Quant-iT™ PicoGreen® dsDNA Reagent and Kits

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Table 1. Contents and Storage Information.

Material	Amount	Concentration	Storage	Stability
Quant-iT™ PicoGreen® dsDNA reagent (Component A)	1 mL in 1 vial (P7589) or in10 vials of 100 µL each (P11496)	Solution in DMSO	• 2–6ºC • Desiccate • Protect from light	When stored as directed, product stable for at least 6 months
20X TE (Component B)	25 mL	200 mM Tris-HCl, 20 mM EDTA, pH 7.5	• Room temperature *	
Lambda DNA standard (Component C)	1 mL	100 µg/mL in TE	• 2–6ºC *	

* For long-term storage, both the 20X TE and lambda DNA standard can be stored at ≤–20ºC.

Number of Labelings: For either the kits or the stand-alone reagent, sufficient reagent is supplied for 200 assays using an assay volume of 2 mL and the protocol described below. Note that the assay volume is dependent on the instrument used to measure fluorescence; with a microplate reader and a 96-well microplate, the assay volume is reduced to 200 µL and 2,000 assays are possible.

Spectral Data: Quant-iT™ PicoGreen® dsDNA reagent 502/523 nm, bound to nucleic acids

Introduction

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 extremely important in a wide variety of biological applications. These include standard molecular biology techniques, such as synthesizing cDNA for library production and purifying DNA fragments for subcloning, as well as diagnostic techniques, such as quantitating DNA amplification products and detecting DNA molecules in drug preparations. The Quant-iT™ PicoGreen® reagent has recently been used to quantitate PCR amplification yields in a method for direct cycle sequencing of PCR products.[1]

The most commonly used technique for measuring nucleic acid concentration is the determination of absorbance at 260 nm (A_{260}). The major disadvantages of the absorbance method are the large relative contribution of nucleotides and single-stranded nucleic acids to the signal, the interference caused by contaminants commonly found in nucleic acid preparations, the inability to distinguish between DNA and RNA, and the relative insensitivity of the assay (an A_{260} of 0.1 corresponds to a 5 µg/mL dsDNA solution). Hoechst (bisbenzimide) dyes are sensitive fluorescent nucleic acid stains that circumvent many of these problems. The Hoechst 33258–based assay is somewhat selective for dsDNA, does not show significant fluorescence enhancement in the presence of proteins, and allows the detection and quantitation of DNA concentrations as low as 10 ng/mL DNA.[2] Molecular Probes' proprietary YO-PRO®-1 and YOYO®-1 nucleic acid stains have also been used to quantitate nucleic acids, allowing the detection of about 0.5 ng/mL DNA in solution.[3,4]

Our Quant-iT™ PicoGreen® dsDNA reagent enables researchers to quantitate as little as 25 pg/mL of dsDNA (50 pg dsDNA in a 2 mL assay volume) with a standard spectrofluorometer and fluorescein excitation and emission wavelengths. This sensitivity exceeds that achieved with the Hoechst 33258–based assay by 400-fold. Using a fluorescence microplate reader, we can detect as little as 250 pg/mL dsDNA (50 pg in a 200 µL assay volume). The standard Quant-iT™ PicoGreen® assay protocol is also simpler than that for Hoechst 33258 because a single concentration of the Quant-iT™ PicoGreen® reagent allows detection over the full dynamic range of the assay. In order to achieve more than two orders of magnitude in dynamic range with Hoechst-based assays, two different dye concentrations are recommended. In contrast, the linear detection range of the Quant-iT™ PicoGreen® assay in a standard fluorometer extends over more than four orders of magnitude in DNA concentration—from 25 pg/mL to 1,000 ng/mL—with a single dye concentration (Figure 1). We have shown that 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.

Our assay protocol was also developed to minimize the fluorescence contribution of RNA and single-stranded DNA (ssDNA) (Figure 2). Although the Hoechst 33258–based method is not significantly affected by the presence of RNA when the assay is carried out in the recommended high-salt buffer, we have found that Hoechst 33258 does exhibit a large fluorescence enhancement with ssDNA under these conditions. Furthermore, when the Hoechst 33258–based assay is carried out in TE alone (10 mM Tris-HCl, 1 mM EDTA, pH 7.5, with no NaCl added), RNA contributes a significant fluorescence signal. 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.

