



Application Note

PicoGreen® dsDNA Quantitation with the Beckman Coulter DTX 800/880 Multimode Detector

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Introduction

Nucleic acid concentrations are measured in most laboratories by the determination of absorbance at 260 nm (A_{260}). The absorbance method, however, is not specific for double stranded DNA (dsDNA) because absorbance measurements are highly influenced by other types of nucleic acids such as single stranded DNA (ssDNA) or ribosomal RNA (rRNA). Besides unspecific compounds and contaminants, the relative insensitivity of the assay (an A_{260} of 0.1 corresponds to a 5 µg/ml dsDNA solution) is an additional disadvantage of the absorbance method.

PicoGreen® dsDNA quantitation reagent is an ultrasensitive fluorescent nucleic acid stain for quantitating 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 PicoGreen reagent has recently been used to quantitate PCR amplification yields in a method for direct cycle sequencing of PCR products.¹ The PicoGreen assay remains linear in the presence of several compounds that commonly contaminate nucleic acid preparations, although the signal intensity may be affected.

This note demonstrates that PicoGreen dsDNA quantitation using the Beckman Coulter DTX800/880 Multimode Detector enables high sensitivity results, with detection limits as low as 5 pg dsDNA/200 µl.

Experimental Protocol

Required Materials

- DTX800 and/or DTX880 Multimode Detectors (Beckman Coulter)
- PicoGreen dsDNA Quantitation Reagent *special packaging* (Molecular Probes, Inc., catalog number P-11496)
- 96 and 384 well format microplates (Beckman Coulter)

Reagent Preparation

The simple buffer, 10 mM Tris-HCl, 1mM EDTA, pH 7.5 (TE), is used for diluting the PicoGreen reagent, for diluting DNA samples and in the assay itself. Because the PicoGreen dye is an extremely sensitive detection reagent for dsDNA, it is imperative that the TE solution used is free of contaminating nucleic acids. The PicoGreen dsDNA quantitation reagent is

delivered in a dimethylsulfoxide (DMSO) solution. The working solution of PicoGreen reagent is made by a 200-fold dilution of the concentrated DMSO solution in TE. It is recommended to prepare 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 PicoGreen reagent is susceptible to photodegradation. For best results, this solution should be used within a few hours of its preparation.

Note: The PicoGreen kit is sufficient for 200 assays using an assay volume of 2 ml. Note that the assay volume is dependent on the instrument used to measure fluorescence. With the Beckman DTX 800/880 Multimode Detectors the assay volume is reduced to 200 μ l and 2000 assays.

Preparation of a DNA Standard Curve

Prepare a 2 μ g/ml stock solution of dsDNA in TE. For a standard curve, bacteriophage lambda or calf thymus DNA 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 plasmid DNA. However, most linear dsDNA molecules yield approximately equivalent signals, regardless of fragment length. To create a high range five-point standard curve from 1 ng/mL to 1 μ g/mL refer to Table 1. For a low range standard curve from 25 pg/mL to 25 ng/mL, prepare a 40-fold dilution of the 2 μ g/mL DNA solution to yield a 50 ng/ml DNA stock solution and dilute as indicated in Table 2. The standard curves for 96 well plates are shown in Figures 1 and 2. Figure 3 shows pipetting schemes as well as low and high range standard curves for 384 well plates.

Protocol for preparing a high-range standard curve				Protocol for preparing a low-range standard curve			
Volume (μ L) of 2 μ g/mL DNA Stock	Volume (μ L) of TE	Volume (μ L) Diluted PicoGreen Reagent	Final DNA Concentration in PicoGreen Assay	Volume (μ L) of 50 ng/mL DNA Stock	Volume (μ L) of TE	Volume (μ L) Diluted PicoGreen Reagent	Final DNA Concentration in PicoGreen Assay
100	0	100	1 μ g/mL	100	0	100	25 ng/mL
10	90	100	100 ng/mL	10	90	100	2.5 ng/mL
1	99	100	10 ng/mL	1	99	100	250 pg/mL
0.1*	99.9*	100	1 ng/mL	0.1*	99.9*	100	25 pg/ml
0	100	100	blank	0	100	100	blank
Table 1				Table 2			

** The manual pipetting of 0.1 μ l or 99.9 μ l is not recommended because of the inaccuracy of pipetting volumes. Therefore, take 1 μ l of the stock solution, make a 1:10 dilution and pipette 1 μ l of this dilution instead of 0.1 μ l of the stock.*

After addition of the PicoGreen working solution to all wells, mix thoroughly and incubate for 2 to 5 minutes at room temperature, protected from light.

