Quant-iT™ dsDNA High-Sensitivity Assay Kit
Catalog no. Q33120

Table 1. Contents and storage

<table>
<thead>
<tr>
<th>Material</th>
<th>Amount</th>
<th>Concentration</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quant-iT™ dsDNA HS reagent (Component A)</td>
<td>1.0 mL</td>
<td>200X in DMSO</td>
<td>• Room temperature</td>
<td>When stored as directed, kit contents are stable for at least 6 months.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Protect from light</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Desiccate</td>
<td></td>
</tr>
<tr>
<td>λ dsDNA HS standards (Component C)</td>
<td>set of 8 (500 µL each)</td>
<td>0, 0.5, 1, 2, 4, 6, 8, and 10 ng/µL</td>
<td>≤6°C</td>
<td></td>
</tr>
<tr>
<td>Quant-iT™ dsDNA HS buffer (Component B)</td>
<td>250 mL</td>
<td>NA</td>
<td>• ≤6°C *</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Protect from light</td>
<td></td>
</tr>
</tbody>
</table>

* 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).

For Research Use Only. Not for use in diagnostic procedures.
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.
Using the Quant-iT™ dsDNA High-Sensitivity Assay Kit with a Fluorescence Microplate Reader

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.

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.
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 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.
Using the Quant-iT™ dsDNA High-Sensitivity Assay Kit with the Qubit® Fluorometer

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.

**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.

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* where n = number of standards plus number of samples

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**Figure 5.** Overview for using the Quant-iT™ dsDNA HS assay in the Qubit® fluorometer.
2.3 Prepare assay tubes according to Table 2, below.

Table 2. Tube setup.

<table>
<thead>
<tr>
<th></th>
<th>Standard assay tubes</th>
<th>User Sample assay tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of working solution (from step 2.2)</td>
<td>190 μL</td>
<td>180–199 μL</td>
</tr>
<tr>
<td>Volume of standard (from kit)*</td>
<td>10 μL</td>
<td>—</td>
</tr>
<tr>
<td>Volume of user sample</td>
<td>—</td>
<td>1–20 μL</td>
</tr>
<tr>
<td>Total volume in each assay tube</td>
<td>200 μL</td>
<td>200 μL</td>
</tr>
</tbody>
</table>

* 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. *

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

* 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.
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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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