A Modulus™ Microplate Fluorometer Method for DNA Quantitation Using

Quant-iT™ PicoGreen® dsDNA Kit

1. INTRODUCTION

The Quant-iT™ PicoGreen® dsDNA Reagent is an ultra-sensitive 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]

Use of the Modulus™ Microplate Fluorometer from Turner BioSystems in combination with Quant-iT™ PicoGreen® dsDNA Reagent allows for direct quantitation of dsDNA in small volume microplates (200 µL per well). The limit of detection for the Modulus™ Microplate is 50 pg DNA in 200 µL.

The linear detection range of the Quant-iT™ PicoGreen® assay in the Modulus™ Microplate 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. These contaminates may include: salts, urea, ethanol, chloroform, detergents, proteins, and agarose. The assay protocol minimizes the fluorescent contribution of RNA and single-stranded DNA (ssDNA). By using Quant-iT™ PicoGreen® dsDNA Reagent in conjunction with the Modulus™ Microplate Fluorometer, researchers may analyze dsDNA in the presence of equimolar concentrations of ssDNA and RNA with minimal effect on the quantitative results.

2. MATERIALS REQUIRED

- Modulus™ Microplate Multimode Reader
- Fluorescence Optical Kit - Blue, 490/515 - 580 nm
- Black 96-well microplates, FluoTrac 200 (E&K Scientific, EK-25076)
- Quant-iT™ PicoGreen® dsDNA Reagent Kit (Molecular Probes, Inc., P-7581) including:
  - **Quant-iT™ PicoGreen® ssDNA Reagent**, (Component A) 1 mL solution in DMSO
  - **20X TE**, (Component B) 25 mL of 200 mM Tris-HCL, 20 mM EDTA, pH 7.5
  - **Lambda DNA Standard,** (Component C) 1 mL of 100 µg/mL in TE

Note: 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 Assay Buffer Preparation

TE assay buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) is used for diluting Quant-iT™ PicoGreen® dsDNA Reagent and samples of dsDNA. Since Quant-iT™ PicoGreen® is highly sensitive, it is of the utmost importance that the TE assay buffer be free of all contaminating nucleic acids. The 20X TE buffer included in the Quant-iT™ OliGreen® ssDNA Quantitation Kit is certified to be free of nuclease and nucleic acids.

Prepare the 1X TE working solution by diluting the concentrated buffer 20-fold with nuclease-free water. Dilute 1 mL of 20X TE in 19 mL of water to make enough assay buffer for one 96-well plate.

3.2 Reagent Preparation

The Quant-iT™ PicoGreen® dsDNA 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 Quant-iT™ PicoGreen® dsDNA Reagent; make a 1:200 dilution of the concentrated dye solution in 1X TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). Use a plastic container for solution preparation as the reagent may adsorb to glass surfaces. Protect the working solution from light by covering it with foil or by keeping it in a dark environment; Quant-iT™ PicoGreen® dsDNA Reagent is susceptible to photodegradation.

Note: For best results, use this solution within a few hours of preparation.

3.3 DNA Standard Curve

1. Prepare a 2-µg/mL stock solution of dsDNA in 1X TE. Bacteriophage lambda or calf thymus DNA are commonly used for determining 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. For example, use long or short linear DNA fragments for quantitating similar-sized restriction fragments, or plasmid for quantitating plasmid DNA. However, most linear dsDNA molecules have been found to yield approximately equivalent signals, regardless of fragment length. In the presence of several compounds that commonly contaminate nucleic acid preparations, Quant-iT™ PicoGreen® assay remains linear, although signal intensity may be affected. Thus, to serve as an effective control, any dsDNA solution used for standard curve preparation should be treated the same way as the experimental samples and contain similar compound levels.

2. For the high-range standard curve, dilute the 2-µg/mL DNA solution as shown in Table 1. For the low-range standard curve, first dilute the 2-µg/mL DNA solution 40-fold with TE buffer to make a 50-ng/mL DNA stock solution and use this to prepare the dilutions shown in Table 2.

3. Add 100 µL of each standard to separate wells of a 96-well assay plate.

Note: It is recommended to perform each standard in duplicate or triplicate to accurately determine standard curve.

3.4 Sample Analysis

1. Dilute each experimental DNA solution in TE to a final volume of 100 µL and add to a microtiter plate. It may be useful to prepare several dilutions of each experimental sample. For example, if a series of DNA samples contain widely differing salt concentrations, they cannot be compared to a single standard curve. To avoid this problem, adjust the concentration of contaminants to be the same in all samples, if possible.

2. Add 100 µL of the Quant-iT™ PicoGreen® dsDNA Reagent (detailed in Section 3.2) to each sample. Incubate for 2 - 5 minutes at room temperature, protected from light.

3. Set up the Modulus™ Microplate Fluorometer as per instructions in the *Operating Manual*. Read the assay plate.

4. