A 20/20^n 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.

Turner BioSystems offers a unique Fluorescent Module for the 20/20^n Luminometer that enables fluorometric analysis. The Blue Fluorescent Module in combination with PicoGreen® dye allows the direct quantitation of dsDNA in as little as 100 µL total volume. The limit of detection for the 20/20^n is less than 45 pg in 100 µL total volume.

The linear detection range of the PicoGreen® assay in the 20/20^n extends over three orders of magnitude in DNA concentration - from 450 pg/mL to 1000 ng/mL (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 fluorescence contribution of RNA and single-stranded DNA (ssDNA). Researchers can quantitate dsDNA in the presence of equimolar concentrations of ssDNA and RNA with minimal effect on the quantitative results.

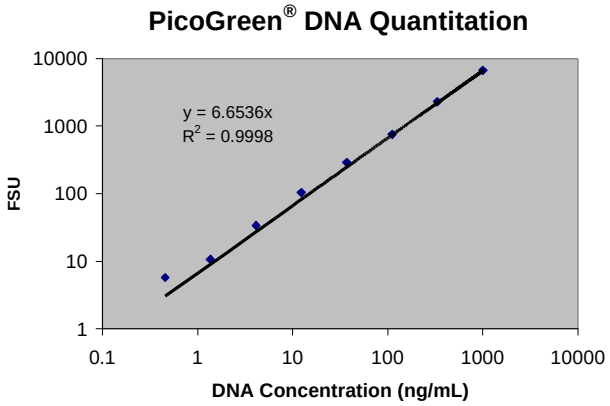

Figure 1. dsDNA and PicoGreen® analyzed on the 20/20^n. 2 µg/mL DNA was serially diluted in 1xTE before the addition of 2x PicoGreen® working solution. After a 5 minute incubation, 100 µL of each sample was transferred to a minicell cuvette and read using the Blue Fluorescent Module.

2. MATERIALS REQUIRED

- 20/20^n Luminometer (P/N 2030-000, 2030-001, 2030-002)
- 20/20^n Blue Fluorescent Module (P/N 2030-041)
- Minicell Cuvettes (P/N 7000-950)
- PicoGreen® dsDNA Quantitation Reagent (Molecular Probes, P-7581)

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.

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 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.

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

3.2 Instrument Set-Up

3.2.1 With the 20/20^n powered OFF, insert the Blue Fluorescent Module according to the operating instructions.

3.2.2 Turn ON the 20/20^n. Allow the 20/20^n 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 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 an equal volume of the DNA stock solution from step 3.2.1 to 2x PicoGreen® working solution, prepared in step 3.1. Mix well in a microcentrifuge tube.

3.3.3 Prepare the blank solution. Add an equal volume of the sample buffer (usually 1xTE without DNA) to 2x PicoGreen® working solution in a separate microcentrifuge tube.

3.3.4 Prepare the samples. Add equal volumes of the sample to 2x PicoGreen® working solution in a separate microcentrifuge tube.

NOTE: Do not mix samples and PicoGreen® in the minicell cuvette.

3.3.5 Transfer 100 µL of the each sample, standard, and blank solution to a minicell cuvette. Incubate for 2-5 minutes at room temperature, protected from light.

NOTE: Do not introduce air bubbles in the minicell cuvette. Air bubbles cause erroneous readings.

3.3.6 Touch “Calibrate” and select ng/mL for the unit of measure.

3.3.7 Using the number pad, enter 1000 for the standard concentration.

NOTE: If you desire to measure picogram levels of DNA, please select pg/mL for the unit of measure. Dilute the DNA stock solution from 3.3.1 10-fold to 200 ng/mL. Add equal volume to PicoGreen®. Enter 100,000 (pg/mL) for the standard concentration.

3.3.8 Insert the minicell cuvette containing the blank solution into the Fluorescent Module. Touch “OK” to start the calibration readings.

3.3.9 Insert the minicell cuvette containing the standard solution into the Fluorescent Module. Touch “OK” to finish the calibration reading.

3.3.10 Measure the sample solutions.

NOTE: It is not necessary to run a standard curve after calibration. All subsequent readings will report in ng/mL final DNA concentration. Remember the final concentration is half of the sample concentration because of the 1:1 addition of the PicoGreen® dye.

3.4 Eliminating Single-Stranded Nucleic Acids from Samples

Double-stranded DNA can be quantitated in the presence of equimolar concentrations of single-stranded nucleic acids with minimal interference. A 10-fold excess of RNA over dsDNA generally produces no more than a 10% change in the fluorescence signal. Somewhat larger distortions are produced by ssDNA, particularly at low DNA concentrations (see Molecular Probes' product information sheet MP7581 for more details). Fluorescence due to PicoGreen® Reagent binding to RNA at high concentrations can be eliminated by treating the sample with DNase-free RNase.[6] 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.[6]

4. REFERENCES

1. *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.

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