

Quant-iT™ 1X dsDNA HS Assay Kit

Catalog No. Q33232

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Rev. C.0

Product information

The Quant-iT™ 1X dsDNA HS (High Sensitivity) Assay Kit makes DNA quantitation easy and accurate. The kit includes a ready-to-use assay buffer and DNA standards. To perform the assay, dilute your sample (any volume from 1–20 µL is acceptable) into the 1X working solution provided, then read the concentration using a fluorescence plate reader. The assay is highly selective for double-stranded DNA (dsDNA) over RNA (Figure 4, page 6) and is accurate for initial sample concentrations from 10 pg/µL to 100 ng/µL, providing a core detection range of 0.2 ng to 100 ng of DNA in the assay tube. The assay is performed at room temperature, and the signal is stable for 3 hours when the samples are protected from light. Common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (Table 2, page 7).

In addition to the Quant-iT™ 1X dsDNA HS Assay Kit described here, we also offer the Quant-iT™ 1X dsDNA BR (Broad Range) Assay Kit (Cat. No. Q33267). The Quant-iT™ 1X dsDNA BR Assay Kit is designed for assaying samples containing 4–2000 ng of DNA. The Qubit™ dsDNA HS Assay – Lambda standard (Cat. No. Q33233) can be used to create the standard dilution series for the Quant-iT™ dsDNA HS assay.

If you would like to use this kit with the Qubit™ Fluorometer, we have included instructions under "Perform the Quant-iT™ 1X dsDNA HS Assay on a Qubit™ Fluorometer" (page 4).

Table 1. Contents and storage

Material	Amount	Concentration	Storage*
Quant-iT™ 1X dsDNA HS Working Solution (Component A)	250 mL	1X	• 2–8°C
Quant-iT™ 1X dsDNA HS Standards (Component B)	500 µL	0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 ng/µL in TE buffer	• Protect from light
Number of labelings: 1000 labelings, with a 200 µL assay volume in a 96-well microplate format. The Quant-iT™ 1X 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)			
* When stored as directed, the kits are stable for at least 6 months from the date of receipt.			

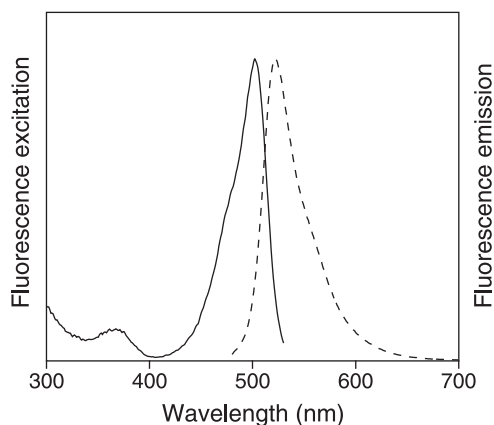


Figure 1. Excitation and emission maxima for the Quant-iT™ 1X dsDNA HS reagent when bound to dsDNA.

Critical assay parameters

Assay temperature The Quant-iT™ 1X dsDNA HS Assay delivers optimal performance when all solutions are at room temperature (18–28°C). Temperature fluctuations can influence the accuracy of the assay (Figure 5, page 6).

To minimize temperature fluctuations, insert all assay tubes into the fluorescence microplate reader only for as much time as it takes for the instrument to measure the fluorescence. Do not hold the assay tubes in your hand before reading because this warms the solution and results in a different reading.

Incubation time To allow the Quant-iT™ 1X dsDNA HS Assay to reach optimal fluorescence, incubate the tubes for 2 minutes after mixing the sample or the standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature when samples are protected from light.

Photostability of Quant-iT™ reagents The Quant-iT™ reagents exhibit high photostability, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings.

Handling and disposal No data are currently available that address the mutagenicity or toxicity of the Quant-iT™ 1X dsDNA HS Reagent (the dye in Component A). This reagent is known to bind nucleic acids. Treat the Quant-iT™ 1X dsDNA HS working solution with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

Perform the Quant-iT™ dsDNA HS Assay on a fluorescence microplate reader

This protocol describes the use of the Quant-iT™ 1X dsDNA HS Assay Kit with a fluorescence microplate reader that is equipped with either a monochromator or excitation and emission filters appropriate for fluorescein or Alexa Fluor™ 488 dye (Figure 1, page 2). Some contaminating substances may interfere with the assay; for more information, see "Contaminants tolerated by the Quant-iT™ 1X dsDNA HS Assay" (page 7). For an overview of this procedure, see Figure 2.

