

# Quant-iT™ Protein Assay Kit

Catalog no. Q33210

**Table 1.** Contents and storage

| Material                                | Amount                 | Concentration  | Storage   | Stability   |
|---|------------------------|--|---|---|
| Quant-iT™ protein reagent (Component A) | 1.0 mL                 | 200X in 1,2-propanediol  | <ul style="list-style-type: none"> <li>• Room temperature</li> <li>• Desiccate</li> <li>• Protect from light</li> </ul> | When stored as directed, kit contents are stable for at least 6 months. |
| BSA standards (Component C)             | set of 8 (500 µL each) | 0, 25, 50, 100, 200, 300, 400, and 500 ng/µL in a solution containing 2 mM azide | ≤6°C  |   |
| Quant-iT™ protein buffer (Component B)  | 250 mL                 | NA   | <ul style="list-style-type: none"> <li>• ≤6°C *</li> <li>• Protect from light</li> </ul>                                |   |

\* 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™ protein assay can be adapted for use in cuvettes or 384-well microplates. The Quant-iT™ protein reagent is a new formulation of the NanoOrange® reagent.

**Approximate fluorescence excitation/emission maxima:** 470/570 nm (see Figure 1, page 2)

## Introduction

The Quant-iT™ Protein Assay Kit makes protein quantification easy and accurate. The kit provides concentrated assay reagent, dilution buffer, and pre-diluted BSA 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 protein. In the range of 0.25–5 µg of protein, the response curve is sigmoidal (pseudolinear from 0.5–4 µg) and exhibits low protein-to-protein variation (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, or DNA, but not detergents, are well tolerated in the assay.

If you would like to use this kit with the Qubit® fluorometer, we have included instructions under *Using the Quant-iT™ Protein Assay Kit with the Qubit® Fluorometer*.

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

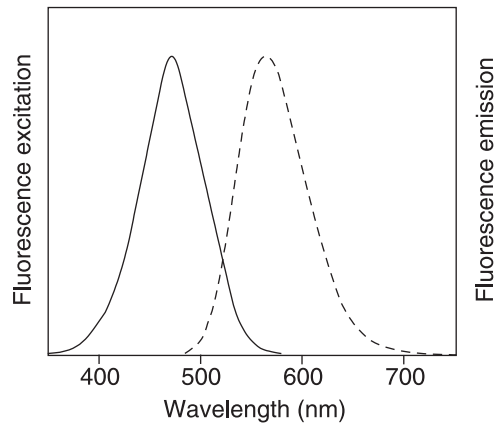


Figure 1. Excitation and emission maxima for the Quant-iT™ protein reagent bound to BSA.

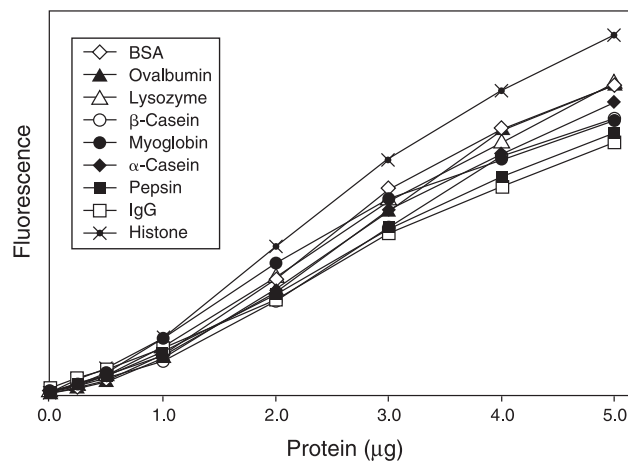


Figure 2. Low protein-to-protein variation in the Quant-iT™ protein assay. Solutions of the following proteins were prepared, diluted, and assayed in the Quant-iT™ protein assay: bovine serum albumin (BSA), chicken-egg ovalbumin, chicken-egg lysozyme, bovine-milk  $\beta$ -casein, equine myoglobin, bovine-milk  $\alpha$ -casein, porcine pepsin, mouse immunoglobulin (IgG), and calf-thymus histone. Fluorescence was measured at 485/590 nm and plotted versus the mass of the protein sample. At 3  $\mu$ g, the fluorescence variation was 12.4%, or 8.7% excluding the basic histone protein. 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™ protein reagent. This reagent is an organic dye and it is provided as a solution in 1,2-propanediol. 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™ Protein Assay Kit from storage and allow the components to equilibrate to room temperature. During all steps, protect the Quant-iT™ protein reagent concentrate and the working solution from light as much as possible.

