Quant-iT™ dsDNA High-Sensitivity Assay Kit

Catalog Number Q33120

Pub. No. MAN0002339 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Quant-iT™ dsDNA High-Sensitivity Assay Kit makes DNA quantification easy and accurate. The assay is highly selective for double-stranded DNA over RNA (see "Assay selectivity" on page 1), and in the range of 0.2–100 ng, the fluorescence signal is linear with DNA. Common contaminants, such as salts, solvents, detergents, or protein are well tolerated in the assay (Table 1).

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 Assay Kit is designed for assaying samples containing 4–1,000 ng of DNA.

Contents and storage

Component	Amount	Concentration	Storage[1]
Quant-iT™ dsDNA HS reagent (Component A)	1.0 mL	200X in DMSO	2°C to 8°C Desiccate Protect from light
Quant-iT™ dsDNA HS buffer (Component B)	250 mL	Not applicable	≤30°C
λ dsDNA HS standards (Component C)	set of 8 (500 µL each)	0, 0.5, 1, 2, 4, 6, 8, and 10 ng/µL	2°C to 8°C[2]
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 "Spectral data" on page 2)			

[1] When stored as directed, kit contents are stable for at least 6 months.
[2] For long-term storage, the dsDNA standards can be stored at ≤–20°C.

Required materials not supplied

- Nuclease-free pipettors and tips
- Microplates for Fluorescence-based Assays, 96-well (Cat. No. M33089)

Critical assay parameters

Assay temperature

Quant-iT™ assays deliver optimal performance when all solutions are at room temperature; temperature fluctuations can influence the accuracy of the assay.

Assay selectivity

The Quant-iT™ dsDNA HS assay is highly selective for double-stranded DNA over RNA (Figure 1).

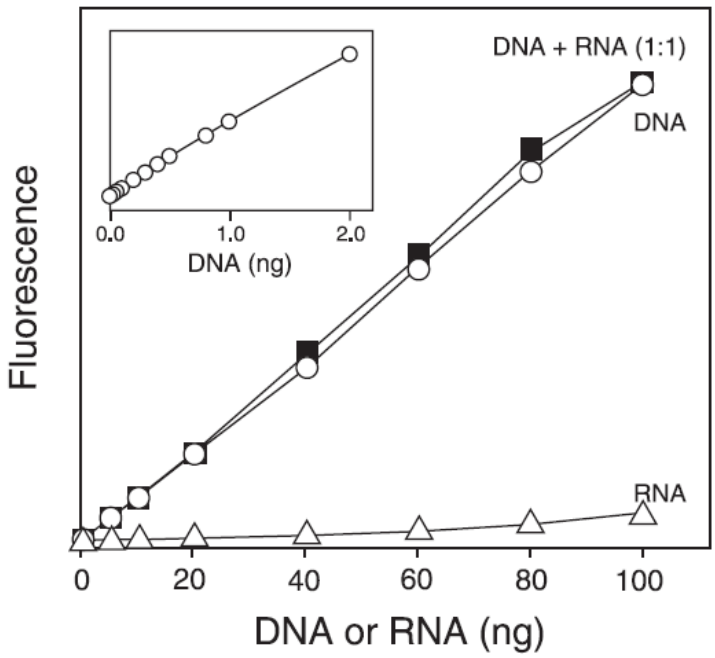

Figure 1 DNA selectivity and sensitivity of the Quant-iT™ dsDNA HS assay.
Triplicate 10 µL samples of λ DNA (O), *E. coli* rRNA (Δ), or a 1:1 mixture of DNA and RNA (■) were assayed in the Quant-iT™ dsDNA HS assay. Fluorescence was measured at 485/530 nm and plotted versus the mass of nucleic acid for 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.

Incubation time

To allow the Quant-iT™ dsDNA HS assay to reach maximum fluorescence, incubate 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 when the samples and standards are protected from light.

