100 ng. For best results at the low end of the standard curve, the line should be forced through the background point (or through zero, if background has been subtracted). When 10 µL volumes of the standards are used, the lowest DNA-containing standard represents 5 ng of DNA; nevertheless, highly accurate determinations of DNA down to 0.2 ng are attained using the standard curve as described above.

To assess the reliability of the assay in the low range, use smaller volumes of the standards; for example, 2 µL volumes for a standard curve ranging from 0–20 ng (Figure 4A, below). Alternatively, dilute the standards in buffer for an even tighter range (Figure 4A, inset). During development of the Quant-iT™ dsDNA HS assay, we were able to detect 0.05 ng of λ DNA under ideal experimental circumstances (using calibrated pipettors, octuplicate determinations, the best microplate readers, and Z-factor[1] analysis). Your results may vary.

If desired, the utility of the Quant-iT™ dsDNA HS assay can be extended beyond 100 ng, up to 200 ng (Figure 4B, below). For standards in this range, use 20 µL volumes of the provided standards. Note that the standard curve may not be linear in the range 160–200 ng, and high levels of RNA may now interfere slightly with the results.

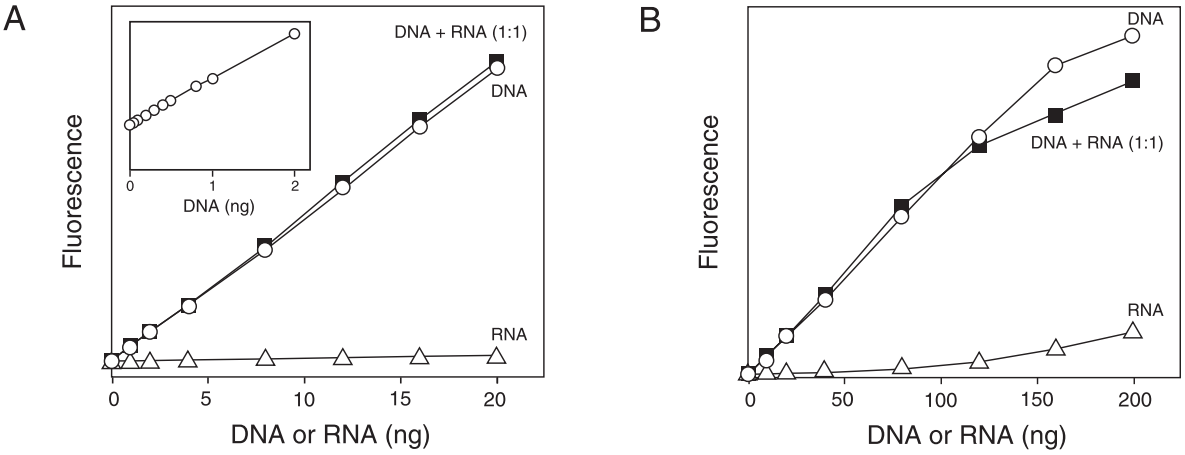

Figure 4. Extended ranges for the Quant-iT™ dsDNA HS assay. Triplicate 2 µL (Panel A) or 20 µL samples (Panel B) of λ DNA (○), *E. coli* rRNA (△), or a 1:1 mixture of DNA and RNA (■) were assayed in the Quant-iT™ dsDNA HS assay. Fluorescence was measured at 485/530 nm and plotted versus the mass of nucleic acid for the DNA alone or RNA alone, or versus the mass of the DNA component in the 1:1 mixture. The inset (Panel A), a separate experiment with octuplicate determinations, shows the sensitivity of the assay for DNA. Background fluorescence has not been subtracted.

The Quant-iT™ dsDNA HS Assay Kit can easily be adapted for use with the Qubit® fluorometer. The protocol below is abbreviated from the Qubit® fluorometer user guide, which is available at **www.lifetechnologies.com/qubit**. Although a step-by-step protocol and critical assay parameters are given here, more detail is available in the Qubit® fluorometer user guide and you are encouraged to familiarize yourself with this manual before you begin your assay. See Figure 5, below, for an overview of the procedure.