## Using the Quant-iT™ Protein Assay Kit with a Fluorescence Microplate Reader

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

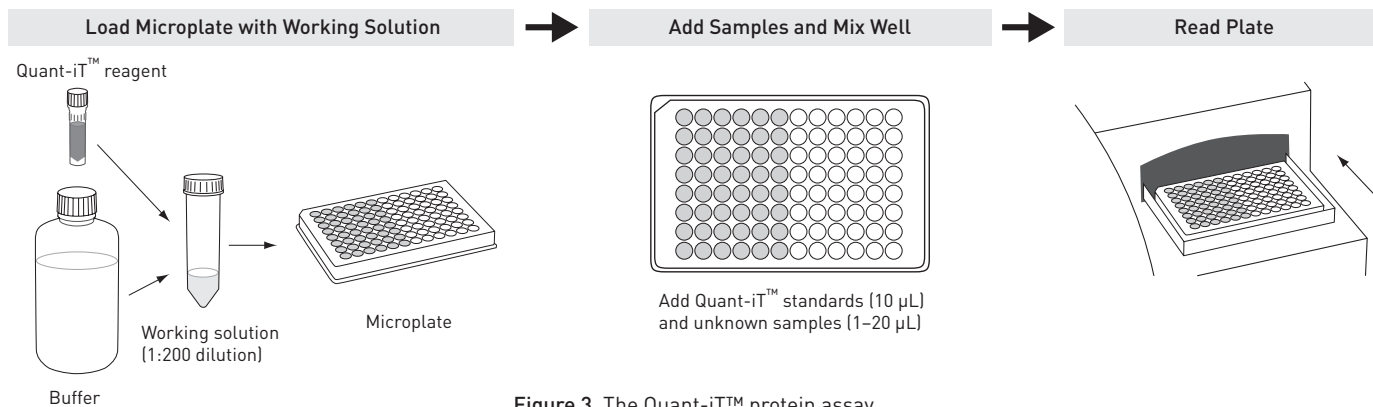


Figure 3. The Quant-iT™ protein assay.

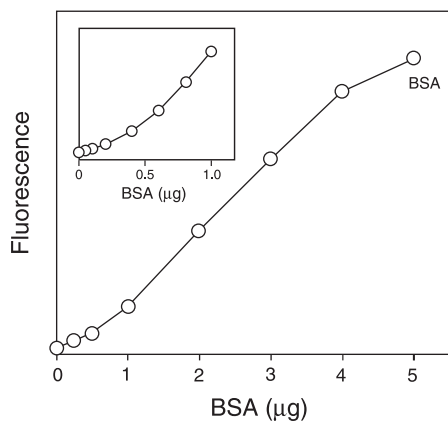
### Assay procedure

- 1.1 Make a working solution by diluting Quant-iT™ protein reagent 1:200 in Quant-iT™ protein buffer. For example, for ~100 assays put 100 µL of Quant-iT™ protein reagent (Component A) and 20 mL of Quant-iT™ protein 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™ protein buffer to make the working solution.
- 1.2 Load 200 µL of the working solution into each microplate well. Diluted Quant-iT™ protein reagent is stable for at least 3 hours at room temperature, protected from light.
- 1.3 Add 10 µL of each BSA standard (Component C) to separate wells and mix well. Duplicates or triplicates of the standards are recommended.
- 1.4 Add 1–20 µL of each unknown protein 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 the *Appendix*.
- 1.5 Measure the fluorescence using a microplate reader (excitation/emission maxima are 470/570 nm; see Figure 1, page 2). The fluorescence signal is stable for 3 hours at room temperature.
- 1.6 Use a standard curve to determine the protein amounts. For the BSA 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™ protein reagent bound to protein results in a sigmoidal standard curve from 0–5 µg (Figure 4, below). For best results fit a curve through the data points of the standards, including the background data point. The curve is pseudo-linear from 0.5–4 µg, and a straight line may be fit to this range of data points. When 10 µL volumes of the standards are used, the lowest BSA-containing standard represents 0.25 ng of protein.

To assess the reliability of the assay in the low range, use smaller volumes of the standards, e.g. 2 µL volumes for a standard curve ranging from 0–1 µg (Figure 4, inset). During development of the Quant-iT™ protein assay, we were able to detect 0.1 µg of BSA under ideal experimental circumstances (using calibrated pipettors, sextuplicate determinations, the best microplate readers, and Z-factor<sup>1</sup> analysis). Your results may vary.



**Figure 4.** Protein sensitivity of the Quant-iT™ protein assay. The provided BSA standards were assayed in the Quant-iT™ protein assay using quadruplicate 10 µL or sextuplicate 2 µL volumes (inset). Fluorescence was measured at 485/590 nm and plotted versus the mass of protein in the sample. The variation [CV] of replicate protein determinations was ≤5% for the 10 µL and ≤10% for the 2 µL samples. Background fluorescence has not been subtracted.