Spectral data

The Quant-iT™ dsDNA HS Reagent has an excitation/emission maxima of 502/523 nm when bound to DNA.

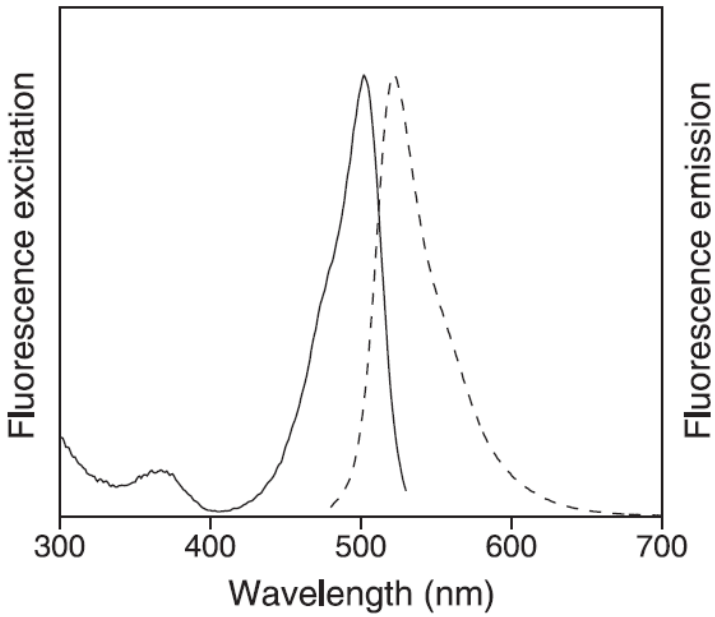

Figure 2 Excitation and emission maxima for the Quant-iT™ dsDNA HS Reagent bound to DNA.

Photostability of the Quant-iT™ reagent

Avoid multiple sequential fluorescence reads to minimize reagent photobleaching.

Effects of contaminating substances

A number of common contaminants have been tested in the Quant-iT™ dsDNA HS assay, and most are well tolerated. 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 1 Effects 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[1]
Sodium chloride	50 mM	500 mM	1 M	OK
Magnesium chloride	5 mM	50 mM	100 mM	OK[2]
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[2]
Chloroform[3]	1%	10%	20%	OK
SDS	0.01%	0.1%	0.2%	OK
Triton™ X-100	0.01%	0.1%	0.2%	OK[2]
dNTPs[4]	100 µM	1 mM	2 mM	OK
BSA	10 mg/mL	100 mg/mL	200 mg/mL	OK[2]
IgG	0.5 mg/mL	5 mg/mL	10 mg/mL	OK

[1] Results are given as OK, usually less than 10% perturbation, or as NR (not recommended).
[2] An acceptable result, but with some distortion of the standard curve; for best results, add the same amount of contaminant to the standard samples.
[3] Immiscible.
[4] A mixture of dATP, dCTP, dGTP, and dTTP.

Prepare and read samples and standards 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. For an overview of this procedure, see Figure 3.