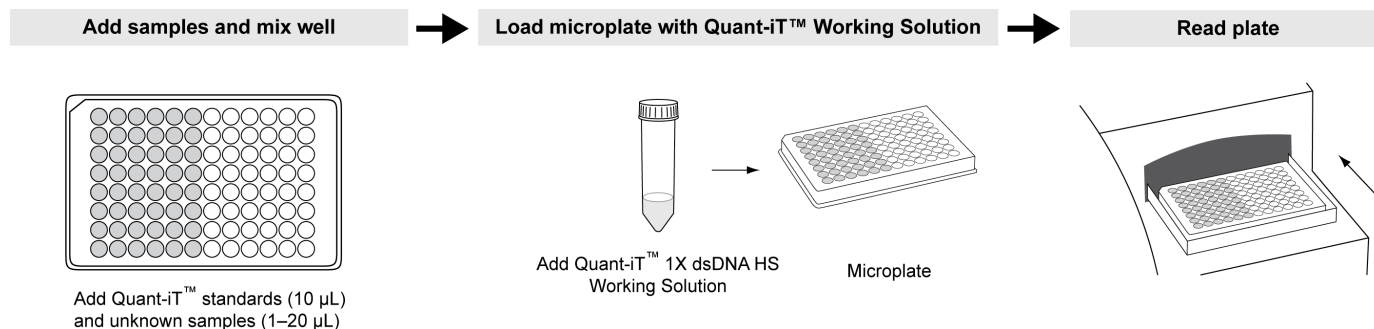


Figure 2. The Quant-iT™ dsDNA High-Sensitivity assay.

Assay procedure

IMPORTANT! For best results, ensure that all materials and reagents are at room temperature.

- 1.1 Add 10 µL of each Quant-iT™ 1X dsDNA HS Standard to separate wells. Duplicates or triplicates of the standards are recommended.
- 1.2 Add 1–20 µL of each unknown DNA sample to separate wells. Duplicates or triplicates of the unknown samples are recommended.
- 1.3 Load 200 µL of the Quant-iT™ 1X dsDNA working solution into each microplate well. This can be done readily using a multichannel pipettor.
- 1.4 If possible, mix your 96-well plate using a plate mixer or using the plate reader for about 3–10 seconds. Following mixing, allow the plate to incubate at room temperature for 2 minutes..
- 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 when protected from light.
- 1.6 Use a standard curve to determine the DNA amounts. For the dsDNA standards, plot amount vs. fluorescence, and fit a straight line to the data points.

Note: Many curve fitting programs will calculate the y-intercept. However, for best results, manually set the y-intercept as the RFU value obtained from the 0 ng/µL dsDNA standard.

Data analysis considerations – standard curves and extended ranges

The fluorescence of the Quant-iT™ 1X 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. Alternatively, dilute the standards in buffer for an even tighter range. During development of the Quant-iT™ 1X 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-factor¹ analysis). Your results may vary.

If desired, the utility of the Quant-iT™ 1X dsDNA HS assay can be extended beyond 100 ng, up to 200 ng. 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.

Perform the Quant-iT™ dsDNA HS Assay on a Qubit™ Fluorometer

The Quant-iT™ 1X dsDNA Assay Kit can be adapted for use with the Qubit™ Fluorometer. The protocol below is abbreviated from the Qubit™ Fluorometer user guide, which is available at thermofisher.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 3 for an overview of the procedure.