**Protein-to-protein variation**

The Quant-iT™ protein assay has been applied to a number of different protein species, and there is minimal protein-to-protein variation in the response (Figure 2, page 2). Only the highly basic protein, histone, behaves aberrantly. Considering all proteins, assayed as 3 µg-samples, the CV in fluorescence signal is only 12.4%, or 8.7% if histone is excluded.

## Using the Quant-iT™ Protein Assay Kit with the Qubit® Fluorometer

The Quant-iT™ Protein 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](http://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.

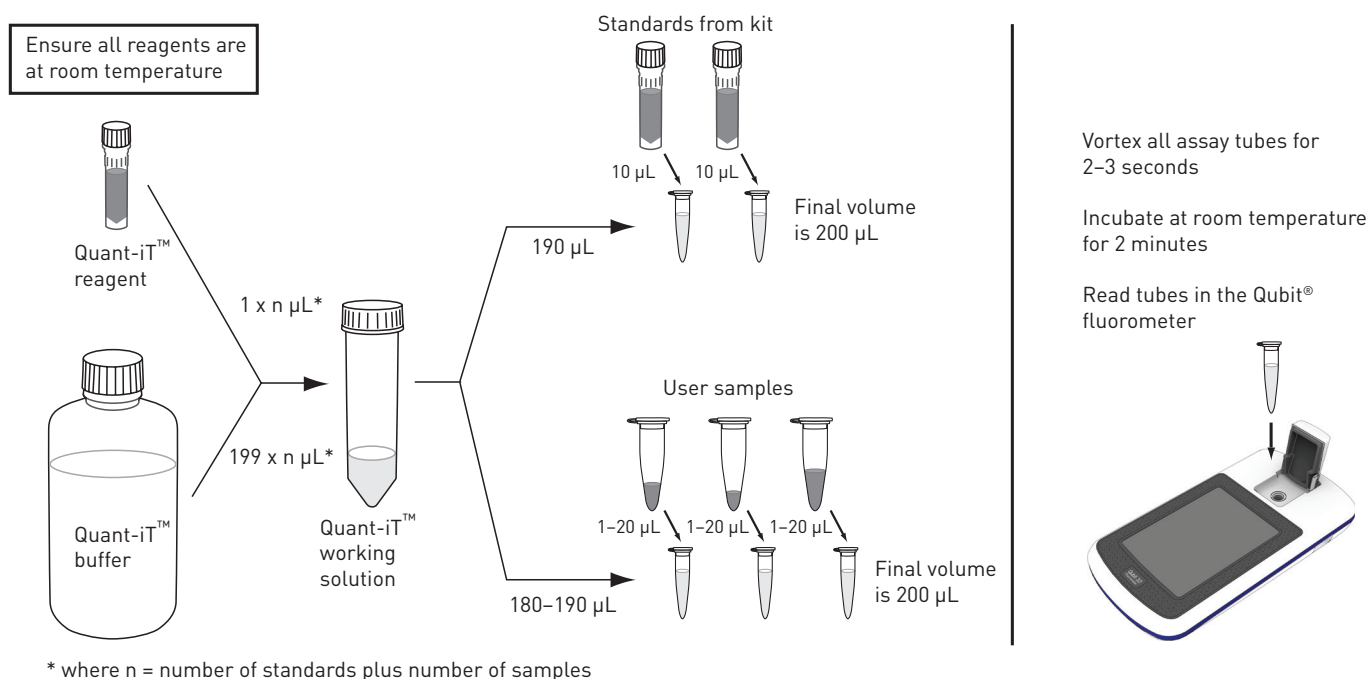


Figure 5. Overview for using the Quant-iT™ protein assay in the Qubit® fluorometer.

**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™ Protein Assay Kit requires three standards for the calibration of the Qubit® Fluorometer. Prepare a dilution of the 0 ng/µL BSA protein standard from the Component C set to generate Standard #1, a dilution of the 200 ng/µL BSA protein standard from the Component C set to generate Standard #2, and a dilution of the 400 ng/µL BSA protein standard from the Component C set to generate Standard #3 (see step 2.3 below).

#### 2.2 Make the Quant-iT™ protein working solution by diluting the Quant-iT™ protein 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, Standard #2 by diluting 10 µL of the 200 ng/µL standard, and Standard #3 by diluting 10 µL of the 400 ng/µL standard. |                      |                         |

2.4 Vortex all tubes for 2–3 seconds.

2.5 Incubate the tubes for 15 minutes at room temperature.

2.6 Calibrate the Qubit® fluorometer using Standard #1, Standard #2, and Standard #3.

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.

Calculate 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 Quant-iT™ protein 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.