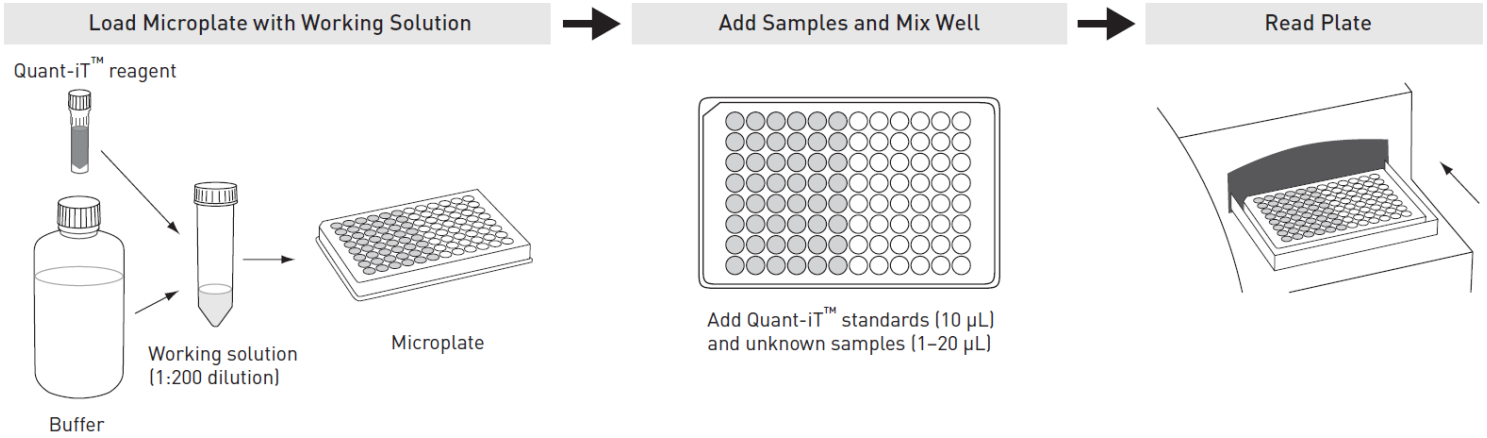

Figure 3 Overivew of the Quant-iT™ dsDNA High-Sensitivity assay.

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

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 combine 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. The Quant-iT™ working solution is stable for at least 3 hours at room temperature, protected from light.

   IMPORTANT! Do not use glass containers. Do not use buffers other than the Quant-iT™ dsDNA HS buffer to make the working solution.

2. Load 200 µL of the working solution into each microplate well.

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.

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.

5. If possible, mix the 96-well plate using a plate mixer or using the plate reader for about 3–10 seconds. After mixing, allow the plate to incubate at room temperature for 2 minutes.

6. Measure the fluorescence using a microplate reader (excitation/emission maxima are 502/523 nm; see "Spectral data" on 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.

7. 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 up to 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). Alternatively, dilute the standards in buffer for an even tighter range (Figure 4A, inset).