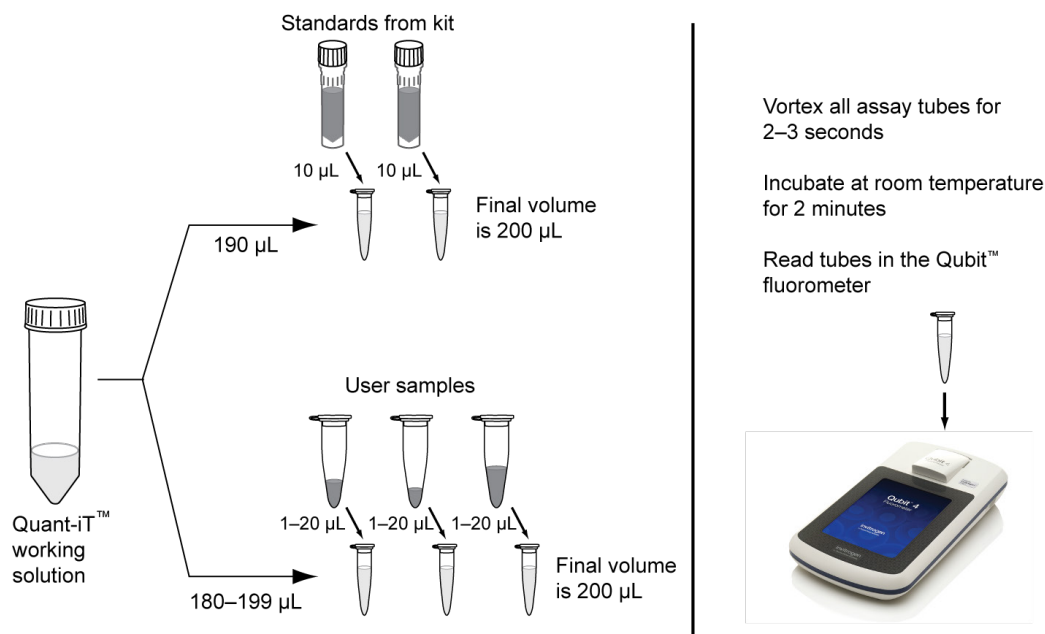


Figure 3. Overview for using the Quant-iT™ 1X dsDNA HS assay in the Qubit™ fluorometer.

Assay procedure **IMPORTANT!** For best results, ensure that all materials and reagents are at room temperature.

- 2.1 Set up the required number of 0.5-mL tubes for standards and samples. The Quant-iT™ 1X dsDNA HS Assay requires 2 standards.

Note: Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit™ assay tubes (Cat. No. Q32856).

- 2.2 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.

- 2.3 Add 10 µL of the 0 ng/µL and the 10 ng/µL Quant-iT™ 1X dsDNA HS Standard to the appropriate tube

- 2.4 Add 1–20 µL of each user sample to the appropriate tube.

- 2.5 Add the Quant-iT™ 1X dsDNA HS Working Solution to each tube such that the final volume is 200 µL.

Note: The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Quant-iT™ working solution, and each sample tube requires anywhere from 180–199 µL.

- 2.6 Mix each sample vigorously by vortexing for 3–5 seconds.

- 2.7 Allow all tubes to incubate at room temperature for 2 minutes, then proceed to read the standards and samples. Follow the procedure appropriate for your instrument:

- Qubit™ Flex Fluorometer
- Qubit™ 4 Fluorometer
- Qubit™ 3 Fluorometer

Note: If you are using the Qubit™ 3 Fluorometer, download the 1X dsDNA algorithm and assay button from thermofisher.com/qubit, then install it onto your Qubit™ Fluorometer.

Appendix

Selectivity of the Quant-iT™ 1X dsDNA HS Assay

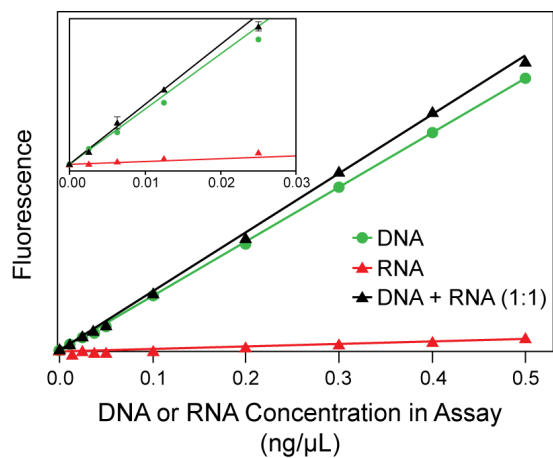


Figure 4. DNA selectivity and sensitivity of the Quant-iT™ 1X dsDNA HS Assay (Cat. No. Q33232). Triplicate 10-μL samples of λ DNA, *E. coli* rRNA, or a 1:1 mixture of DNA and RNA were assayed with the Quant-iT™ 1X dsDNA HS Assay. Fluorescence was measured at 502/532 nm and plotted versus the concentration of the RNA or DNA sample 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 is an expanded view of the low range of the assay showing the extreme sensitivity of the assay for DNA. Background fluorescence has not been subtracted.

