Quant-iT™ PicoGreen™ dsDNA Reagent and Kit

Catalog Numbers P11496, P7589, P11495, P7581

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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™ PicoGreen™ dsDNA Reagent is an ultrasensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in solution. Detecting and quantitating small amounts of DNA is important in many applications including synthesizing cDNA for library production, purifying DNA fragments for subcloning, and quantifying DNA libraries for next-generation sequencing.

The Quant-iT™ PicoGreen™ dsDNA Reagent enables researchers to quantitate as little as 250 pg/mL of dsDNA (50 pg dsDNA in a 200 µL assay volume) with a fluorescence microplate reader using fluorescein excitation and emission wavelengths. The linear detection range of the Quant-iT™ PicoGreen™ assay in a standard fluorometer extends over more than 4 orders of magnitude in DNA concentration (25 pg/mL to 1,000 ng/mL) with a single dye concentration (Figure 1). This linearity is maintained in the presence of several compounds that commonly contaminate nucleic acid preparations, including salts, urea, ethanol, chloroform, detergents, proteins, and agarose.

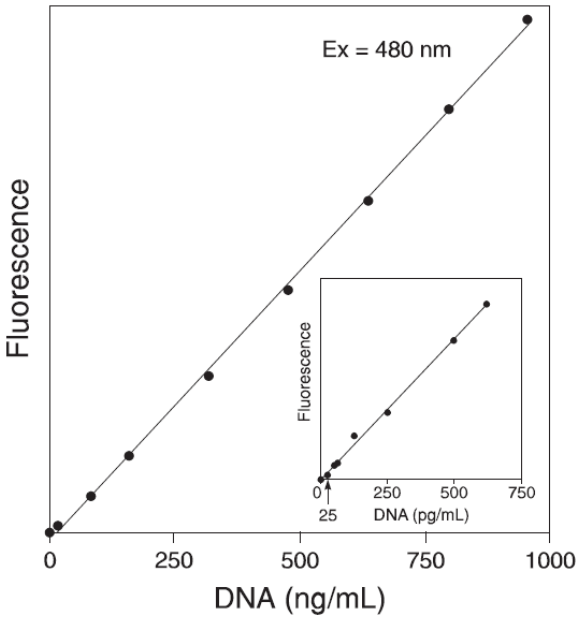

Figure 1 Dynamic range and sensitivity of the Quant-iT™ PicoGreen™ dsDNA assay.
Calf thymus DNA was added to cuvettes containing the Quant-iT™ PicoGreen™ dsDNA Reagent diluted in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5 (TE). The samples were excited at 480 nm and the fluorescence emission intensity was measured at 520 nm using a spectrofluorometer. Fluorescence emission intensity was then plotted versus DNA concentration; the inset shows an enlargement of the results obtained with DNA concentrations between 0 and 750 pg/mL.

The Quant-iT™PicoGreen™ dsDNA assay was developed to minimize the fluorescence contribution of RNA and single-stranded DNA (ssDNA) (Figure 2). Using the Quant-iT™ PicoGreen™ dsDNA Reagent and the recommended assay protocol, researchers can quantitate dsDNA in the presence of equimolar concentrations of ssDNA and RNA with minimal effect on the quantitation results.

