The *Pico*fluor™ Method for DNA Quantitation Using PicoGreen®

1. INTRODUCTION

PicoGreen dsDNA Quantitation Reagent is an ultra-sensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in molecular biological procedures such as cDNA synthesis for library production and DNA fragment purification for subcloning, as well as diagnostic applications, such as quantitating DNA amplification products[1,2] and primer extension assays.[3,4]

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, single-stranded nucleic acids and proteins 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 (bis-benzimide) 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 5.0 ng/mL DNA.[5]

The *Pico*fluor Handheld Fluorometer used in conjunction with Molecular Probes' PicoGreen dsDNA Quantitation Reagent enables researchers to quantitate as little as 1 ng/mL of dsDNA. This sensitivity exceeds that achieved with the Hoechst 33258. The standard PicoGreen assay protocol is also simpler than the Hoechst 33258 method, because a single concentration of the PicoGreen Reagent allows detection over the full dynamic range of the assay. In order to obtain the full dynamic range with Hoechst-based assays, two different dye concentrations are recommended. In contrast, the linear detection range of the PicoGreen assay in the *Pico*fluor Fluorometer extends four orders of magnitude in DNA concentration - from 1 ng/mL to 1000 ng/mL with a single dye concentration (see Figures 1 and 2). This linearity is maintained in the presence of several compounds commonly found to contaminate nucleic acid preparations, including salts, urea, ethanol, chloroform, detergents, proteins and agarose. The assay protocol has been developed to minimize the fluorescence contribution of RNA and single-stranded DNA (ssDNA). Using the PicoGreen dsDNA Quantitation Reagent and the *Pico*fluor Fluorometer, researchers can quantitate dsDNA in the presence of equimolar concentrations of ssDNA and RNA with minimal effect on the quantitation results.

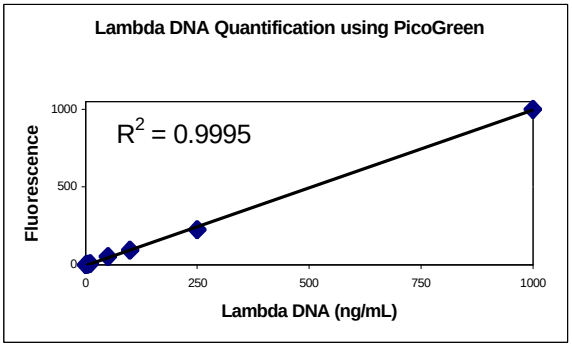

Figure 1. *DNA Standard calibration plot.*

2. MATERIALS REQUIRED

- *Pico*fluor™ Fluorometer with blue optical configuration (P/N 8000-003 or 8000-004)
- 10 × 10 mm methacrylate fluorescence cuvettes (P/N 7000-959)
- PicoGreen dsDNA Quantitation Reagent, supplied by Molecular Probes, Inc., Eugene, Oregon, catalog number P-7581. A single 1-

mL unit of the reagent concentrate is sufficient for 200 assays using an assay volume of 2 mL and the protocol described in section 3. Handling, storage and use of the reagent should be performed in accordance with the product information sheet supplied by Molecular Probes, Inc.

3. EXPERIMENT PROTOCOL

3.1 Reagent Preparation

The PicoGreen dsDNA Quantitation Reagent is supplied as a 1-mL concentrated dye solution in anhydrous dimethylsulfoxide (DMSO). On the day of the experiment, prepare an aqueous working solution of the PicoGreen Reagent by making a 1:200 dilution of the concentrated DMSO solution in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5 (TE). To prepare enough working solution to assay 20 samples, add 100 µL PicoGreen dsDNA Quantitation Reagent to 20.0 mL TE. Preparing this solution in a plastic container is recommended, 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.***

3.2 DNA Standard Curve

3.2.1 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. Calf thymus DNA is commonly used for a standard curve, although any purified dsDNA preparation may be used. It is preferable to prepare the standard curve with DNA similar to the type being assayed; long or short linear DNA fragments for quantitating similar-sized restriction fragments; plasmid for quantitating plasmid DNA. However, most linear dsDNA molecules have been found to yield approximately equivalent signals, regardless of fragment length. The PicoGreen assay remains linear in the presence of several compounds that commonly contaminate nucleic acid preparations, although the signal intensity may be affected. 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 generate a single-replicate, five-point standard curve from 1 ng/mL to 1000 ng/mL, proceed to step 3.2.2.

Note: Only one standard is necessary for proper calibration. Additional concentrations are optional and may be useful for checking the linearity of the assay.

3.2.2 For the DNA standard curve, dilute the 2 µg/mL DNA stock solution into acrylic cuvettes as shown in Table 1. Then add 1.0 mL of the aqueous working solution of PicoGreen Reagent (prepared in section 3.1) to each cuvette. Mix well and incubate for 2 to 5 minutes at room temperature, protected from light.

Volume (µL) of 2 µg/mL DNA Stock	Volume (µL) of TE	Volume (µL) of Diluted PicoGreen Reagent	Final DNA Concentration in PicoGreen Assay
1000	0	1000	1000 ng/mL
500	500	1000	500 ng/mL
100	900	1000	100 ng/mL
10	990	1000	10 ng/mL
1	999	1000	1ng/mL
0	1000	1000	blank

Table 1. *Protocol for preparing standard curve.*

3.2.3 After incubation calibrate the *Pico*fluor Fluorometer. Press “STD VAL” and enter “999” for the highest standard (1000 ng/mL).Press the “CAL” button and then “Enter”. Next, insert blank from chart above and press “Enter”. After blank is read insert the most fluorescent sample (1 µg/mL DNA) and press “Enter”. After the standard is read, press “Enter”.

3.2.4 Measure the fluorescence of the remaining samples***. To equalize any photobleaching effects, insert samples into the fluorometer for approximately equal time periods.*** The readout is the actual concentration of the sample as it relates to the standard unit of measure (ng/mL).

4. REFERENCES

1. Nucleic Acids Res. 24, 2623 (1996)
2. BioTechniques 21, 372 (1996)
3. BioTechniques 21, 664 (1996)
4. Proc. Natl. Acad. Sci. USA 93, 6091 (1996)
5. Anal. Biochem. 102, 344 (1980)
6. *Molecular Cloning: A Laboratory Manual, Second Edition*, J. Sambrook, E.F. Fritsch and T. Maniatis, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

5. WARNINGS AND PRECAUTIONS

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.

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