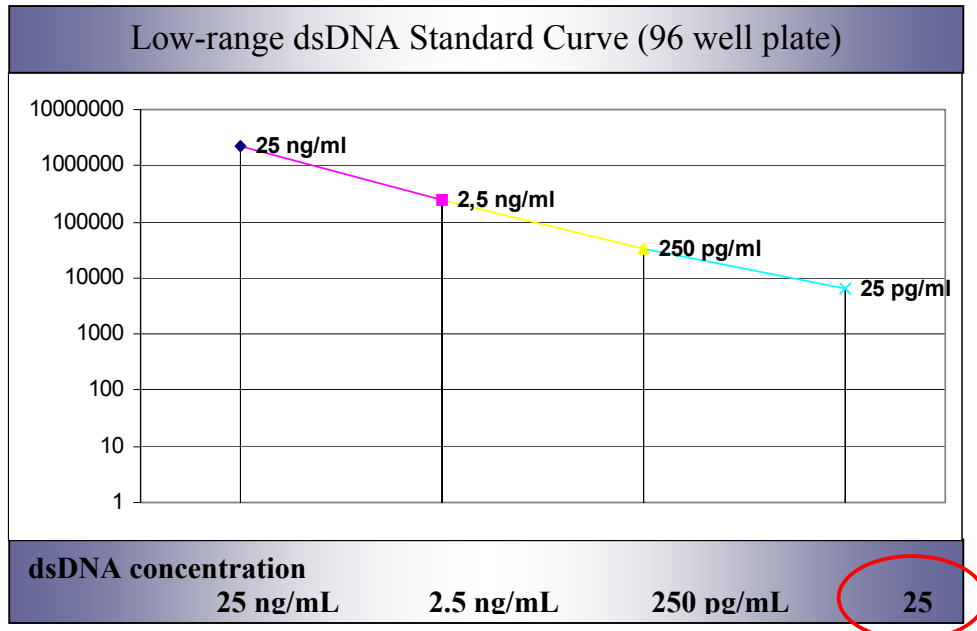


Fig. 1

Note: 25 pg/ml dsDNA concentration is detectable in a 200 µl assay volume (5 pg dsDNA in 200 µL) and delivers fluorescence counts 6.3 % above background level.