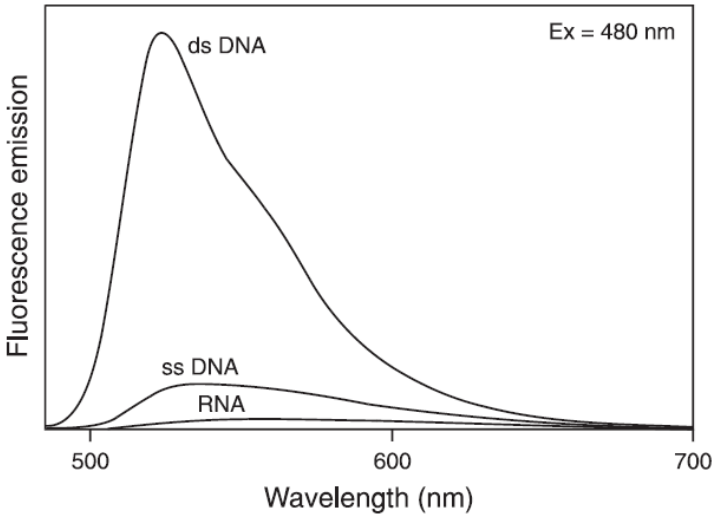

Figure 2 Fluorescence enhancement of the Quant-iT™ PicoGreen™ dsDNA Reagent upon binding dsDNA, ssDNA, and RNA. Samples containing 500 ng/mL calf thymus DNA, M13 ssDNA, or *E. coli* ribosomal RNA were added to cuvettes containing the Quant-iT™ PicoGreen™ dsDNA Reagent in TE. Samples were excited at 480 nm and the fluorescence emission spectra were collected using a spectrofluorometer. Emission spectra for samples containing dye and nucleic acids, as well as for dye alone (baseline), are shown.

Contents and storage

Component	Quant-iT™ PicoGreen™ dsDNA Reagents[1]		Quant-iT™ PicoGreen™ dsDNA Assay Kits		Concentration	Storage[2]
	Cat. No. P7581	Cat. No. P11495	Cat. No. P7589	Cat. No. P11496		
Quant-iT™ PicoGreen™ dsDNA Reagent (Component A)	1 mL	10 x 100 µL	1 mL	10 x 100 µL	200X in DMSO	2°C to 8°C[3] Desiccate Protect from light
20X TE (Component B)	Not applicable	Not applicable	25 mL	25 mL	200 mM Tris-HCl, 20 mM EDTA, pH 7.5	≤30°C
Lambda DNA standard (Component C)	Not applicable	Not applicable	1 mL	1 mL	100 µg/mL in TE	2°C to 8°C[3]
Number of labelings: 2,000 with an assay volume of 200 µL in a 96-well microplate format. The Quant-iT™ PicoGreen™ dsDNA assay can be adapated for use in cuvettes or 384-well microplates.						
Approximate fluorescence excitation/emission maxima: 502/523 nm, bound to nucleic acid.						

[1] Stand-alone reagents do not include Components B and C.
[2] When stored as directed, products are stable for at least 6 months.
[3] For long-term storage, the Quant-iT™ PicoGreen™ dsDNA Reagent and lambda DNA standard can be stored at ≤–20°C.

Required materials not supplied

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

Prepare the assay buffer

Prepare a 1X TE working solution by diluting the concentrated buffer 20-fold with sterile, distilled, DNase-free water.

IMPORTANT! TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) is used to prepare the Quant-iT™ PicoGreen™ dsDNA Reagent working solution, and to dilute the dsDNA standards and samples. Because the Quant-iT™ PicoGreen™ dye is an extremely sensitive detection reagent for dsDNA, the TE solution used must be free of contaminating nucleic acids. The 20X TE buffer included in the Quant-iT™ PicoGreen™ dsDNA Assay Kit is certified to be nucleic acid–free and DNase-free.

Prepare the reagent

On the day of the experiment, allow the Quant-iT™ PicoGreen™ dsDNA Reagent to warm to room temperature before opening the vial, then prepare an aqueous working solution of the Quant-iT™ PicoGreen™ dsDNA Reagent by diluting the concentrated DMSO solution 200-fold in TE. For microplate assays of a total 200 µL assay volume, you need 100 µL of the Quant-iT™ PicoGreen™ dsDNA Reagent working solution per sample.

For example, to prepare enough working solution to assay 100 samples in 200 µL volumes, add 50 µL Quant-iT™ PicoGreen™ dsDNA Reagent to 9.95 mL TE.

Note: We recommend preparing this solution in a plastic container rather than glass, as the reagent may adsorb to glass surfaces. Protect the working solution from light, as the Quant-iT™ PicoGreen™ dsDNA Reagent is susceptible to photodegradation. **For best results, use the working solution within a few hours of preparation.**

Prepare the DNA standard curve

1. Prepare a 2 µg/mL stock solution of dsDNA in TE. Determine the DNA concentration on the basis of absorbance at 260 nm (A_{260}) in a cuvette with a 1 cm pathlength; an A_{260} of 0.04 corresponds to 2 µg/mL dsDNA solution.