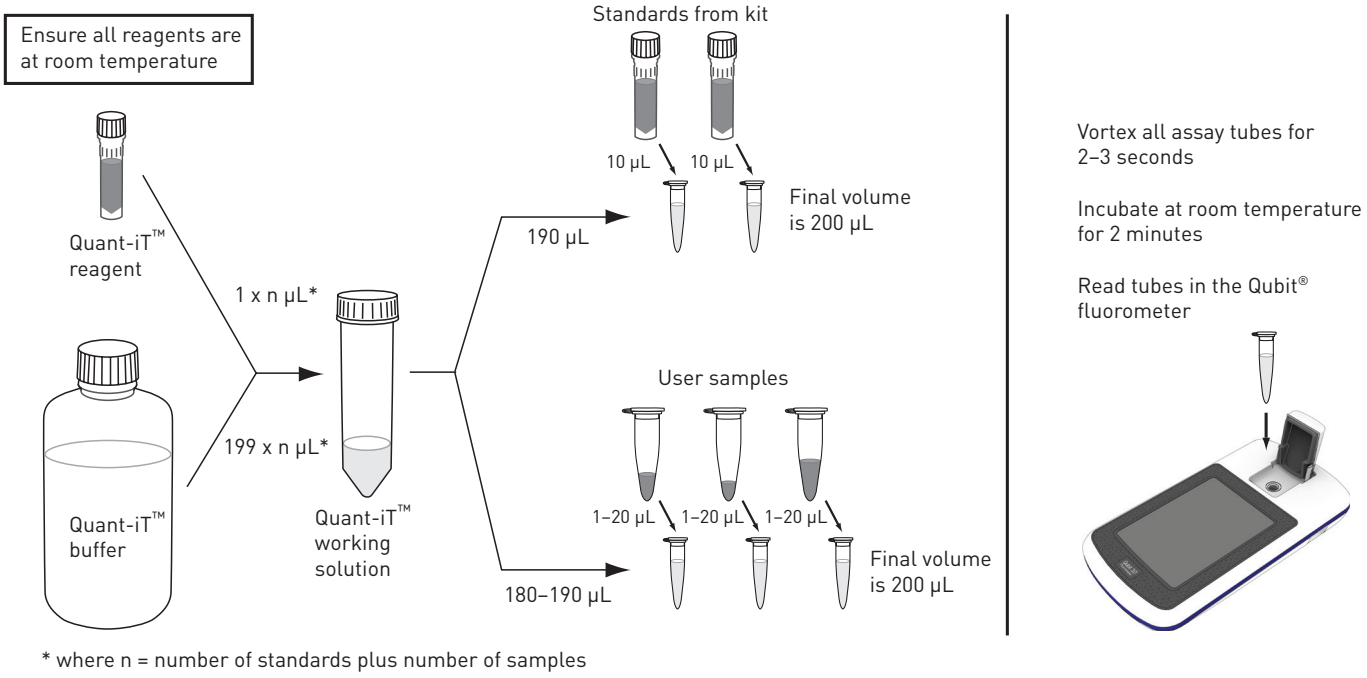

Figure 5. Overview for using the Quant-iT™ dsDNA HS assay in the Qubit® fluorometer.

IMPORTANT! Ensure all assay reagents are at room temperature before you begin. 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).

Assay procedure

2.1 Label the lids of the assay tubes* you will need for the standards and user samples.

Note: The Quant-iT™ dsDNA HS Assay Kit requires two standards for calibration. Prepare a dilution of the 0 ng/µL λ dsDNA HS standard from the Component C set to generate Standard #1, and a dilution of the 10 ng/µL λ dsDNA HS standard from the Component C set to generate Standard #2 (see step 2.3 below).

2.2 Make the Quant-iT™ dsDNA HS working solution by diluting the Quant-iT™ dsDNA HS reagent 1:200 in Quant-iT™ buffer.

2.3 Prepare assay tubes according to Table 2, below.

Table 2. Tube setup.

	Standard assay tubes	User Sample assay tubes
Volume of working solution (from step 2.2)	190 µL	180–199 µL
Volume of standard (from kit)*	10 µL	—
Volume of user sample	—	1–20 µL
Total volume in each assay tube	200 µL	200 µL

* Prepare Standard #1 by diluting 10 µL of the 0 ng/µL standard, and Standard #2 by diluting 10 µL of the 10 ng/µL standard.

2.4 Vortex all tubes for 2–3 seconds.

2.5 Incubate the tubes for 2 minutes at room temperature.

2.6 Calibrate the Qubit® fluorometer using Standard #1 and Standard #2.

2.7 Read the user samples in the Qubit® fluorometer.

2.8 *For Qubit® 2.0 Fluorometer users:* Multiply the readout from the Qubit® 2.0 Fluorometer by the value given by the dilution factor (see the Qubit® 2.0 Fluorometer user guide) to determine the concentration of your original sample. Alternatively, choose **Calculate Sample Concentration** to have the Qubit® 2.0 Fluorometer perform this multiplication for you. For more information, refer to the Qubit® 2.0 Fluorometer user guide.