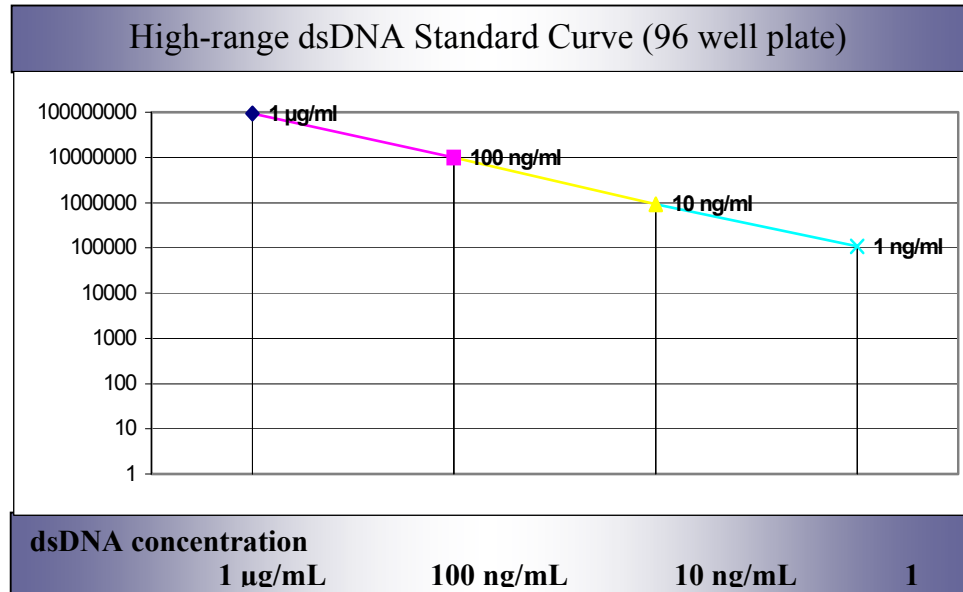


Fig. 2

dsDNA Concentration	dsDNA Volume	PicoGreen*	End Volume	dsDNA End Concentration
50 ng/ml	40 µl	40 µl	80 µl	25 ng/ml 2 ng dsDNA (80 µl)
1:10 dilution	40 µl	40 µl	80 µl	2.5 ng/ml 200 pg dsDNA (80 µl)
1:100 dilution	40 µl	40 µl	80 µl	250 pg/ml 20 pg dsDNA (80 µl)
1:1000 dilution	40 µl	40 µl	80 µl	25 pg/ml 2 pg dsDNA (80 µl)

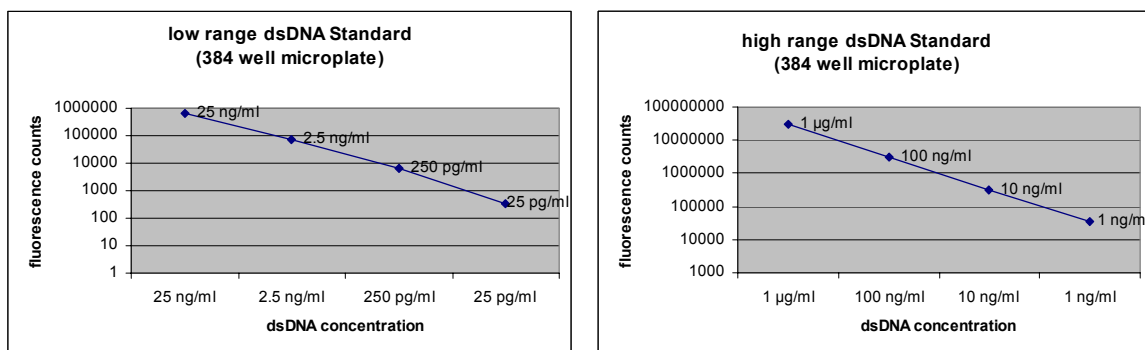


Figure 3: Standard curves and pipetting scheme for 384 well plates

Reading Parameters

After incubation, measure the sample fluorescence using the Beckman Coulter DTX 800/880 Multimode Detector and Multimode Detection Software with the appropriate detection method, excitation filter (485nm), emission filter (535nm), and integration time (1 second) as per Figure 4. The DTX 800/880 also offers the capability of detecting fluorescence over many cycles of repeated detections, which enables fluorescence kinetic measurements in special applications.