   The lambda DNA standard, provided at 100 µg/mL in the Quant-iT™ PicoGreen™ dsDNA Assay Kit, is diluted 50-fold in TE to make the 2 µg/mL working solution. For example, 3 µL of the DNA standard mixed with 147 µL of TE is sufficient for the standard curve described in step 2.

   Note: For a standard curve, we commonly use bacteriophage lambda or calf thymus DNA, although any purified dsDNA preparation may be used. It is sometimes preferable to prepare the standard curve with DNA similar to the type being assayed; e.g., long or short linear DNA fragments when quantitating similar-sized restriction fragments or plasmid when quantitating plasmid DNA. However, most linear dsDNA molecules yield approximately equivalent signals, regardless of fragment length.

   Note: The dsDNA solution used to prepare the standard curve should be treated the same way as the experimental samples and should contain similar levels of contaminants. See “Effects of common contaminants” on page 4 for a list of contaminants tested in the Quant-iT™ PicoGreen™ assay.

2. For the **high-range** standard curve from 10 ng/mL to 1 µg/mL, dilute the 2 µg/mL DNA stock solution into microplate wells as shown in Table 1. For the **low-range** standard curve from 250 pg/mL to 25 ng/mL, dilute the 2 µg/mL DNA solution (prepared in step 1) 40-fold in TE to yield a 50 ng/mL DNA stock solution, then prepare the dilution series shown in Table 2.

   Table 1 Protocol for preparing a high-range standard curve.

Volume of TE buffer	Volume of 2 µg/mL DNA stock	Volume of diluted Quant-iT™ PicoGreen™ dsDNA Reagent	Final DNA concentration in assay
0 µL	100 µL	100 µL	1 µg/mL
90 µL	10 µL	100 µL	100 ng/mL
99 µL	1 µL	100 µL	10 ng/mL
100 µL	0 µL	100 µL	blank

   Table 2 Protocol for preparing a low-range standard curve.

Volume of TE buffer	Volume of 50 ng/mL DNA stock	Volume of diluted Quant-iT™ PicoGreen™ dsDNA Reagent	Final DNA concentration in assay
0 µL	100 µL	100 µL	25 ng/mL
90 µL	10 µL	100 µL	2.5 ng/mL
99 µL	1 µL	100 µL	250 pg/mL
100 µL	0 µL	100 µL	blank

3. Add 100 µL of the aqueous working solution of the Quant-iT™ PicoGreen™ dsDNA Reagent (prepared in “Prepare the reagent” on page 3) to each well. Mix well and incubate for 2–5 minutes at room temperature, protected from light.

4. Measure the sample fluorescence using a fluorescence microplate reader and standard fluorescein wavelengths (excitation ~480 nm, emission ~520 nm).

   Note: To ensure that the sample readings remain in the detection range, the instrument’s gain should be set so that the sample containing the highest DNA concentration yields a fluorescence intensity near the microplate reader’s maximum. For optimal

detection sensitivity, the instrument gain can be increased for the low-range assay relative to the high-range assay. To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

5. Subtract the fluorescence value of the reagent blank from that of each of the samples. Use corrected data to generate a standard curve of fluorescence versus DNA concentration (Figure 1).

Analyze samples

1. Dilute the experimental DNA solution in TE to a final volume of 100 µL in microplate wells.

   Note: You can alter the amount of sample diluted, provided that the final volume remains 100 µL. A higher dilution of the experimental sample may diminish the interfering effect of certain contaminants. However, extremely small sample volumes should be avoided because they are difficult to pipet accurately. See "Eliminate single-stranded nucleic acids from samples" on page 5 for information on eliminating RNA and ssDNA from the sample.

2. Add 100 µL of the aqueous working solution of the Quant-iT™ PicoGreen™ dsDNA Reagent to each sample. Incubate for 2–5 minutes at room temperature, protected from light.