Note: The Qubit® 3.0 Fluorometer performs this calculation automatically.

Appendix: Critical Assay Parameters

Assay temperature

The Quant-iT™ dsDNA HS assay for the Qubit® fluorometer delivers optimal performance when all solutions are at room temperature. The Quant-iT™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay. To minimize temperature fluctuations, store the Quant-iT™ dsDNA HS reagent and the Quant-iT™ dsDNA HS 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, as 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, as this will warm the solution and result in a low reading.

Incubation time

To allow the Quant-iT™ dsDNA HS assay to reach maximum fluorescence, incubate the assay tubes 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 Quant-iT™ reagent

The Quant-iT™ dsDNA HS reagent exhibits high photostability in the Qubit® 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® fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature. 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, you should remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Assay tubes to use with the Qubit® Fluorometer

Use only thin-wall, clear 0.5 mL PCR tubes with the Qubit® Fluorometer. Acceptable tubes include Qubit® assay tubes (Cat. no. Q32856, 500 tubes) or Axygen® PCR-05-C tubes (VWR, part number 10011-830). The assay volume must be 200 µL for an accurate read.

Calibrating the Qubit® Fluorometer

When quantitating your samples using the Qubit® fluorometer, you have the choice to calibrate the instrument using freshly prepared calibration solutions or to apply the values from a previously run calibration. *Using the Quant-iT™ dsDNA High-Sensitivity Assay Kit with the Qubit® Fluorometer*, page 5, describes the preparation of fresh calibration standards. Consult the instruction manual for the Qubit® fluorometer for guidance on choosing a calibration mode.

Contaminating substances

A number of common contaminants have been tested in the Quant-iT™ dsDNA HS assay, and most are well tolerated (Table 3, below). For untested contaminating substances and in general, the standards should be assayed under the same conditions as the unknowns for highest accuracy. For example, if the experimental samples are in an unusual buffer and if 10 µL volumes of these samples are used, then add 10 µL volumes of the unusual buffer (lacking DNA) to the assays of the standards.

Table 3. Effect of Contaminants in the Quant-iT™ dsDNA High-Sensitivity Assay. *

Contaminant	Final Concentration in the Assay	Concentration in 20 µL Sample	Concentration in 10 µL Sample	Result
Sodium chloride	50 mM	500 mM	1 M	OK
Magnesium chloride	5 mM	50 mM	100 mM	OK †
Sodium acetate	30 mM	300 mM	600 mM	OK
Ammonium acetate	50 mM	500 mM	1 M	OK
Ethanol	1%	10%	20%	OK
Phenol	0.1%	1%	2%	OK †
Chloroform ‡	1%	10%	20%	OK
SDS	0.01%	0.1%	0.2%	OK
Triton® X-100	0.01%	0.1%	0.2%	OK †
dNTPs §	100 µM	1 mM	2 mM	OK
BSA	10 mg/mL	100 mg/mL	200 mg/mL	OK †
IgG	0.5 mg/mL	5 mg/mL	10 mg/mL	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 less than 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.

Reference

1. J Biomol Screen 4, 67-73 (1999).

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

Cat. no.	Product Name	Unit Size
Q33120	Quant-iT™ dsDNA Assay Kit, High Sensitivity, 1000 assays *0.2–100 ng*	1 kit
Related products		
Q33130	Quant-iT™ dsDNA Assay Kit, Broad Range, 1000 assays *2–1000 ng*	1 kit
Q10213	Quant-iT™ RNA Assay Kit, Broad Range, 1000 assays *20–1000 ng*	1 kit
Q33140	Quant-iT™ RNA Assay Kit, 1000 assays *5–100 ng*	1 kit
Q32882	Quant-iT™ microRNA Assay Kit, 1000 assays *5–500 ng*	1 kit
Q33210	Quant-iT™ Protein Assay Kit, 1000 assays *0.25–5 µg*	1 kit
O11492	Quant-iT™ OliGreen® ssDNA Assay Kit *2000 assays*	1 kit

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**.

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 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.lifetechnologies.com/termsandconditions.** If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support.**

Disclaimer
LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Important Licensing Information
This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

16 February 2015