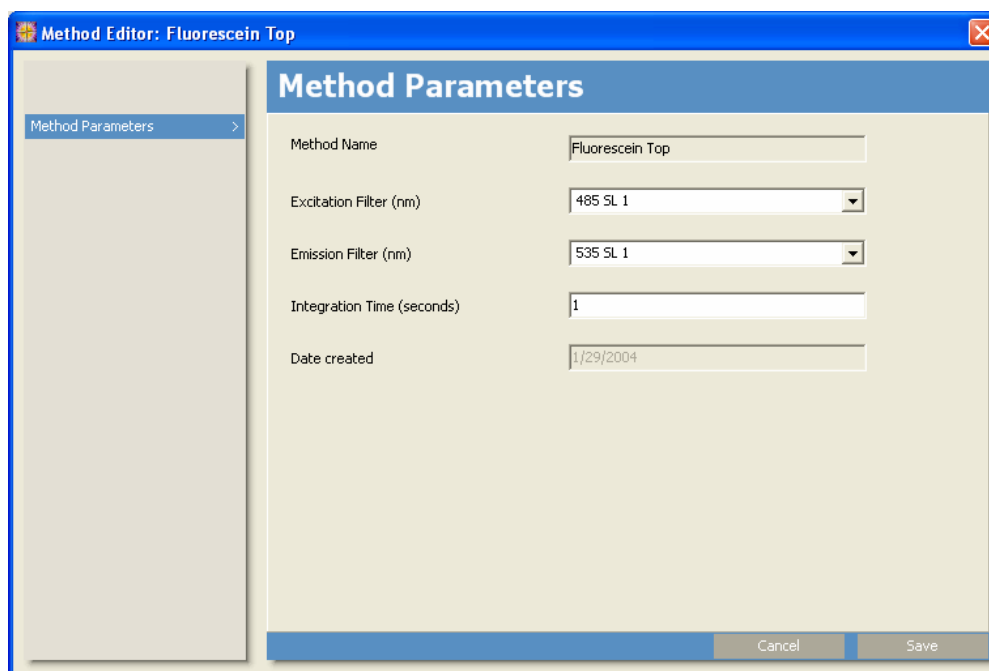


Fig. 4: Software Settings and Reading Parameters

Sample Analysis

Dilute the experimental DNA solutions in TE to a final volume of 100 µl each in a 96-well microplate. You may alter the amount of sample diluted, provided that the final volume remains 100µl per sample. However, extremely small sample volumes should be avoided because they are difficult to manually pipet accurately. After diluting your probe, mix with 100 µl PicoGreen (200-fold working dilution). Incubate the mixture 2-5 minutes in the dark and measure the fluorescence with the DTX 800/880 Multimode Detector.

Eliminating Single-Stranded Nucleic Acids from Samples

dsDNA can be quantitated in the presence of equimolar concentrations of single-stranded nucleic acids with minimal interference. Fluorescence due to PicoGreen reagent binding to

RNA at high concentrations can be eliminated by treating the sample with DNase-free RNase.¹ 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.²

Results Discussion

Reliable, high precision assay data produced by the DTX 800/880 in combination with PicoGreen reagent, allows you to go beyond detection limits of 250 pg/ml (50 pg in a 200 µL assay using a standard fluorescence microplate reader and PicoGreen) as stated by Molecular Probes (Product Information MP07581).

The detection limit of dsDNA for the DTX 880 in 96 well plates is 25 pg/200 µl; in 384 well plates 2pg/80 µl can be detected. The detection limit of dsDNA for the DTX 800 in 96 well plates is 50pg/200µl; in 384 well plates 10 pg/80 µl can be detected. Due to focusing optics, the performance of the DTX 880 is increased compared to the DTX 800. Since the fluorescence signal at the detection limit of 25 pg/ml is 6.3 % above background signal, scientists can detect as little as 5 pg dsDNA in a 200 µl assay volume (Fig. 1). CV values of replicates for standard curves (for 96 and 384 well plates) are around and below 5% (Table 3).

	1 µg/ml	100 ng/ml	10 ng/ml	1 ng/ml
High range 384 microplate	3.6%	2.1%	2.7%	1.8%
High range 96 microplate	5.0%	1.4%	2.5%	0.2%
	25 ng/ml	2.5 ng/ml	250 pg/ml	25 pg/ml
Low range 384 microplate	3.3%	1.6%	2.4%	1.4%
Low range 96 microplate	0.7%	0.3%	5.2%	0.4%

Table 3: CV values calculated by using 3 replicates

The PicoGreen assay shows a linear detection range over more than four orders of magnitude in a DNA concentration - from 25 pg/mL to 1000 ng/mL - with a single dye concentration. We also demonstrated a linear detection range over four orders of magnitude with a single dye concentration, however, in a minimal assay volume of 200 µl. High-throughput dsDNA measurements are therefore feasible using the DTX 800/880 in combination with PicoGreen dye and 96-well microplates. Using the PicoGreen dsDNA quantitation reagent and the recommended assay protocol in a DTX 800/880 Multimode Detector, researchers can quantitate low levels of dsDNA in the presence of equimolar concentrations of ssDNA and RNA with minimal effect on the quantitation results.

Warnings and Precautions

We must caution that no data are available addressing the mutagenicity or toxicity of PicoGreen dsDNA quantitation 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. As with all nucleic acid reagents, solutions of PicoGreen reagent should be poured through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dye.

The PicoGreen dsDNA Quantitation Reagent is the subject of patent applications filed by Molecular Probes, Inc. and is not available for resale or other commercial uses without a specific agreement from Molecular Probes, Inc. PicoGreen is a registered trademark of Molecular Probes, Inc.

References

1. BioTechniques 20, 676 (1996);
2. Sambrook, J., Fritsch, E.F. and Maniatis, T., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press (1989).