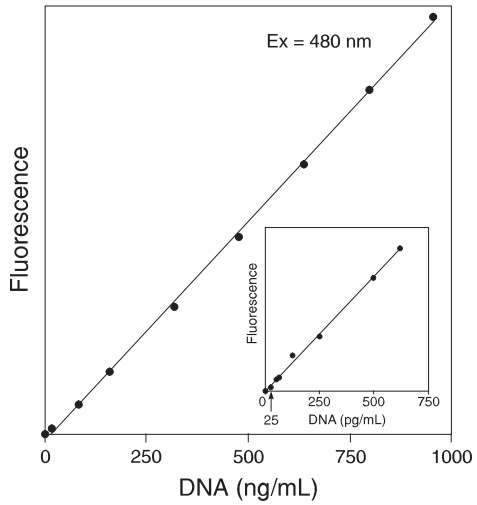

Figure 1. Dynamic range and sensitivity of the Quant-iT™ PicoGreen® dsDNA assay. Calf thymus DNA was added to cuvettes containing Quant-iT™ PicoGreen® dsDNA reagent diluted in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5 (TE). The samples were excited at 480 nm and the fluorescence emission intensity was measured 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 zero and 750 pg/mL.

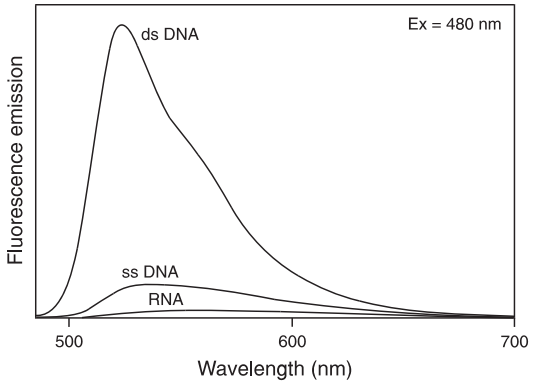

Figure 2. Fluorescence enhancement of Quant-iT™ PicoGreen® reagent upon binding dsDNA, ssDNA, and RNA. Samples containing 500 ng/mL calf thymus DNA, M13 ssDNA, or *E. coli* ribosomal RNA were added to cuvettes containing Quant-iT™ PicoGreen® reagent in TE. Samples were excited at 480 nm and the fluorescence 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.

Before You Begin

The Quant-iT™ PicoGreen® reagent supplied in the kits is exactly the same as the reagent sold separately.

Handling and Disposal

Allow the Quant-iT™ PicoGreen® reagent to warm to room temperature before opening the vial.

Caution:

No data are available addressing the mutagenicity or toxicity of Quant-iT™ PicoGreen® dsDNA reagent. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. Dispose of Quant-iT™ PicoGreen® reagent in accordance with local regulations.

Preparing the Assay Buffer

TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) is used below for diluting the Quant-iT™ PicoGreen® reagent, for diluting DNA samples, and in the assay itself. Because the Quant-iT™ PicoGreen® dye is an extremely sensitive detection reagent for dsDNA, it is imperative that the TE solution used be free of contaminating nucleic acids. The 20X TE buffer included in the Quant-iT™ PicoGreen® dsDNA Assay Kits is certified to be nucleic acid–free and DNase-free. Prepare a 1X TE working solution by diluting the concentrated buffer 20-fold with sterile, distilled, DNase-free water.

Preparing the Reagent

On the day of the experiment, prepare an aqueous working solution of the Quant-iT™ PicoGreen® reagent by making a 200-fold dilution of the concentrated DMSO solution in TE. For example, to prepare enough working solution to assay 20 samples in a 2 mL final volume, add 100 µL Quant-iT™ PicoGreen® dsDNA reagent to 19.9 mL TE. 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 by covering it with foil or placing it in the dark, as the Quant-iT™ PicoGreen® reagent is susceptible to photodegradation. *For best results, this solution should be used within a few hours of its preparation.*

Experimental Protocol

DNA Standard Curve

1.1 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. For a standard curve, we commonly use bacteriophage lambda or calf thymus DNA, although any purified dsDNA preparation may be used. The lambda DNA standard, provided at 100 µg/mL in the Quant-iT™ PicoGreen® Kits, can simply be diluted 50-fold in TE to make the 2 µg/mL working solution. For example, 30 µL of the DNA standard mixed with 1.47 mL of TE will be sufficient for the standard curve described below. 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, we have found that most linear dsDNA molecules yield approximately equivalent signals, regardless of fragment length. Our results have shown that 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 2). Thus, to serve as an effective control, 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 such compounds. To create a five-point standard curve from 1 ng/mL to 1 µg/mL, proceed to step 1.2. For a low-range standard curve from 25 pg/mL to 25 ng/mL, prepare a 40-fold dilution of the 2 µg/mL DNA solution to yield a 50 ng/mL DNA stock solution and proceed to step 1.5.