## Appendix: Critical Assay Parameters

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|  |  |
|--|--|
| Assay temperature                              | The Quant-iT™ protein 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™ protein reagent and the Quant-iT™ protein 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. <b>Do not hold the assay tubes in your hand before reading, as this will warm the solution and result in a low reading.</b> |
| Incubation time                                | In order to allow the Quant-iT™ protein assay to reach maximum fluorescence, incubate the assay tubes for 15 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™ protein 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 quantifying 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. <i>Using the Quant-iT™ Protein Assay Kit with the Qubit® Fluorometer</i> (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™ protein assay, and most are well tolerated; however, samples containing detergents are not recommended (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 protein) to the assays of the standards.

**Table 3.** Effect of Contaminants in the Quant-iT™ Protein Assay. \*

| Contaminant                     | Final Concentration in the Assay | Concentration in 20 µL Sample | Concentration in 10 µL Sample | Result |
|---------------------------------|----------------------------------|-------------------------------|-------------------------------|--------|
| Sodium chloride                 | 20 mM                            | 200 mM                        | 400 mM                        | OK †   |
| Magnesium chloride              | 2 mM                             | 20 mM                         | 40 mM                         | OK     |
| Potassium chloride ‡            | 20 mM                            | 200 mM                        | 400 mM                        | OK     |
| Calcium chloride ‡              | 2 mM                             | 20 mM                         | 40 mM                         | OK †   |
| Ammonium sulfate                | 5 mM                             | 50 mM                         | 100 mM                        | OK †   |
| DTT                             | 1 mM                             | 10 mM                         | 20 mM                         | OK     |
| β-Mercaptoethanol               | 1 mM                             | 10 mM                         | 20 mM                         | OK     |
| EDTA                            | 1 mM                             | 10 mM                         | 20 mM                         | OK     |
| Sodium azide                    | 1 mM                             | 10 mM                         | 20 mM                         | OK     |
| HEPES, pH 7.4                   | 5 mM                             | 50 mM                         | 100 mM                        | OK     |
| Potassium phosphate, pH 7.4     | 5 mM                             | 50 mM                         | 100 mM                        | OK     |
| NaCl/K-PO <sub>4</sub> , pH 7.4 | 1/15 mM                          | 10/150 mM                     | 20/300 mM                     | OK †   |
| Sucrose                         | 50 mM                            | 500 mM                        | 1 M                           | OK     |
| Sucrose                         | 100 mM                           | 1 M                           | 2 M                           | NR     |
| Glycerol                        | 1%                               | 10%                           | 20%                           | OK †   |
| Imidazole                       | 1.25 mM                          | 12.5 mM                       | 25 mM                         | OK     |
| SDS                             | 0.01%                            | 0.1%                          | 0.2%                          | OK †   |
| SDS                             | 0.02%                            | 0.2%                          | 0.4%                          | NR     |
| Tween® 20                       | 0.001%                           | 0.01%                         | 0.02%                         | NR     |
| Triton® X-100                   | 0.001%                           | 0.01%                         | 0.02%                         | NR     |
| Amino acids §                   | 100 µg/mL                        | 1 mg/mL                       | 2 mg/mL                       | OK     |
| dNTPs **                        | 100 µM                           | 1 mM                          | 2 mM                          | OK †   |
| DNA                             | 5 µg/mL                          | 50 µg/mL                      | 100 µg/mL                     | OK †   |
| DNA                             | 10% ††                           | 10% ††                        | 10% ††                        | OK     |
| DNA                             | 50% ††                           | 50% ††                        | 50% ††                        | NR     |

\*BSA 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 with some distortion of the standard curve. For best results, add the same amount of contaminant to the standard samples.

‡ A precipitate was observed.

§ A mixture of 19 amino acids.

\*\* A mixture of dATP, dCTP, dGTP, and dTTP.

†† For each data point, the DNA mass was a fixed percentage of the protein mass.



## Reference

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

## Product List

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

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| Cat. no.                | Product name  | Unit size |
|-------------------------|---|-----------|
| Q33210                  | Quant-iT™ Protein Assay Kit, 1000 assays *0.25-5 µg* . . . . .                  | 1 kit     |
| <i>Related products</i> |   |           |
| Q10213                  | Quant-iT™ RNA Assay Kit, Broad Range, 1000 assays *20-1000 ng* . . . . .        | 1 kit     |
| Q33120                  | Quant-iT™ dsDNA Assay Kit, High Sensitivity, 1000 assays *0.2-100 ng* . . . . . | 1 kit     |
| Q33130                  | Quant-iT™ dsDNA Assay Kit, Broad Range, 1000 assays *2-1000 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     |
| O11492                  | Quant-iT™ OliGreen® ssDNA Assay Kit *2000 assays* . . . . .                     | 1 kit     |

## Purchaser Notification

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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.

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- Search through frequently asked questions (FAQs)
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- 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

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