A TBS-380 Mini-Fluorometer 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 conventional technique for measuring nucleic acid concentrations is the determination of absorbance at 260 nm (A_{260}). The major disadvantages of the absorbance method are the relative large 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 10 ng/mL DNA.[5]

The Turner BioSystems TBS-380 Mini-Fluorometer used in conjunction with Molecular Probes' PicoGreen® dsDNA Quantitation Reagent enables researchers to quantitate as little as 500 pg/mL of dsDNA in 2 mL assay volume. When the Minicell adaptor is used, researchers can quantitate 50 pg dsDNA in a 2 µL assay volume. This sensitivity exceeds that achieved with the Hoechst 33258 - based assay by 20-fold.

The linear detection range of the PicoGreen® assay in the TBS-380 Mini-Fluorometer extends over three orders of magnitude in DNA concentration - from 500 pg/mL to 500 ng/mL - with a single dye concentration (see figures). 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 TBS-380 Mini-Fluorometer, researchers can quantitate dsDNA in the presence of equimolar concentrations of ssDNA and RNA with minimal effect on the quantitative results.

2. MATERIALS REQUIRED

- TBS-380 Mini-Fluorometer (P/N 3800-003).
- 10 × 10 mm square polystyrene disposable cuvettes (P/N 7000-957).
- Minicell Adaptor Kit (P/N 3800-928).
- 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. EXPERIMENTAL 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 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). To prepare enough working solution to assay 20 samples, add 100 µL PicoGreen® dsDNA Quantitation Reagent to 20.0 mL of 1xTE. 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 1 µg/mL stock solution of dsDNA in 1xTE. 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 0.02 corresponds to 1 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.

3.2.2 To generate a single-replicate, eight-point standard curve from 0.5 ng/mL to 500 ng/mL (as shown in Table 1), prepare a series of DNA solution at 2X final concentration, mix equal volume of the 2X DNA solution with the 2X PicoGreen® working solution into 10x10 mm disposable cuvettes. For blank, mix equal volume of 1X TE with the 2X PicoGreen® working solution. Incubate for 2 to 5 minutes at room temperature, protected from light.

2X DNA solution concentration (ng/mL)	Volume of the 2X DNA solution (mL)	Volume of the 2X PicoGreen® solution (mL)	Final DNA concentration in PicoGreen® Assay (ng/mL)
1000	1	1	500
200	1	1	100
50	1	1	25
20	1	1	10
5	1	1	2.5
2	1	1	1
1	1	1	0.5
0	1	1	blank

Table 1. *DNA standard curve for* ***10x10 mm cuvette****.*

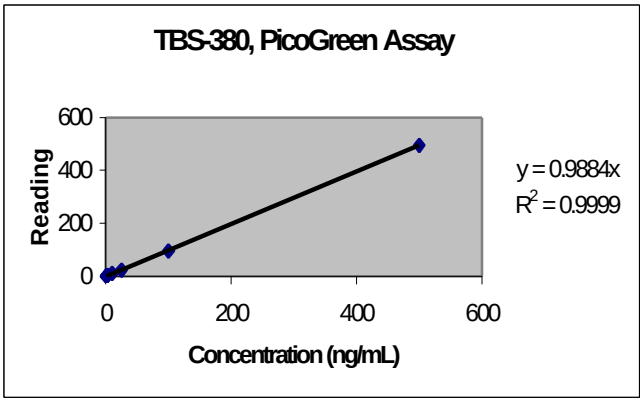
3.2.3 PicoGreen® Assay can also be performed in lower assay volume when Minicell Adaptor is used. The assay volume ranges from 50 µL to 200 µL. To generate standard curve from 1 ng/mL to 100 ng/mL (as shown in Table 2), prepare a series of DNA solution at 2X final concentration, mix equal volume of the 2X DNA solution with the 2X PicoGreen® working solution. Mix well and transfer at least 50 µL of the mix to the Minicell cuvette. Be sure not to introduce any air bubbles in your samples. Slight tapping on the outside cuvette wall will often help dissipate bubbles. Incubate for 2 to 5 minutes at room temperature, protected from light.

2X DNA solution concentration (ng/mL)	Volume of the 2X DNA solution (µL)	Volume of the 2X PicoGreen® solution (µL)	Final DNA concentration in PicoGreen® Assay (ng/mL)
200	30	30	100
50	30	30	25
20	30	30	10
5	30	30	2.5
2	30	30	1
0	30	30	blank

Table 2. *DNA standard curve for* ***Minicell cuvette.***

3.2.4 After incubation, measure the sample fluorescence in the TBS-380 Mini-Fluorometer. Select the BLUE channel. Calibrate the instrument with the most fluorescent sample.

3.2.5 Measure the fluorescence of the remaining samples**.** The TBS-380 Mini-Fluorometer will give a direct concentration read out, and data may be used to generate a standard curve of reading versus DNA



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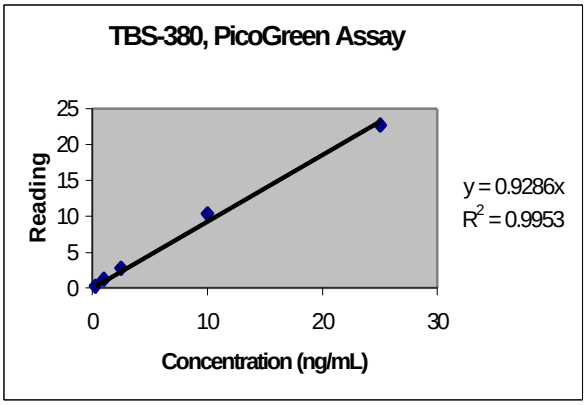

Figure 1A . *PicoGreen® Standard plot*

Figure 1B: *Enlargement of lower left segment of Figure 1A*

Figures 1A and 1B: PicoGreen Assay for quantitation of l DNA on the TBS-380 Mini-Fluorometer. Figure 1B shows an enlargement of the area in the lower left corner of Figure 1A.

3.3 Sample Analysis

3.3.1 Dilute the unknown DNA samples in 1X TE to a desired volume (1.0 mL for 10x10mm cuvette or 25-100 µL for Minicell). A higher dilution of the experimental sample will ensure that any contaminants are maximally diluted. However, extremely small sample volumes should be avoided because they are difficult to pipet accurately. See Section 3.4 for information on eliminating RNA and ssDNA from the sample.

3.3.2 Add equal volume of the 2X working solution of the PicoGreen® Reagent (prepared in section 3.1) to each sample, mix well and transfer the mix to the proper cuvette. Incubate for 2 to 5 minutes at room temperature, protected from light.

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

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

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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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