1.2 For the **high-range** standard curve, dilute the 2 µg/mL DNA stock solution into disposable cuvettes (or plastic test tubes for transfer to quartz cuvettes) as shown in Table 3. Then add 1.0 mL of the aqueous working solution of Quant-iT™ PicoGreen® reagent (prepared in *Reagent Preparation*) to each cuvette. Mix well and incubate for 2 to 5 minutes at room temperature, protected from light.

1.3 After incubation, measure the sample fluorescence using a spectrofluorometer or fluorescence microplate reader and standard fluorescein wavelengths (excitation ~480 nm, emission ~520 nm). To ensure that the sample readings remain in the detection range of the fluorometer, the instrument's gain should be set so that the sample containing the highest DNA concentration yields a fluorescence intensity near the fluorometer's maximum. To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

1.4 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 (see Figure 1).

1.5 For the **low-range** standard curve—from 25 pg/mL to 25 ng/mL—dilute the 50 ng/mL DNA stock solution (prepared in step 1.1) into disposable cuvettes (or plastic test tubes for transfer to quartz cuvettes) as shown in Table 4. Add 1.0 mL of the aqueous working solution of Quant-iT™ PicoGreen® reagent (prepared in *Reagent Preparation*) to each cuvette. Mix well and incubate for 2 to 5 minutes at room temperature, protected from light. Continue with steps 1.3 and 1.4. Adjust the fluorometer gain to accommodate the lower fluorescence signals.

Table 2. Effects of several compounds that commonly contaminate nucleic acid preparations on the signal intensity of the Quant-iT™ PicoGreen® dsDNA assay.

Compound	Maximum Acceptable Concentration	% Signal Change*
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
Other Compounds		
Polyethylene glycol	2%	8% increase
Agarose	0.1%	4% increase

* 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 CytoFluor microplate reader. Samples were excited at 485 nm and fluorescence intensity was measured at 520 nm.

Table 3. Protocol for preparing a high-range standard curve.

Volume (µL) of TE	Volume (µL) of 2 µg/mL DNA Stock	Volume (µL) of Diluted Quant-iT™ PicoGreen® Reagent	Final DNA Concentration in Quant-iT™ PicoGreen® Assay
0	1,000	1,000	1 µg/mL
900	100	1,000	100 ng/mL
990	10	1,000	10 ng/mL
999	1	1,000	1 ng/mL
1,000	0	1,000	blank

Sample Analysis

2.1 Dilute the experimental DNA solution in TE to a final volume of 1.0 mL in disposable cuvettes or test tubes. You may alter the amount of sample diluted, provided that the final volume remains 1.0 mL. 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 *Eliminating Single-Stranded Nucleic Acids from Samples* (below) for information on eliminating RNA and ssDNA from the sample.

2.2 Add 1.0 mL of the aqueous working solution of the Quant-iT™ PicoGreen® reagent to each sample. Incubate for 2 to 5 minutes at room temperature, protected from light.

2.3 Measure the fluorescence of the sample using instrument parameters that correspond to those used when generating your standard curve (see step 1.3). To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

2.4 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 *DNA Standard Curve*.

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

Eliminating Single-Stranded Nucleic Acids from Samples

We have found that dsDNA can be quantitated in the presence of equimolar concentrations of single-stranded nucleic acids with minimal interference. Table 5 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 Quant-iT™ PicoGreen® reagent binding to RNA at high concentrations can be eliminated by treating the sample with DNase-free RNase.[5] 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.[5]

Table 4. Protocol for preparing a low-range standard curve.

Volume (µL) of TE	Volume (µL) of 50 ng/mL DNA Stock	Volume (µL) of Diluted Quant-iT™ PicoGreen® Reagent	Final DNA Concentration in Quant-iT™ PicoGreen® Assay
0	1,000	1,000	25 ng/mL
900	100	1,000	2.5 ng/mL
990	10	1,000	250 pg/mL
999	1	1,000	25 pg/mL
1,000	0	1,000	blank

Table 5. Sensitivity of the Quant-iT™ PicoGreen® dsDNA assay for quantitating dsDNA in the presence of single-stranded nucleic acids.

[dsDNA]*	[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/L	(3X)
100 pg/mL	1 ng/mL	(10X)	1 ng/mL	(10X)
50 pg/mL	500 pg/mL	(10X)	500 pg/mL	(10X)

* 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.

References

1. Biotechniques 20, 676 (1996); **2.** Anal Biochem 102, 344 (1980); **3.** Anal Biochem 208, 144 (1993); **4.** Biophys J 61, A314 (1992); **5.** Sambrook, J., Fritsch, E.F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor Laboratory Press (1989).

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	Cat #	Product Name	Unit Size
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Buy Now	P11496	Quant-iT™ PicoGreen® dsDNA Assay Kit *10 × 100 µL*	1 kit
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