3. Measure the fluorescence of the samples using the same instrument parameters used to generate the standard curve (see step 4). To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

4. Subtract the fluorescence value of the reagent blank from that of each of the samples. Determine the DNA concentration of the sample from the standard curve generated in "Prepare the DNA standard curve" on page 3.

5. The assay can be repeated using a different dilution of the sample to confirm the quantitation results.

Effects of common contaminants

The Quant-iT™ PicoGreen™ assay remains linear in the presence of several compounds that commonly contaminate nucleic acid preparations, although the signal intensity may be affected (Table 3). For the highest accuracy, the standards should be prepared under the same conditions as the experimental samples and contain similar levels of contaminants.

Table 3 Effects of common contaminants on the signal intensity of the assay.

Compound	Maximum acceptable concentration	% Signal change[1]
Salts		
Ammonium acetate	50 mM	3% decrease
Sodium acetate	30 mM	3% increase
Sodium chloride	200 mM	30% decrease
Zinc chloride	5 mM	8% decrease
Magnesium chloride	50 mM	33% decrease
Urea	2 M	9% increase
Organic solvents		
Phenol	0.1%	13% increase
Ethanol	10%	12% increase
Chloroform	2%	14% increase
Detergents		
Sodium dodecyl sulfate	0.01%	1% decrease
Triton™ X-100	0.1%	7% increase
Proteins		
Bovine serum albumin	2%	16% decrease
IgG	0.1%	19% increase

Compound	Maximum acceptable concentration	% Signal change[1]
Other compounds		
Polyethylene glycol	2%	8% increase
Agarose	0.1%	4% increase

[1] The compounds were incubated at the indicated concentrations with Quant-iT™ PicoGreen™ reagent in the presence of 500 ng/mL calf thymus DNA. All samples were assayed in a final volume of 200 µL in 96-well microplates using a fluorescence microplate reader. Samples were excited at 485 nm and fluorescence intensity was measured at 520 nm.

Eliminate single-stranded nucleic acids from samples

Using the Quant-iT™ PicoGreen™ dsDNA Assay Kit, dsDNA can be quantitated in the presence of equimolar concentrations of single-stranded nucleic acids with minimal interference. Table 4 shows the concentrations of RNA or ssDNA that, for a given dsDNA concentration, result in less than a 10% change in the signal intensity using the Quant-iT™ PicoGreen™ assay protocol. Fluorescence due to the Quant-iT™ PicoGreen™ dsDNA Reagent binding to RNA at high concentrations can be eliminated by treating the sample with DNase-free RNase. The use of RNase A/RNase T1 with S1 nuclease will eliminate all single-stranded nucleic acids and ensure that the entire sample fluorescence is due to dsDNA.

Table 4 Sensitivity of the Quant-iT™ PicoGreen™ dsDNA assay for quantitating dsDNA in the presence of single-stranded nucleic acids.

dsDNA[1]	RNA (amount relative to dsDNA)		ssDNA (amount relative to dsDNA)	
1 µg/mL	10 µg/mL	(10X)	300 ng/mL	(0.3X)
500 ng/mL	500 ng/mL	(1X)	50 ng/mL	(0.1X)
10 ng/mL	100 ng/mL	(10X)	30 ng/mL	(3X)
5 ng/mL	50 ng/mL	(10X)	15 ng/mL	(3X)
100 pg/mL	1 ng/mL	(10X)	1 ng/mL	(10X)
50 pg/mL	500 pg/mL	(10X)	500 pg/mL	(10X)

[1] For several concentrations of dsDNA, we show the concentration of RNA or ssDNA that results in no more than a 10% increase in the sample's signal intensity.

Related products

Table 5 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 6 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

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Revision history: Pub. No. MAN0001931

Revision	Date	Description
A.0	15 March 2022	The format and content were updated. The version numbering was reset to A.0 in conformance with internal document control.
1.00	10 June 2008	New document for the Quant-iT™ PicoGreen™ dsDNA Assay Kit and Quant-iT™ PicoGreen™ dsDNA Reagent.

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