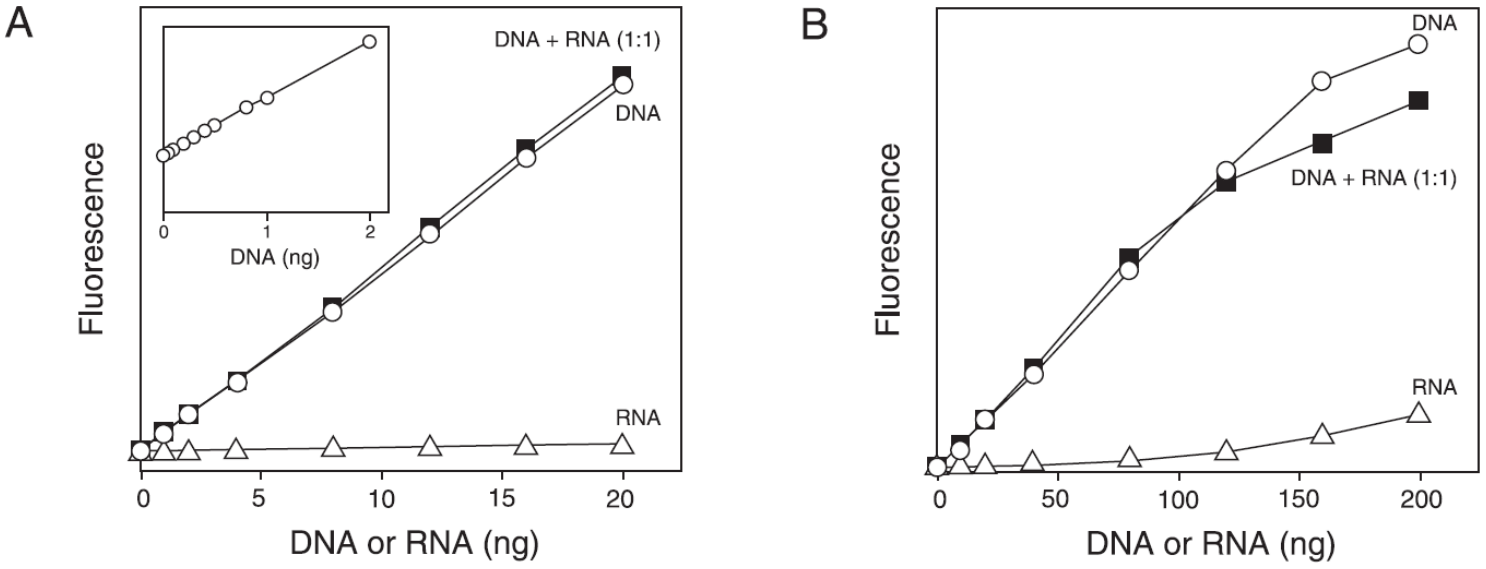

Figure 4 Extended ranges for the Quant-iT™ dsDNA HS assay.
Triplicate 2 µL (Panel A) or 20 µL samples (Panel B) of λ DNA (O), *E. coli* rRNA (Δ), or a 1:1 mixture of DNA and RNA (■) were assayed in the Quant-iT™ dsDNA HS assay. Fluorescence was measured at 485/530 nm and plotted versus the mass of nucleic 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.

If desired, the utility of the Quant-iT™ dsDNA HS assay can be extended beyond 100 ng, up to 200 ng (Figure 4B). 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.

Related products

Table 2 Bulk Reagents and Kits

Product	Quantity	Cat. No.
Quant-iT™ PicoGreen™ dsDNA Assay Kit	1 mL assay kit 10 x 100 µL	P7589 P11496
Quant-iT™ PicoGreen™ dsDNA Reagent	1 mL reagent 10 x 100 µL	P7581 P11495
TE Buffer (20X), RNase-free	100 mL	T11493
Quant-iT™ RiboGreen™ RNA Assay Kit	1 mL assay kit	R11490
Quant-iT™ RiboGreen™ RNA Reagent	1 mL reagent	R11491
Quant-iT™ RediPlate™ 96 RiboGreen™ RNA Quantitation Kit	1 plate	R32700
Quant-iT™ OliGreen™ ssDNA Assay Kit	1 mL assay kit	O11492
Quant-iT™ OliGreen™ ssDNA Assay Reagent	1 mL reagent	O7582

Table 3 Microplate Reader Assays

Product	Dynamic Range	Quantity	Cat. No.
Quant-iT™ 1X dsDNA Assay Kit, High Sensitivity	200 pg–100 ng	1,000 reactions	Q33232
Quant-iT™ 1X dsDNA Assay Kit, Broad-Range	4 ng–2 µg	1,000 reactions	Q33267
Quant-iT™ DNA Assay Kit, High Sensitivity	200 pg–100 ng	1,000 reactions	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	4 ng–1 µg	1,000 reactions	Q33130
Quant-iT™ RNA Assay Kit	5–100 ng	1,000 reactions	Q33140
Quant-iT™ RNA Reagent	5–100 ng	1,000 reactions	Q32884
Quant-iT™ RNA Assay Kit, Broad Range	20 ng–1 µg	1,000 reactions	Q10213
Quant-iT™ RNA XR Assay Kit	200 ng–10 µg	1,000 reactions	Q33225
Quant-iT™ microRNA Assay Kit	1–100 ng	1,000 reactions	Q32882
Quant-iT™ Protein Assay Kit	250 ng–5 µg	1,000 reactions	Q33210
Microplates for Fluorescence-based Assays, 96-well	—	10 plates	M33089

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 at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0002339

Revision	Date	Description
B.0	8 March 2022	The format and content were updated.
A.0	16 February 2015	New document for the Quant-iT™ dsDNA High-Sensitivity Assay Kit.