Effect of temperature on the Quant-iT™ 1X dsDNA HS Assay

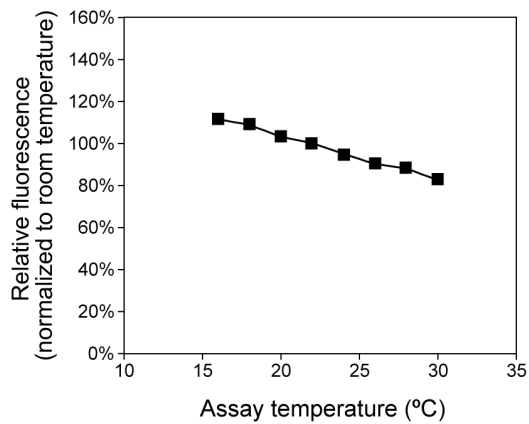


Figure 5. Plot of fluorescence vs. temperature for the Quant-iT™ 1X dsDNA HS Assay. The Quant-iT™ assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

Contaminants tolerated by
the Quant-iT™ 1X dsDNA HS
Assay

Note: While the contaminant tolerances of the Quant-iT™ 1X dsDNA HS assay and the Quant-iT™ dsDNA HS assay are largely similar, they are not identical.

Table 2. Effect of contaminants in the Quant-iT™ 1X dsDNA HS Assay*

Contaminant	Final concentration in the assay	Concentration in 10-µL sample	Concentration in 1-µL sample	Result
Sodium chloride	50 mM	1 M	10 M	OK
Magnesium chloride	1 mM	20 mM	200 mM	OK
Sodium acetate	30 mM	600 mM	6 M	OK
Ammonium acetate	25 mM	500 mM	NA	OK
Sodium azide	1 mM	20 mM	200 mM	OK
Ethanol	1%	20%	NA**	OK
Phenol	0.1%	2%	20%	OK
Chloroform†	1%	20%	NA**	OK
SDS	0.002%	0.04%	0.4%	OK
BSA	1X‡	1X‡	1X‡	OK
IgG	1X‡	1X‡	1X‡	OK
ssDNA	1X‡	1X‡	1X‡	OK
RNA	1X‡	1X‡	1X‡	OK
dNTPs§	1X‡	1X‡	1X‡	OK

* DNA standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 10 µL or 1 µL sample volumes are also listed. In all cases, results are given as OK, usually less than 10% perturbation. For best results, add the same amount of contaminant to the standard samples.

** User sample would require greater than 100% of listed contaminant.

† Immiscible.

‡ 1X indicates a concentration equal to the concentration of dsDNA.

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

NA: Not available.

Reference

1. J Biomol Screen 4, 67–73 (1999).

Ordering information

Cat. No.	Product name	Unit size
Q33232	Quant-iT™ 1X dsDNA HS Assay Kit.	1 kit
Related products		
Q33267	Quant-iT™ 1X dsDNA BR Assay Kit.	1 kit
Q33120	Quant-iT™ dsDNA Assay Kit, High Sensitivity.	1 kit
Q33130	Quant-iT™ dsDNA Assay Kit, Broad Range.	1 kit
Q10213	Quant-iT™ RNA Assay Kit, Broad Range.	1 kit
Q33140	Quant-iT™ RNA Assay Kit, 1000 assays.	1 kit
Q32882	Quant-iT™ microRNA Assay Kit, 1000 assays.	1 kit
Q33210	Quant-iT™ Protein Assay Kit, 1000 assays.	1 kit
O11492	Quant-iT™ OliGreen™ ssDNA Assay Kit.	1 kit
Q33233	Qubit™ 1X dsDNA Assay- Lambda Standard.	1 kit
Q33238	Qubit™ 4 Fluorometer with WiFi.	1 each
Q33327	Qubit™ Flex Fluorometer.	1 each
Q33252	Qubit™ Flex Assay Tube Strips.	125 tube strips
M33089	Microplates for fluorescence-based assays, 96-well (black-walled, clear bottom).	10 plates

Limited Product Warranty

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Revision history: Pub. No. MAN0017526

Revision	Date	Description
C.0	30 September 2021	Correct the amount of standard supplied with the kit, update Related products.
B.0	08 December 2020	Correcting assay range to 0.2 ng-100 ng.
A.0	18 December 2017	New document.

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