A Modulus™ Method for DNA Quantitation Using PicoGreen®

1. INTRODUCTION

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

The Modulus™ Single Tube Fluorometer from Turner BioSystems in combination with PicoGreen® dsDNA reagent allows the direct quantitation of dsDNA in as little as 100 µL total volume. The limit of detection for the Modulus™ Single Tube is 45 pg DNA in 100 µL.

The linear detection range of the PicoGreen assay in the Modulus™ Single Tube extends for nearly four orders of magnitude in DNA concentration (Figure 1). 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 minimizes the fluorescent contribution of RNA and single-stranded DNA (ssDNA). Using the PicoGreen dsDNA Quantitation Reagent and the Modulus Fluorometer, researchers may analyze dsDNA in the presence of equimolar concentrations of ssDNA and RNA with minimal effect on the quantitative results.

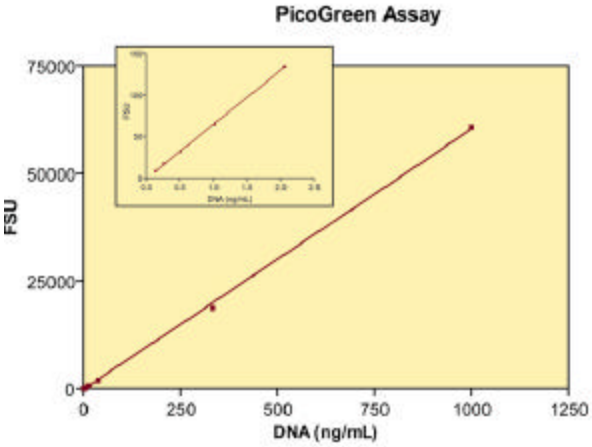

Figure 1. *dsDNA and PicoGreen analyzed using the Modulus and the Blue Fluorescence Optical Kit. 2000 ng/mL of lamda dsDNA was serially diluted in 1XTE before the addition of the PicoGreen working solution. After a 5-minute equilibration period, 100 µL of each sample was transferred to a minicell cuvette. Triplicates of each dilution were read on the Modulus ä Single Tube using the Blue Fluorescence Optical Kit and the Minicell Adaptor.*

2. MATERIALS REQUIRED

- Modulus™ Single Tube Fluorometer (P/N 9200-000 or 9200-002)
- Blue Fluorescence Optical Kit (P/N 9200-040)
- PicoGreen dsDNA Quantitation Reagent (Molecular Probes, P-7581)
- 10 x 10 Methacrylate Cuvettes (P/N 7000-959)

Or

- Minicell Cuvettes (P/N 7000-950) and Minicell Adaptor (P/N 9200-928)

3. EXPERIMENTAL PROTOCOL

3.1 Reagent Preparation

NOTE: Handling, storage and use of the reagent should be performed in accordance with the product information sheet supplied by Molecular Probes, Inc.

Determine the amount of total 1XTNE needed for the assay. Dilute 20XTNE to 1XTNE.
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 a 2X working solution of the PicoGreen Reagent by making a 1:200 dilution of the concentrated dye solution in 1XTE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). Preparing this solution in a plastic container 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. The PicoGreen Reagent is susceptible to photodegradation.

NOTE: For best results, this solution should be used within a few hours of its preparation.

3.2 Instrument Set-Up

3.2.1 Power the Modulus™ OFF. Insert the Blue Fluorescence Optical Kit according to the *Modulus Operating Manual*.

3.2.2 Turn ON the Modulus. Allow the Modulus a 5-minute warm up period before calibration.

3.3 Calibration

3.3.1 Prepare a 2 µg/mL stock solution of dsDNA in 1XTE. 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.

3.3.2 Prepare the standard solution. Add equal volume of the DNA stock solution to 2XPicoGreen working solution. Mix well. The final concentration of the standard is 1000 ng/mL.

NOTE: It is possible but not necessary to calibrate the Modulus™ Single Tube with as many as 5 standards.

3.3.3 Prepare the blank solution. Add equal volume the sample buffer (usually 1XTE without DNA) to 2XPicoGreen working solution. Mix well.

3.3.4 Prepare the samples. Add equal volumes of the sample to 2XPicoGreen working solution. Mix well.

NOTE: Do not mix standards or samples with PicoGreen in the minicell cuvette. Instead, mix the dye and the samples in a separate microfuge tube.

3.3.5 Transfer the mixed sample-dye solution to the appropriate cuvette. For 10 x 10 mm methacrylate cuvettes, the minimum volume is 2 mL. For the minicell cuvette, the minimum volume is 75 µL. Incubate for 2–5 minutes at room temperature, protected from light**.**

NOTE: Do not introduce air bubbles into the cuvette during the transfer.

3.3.6 Calibrate the Modulus with 1000 ng/mL.

NOTE: To optimize the accuracy with a single-point calibration, use a standard that is at or near the concentration of a typical sample. For example, if a typical sample is 300 ng/mL DNA, use a standard of 500 ng/mL DNA.

3.3.7 Save the calibration for future use (optional).

3.4 Sample Analysis

3.4.1 Insert the sample into the Modulus™ Single Tube and press “Measure Fluorescence.”

NOTE: It is not necessary to run a standard curve after calibration. All subsequent readings will report in ng/mL final DNA concentration.

3.4.2 The final concentration of the sample appears on the touchscreen.

NOTE: Remember the final concentration is at least half of the sample concentration because of the 1:1 addition of the PicoGreen dye. In some cases, additional calculations are necessary to account for sample dilution. For an example, see Table 1.

Sample (µL)	Diluent (µL)	Sample Dilution Factor	2X PicoGreen dye (µL)	Dye dilution factor	Reading (ng/mL)	Actual Sample Conc. (ng/mL)
10 x 10 mm Square Cuvette						
5	995	1:200	1000	1:2	410	164,000
10	990	1:100	1000	1:2	125	25,000
2	998	1:500	1000	1:2	740	740,000
Minicell Cuvette						
2	48	1:25	50	1:2	60	3,000
5	95	1:20	100	1:2	290	11,600
10	90	1:10	100	1:2	852	17,040

Table 1. *The effect of various dilution factors on the fluorescent reading. For example only.*

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. PATENTS AND TRADEMARKS

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.

Modulus is a trademark of Turner BioSystems, Inc.

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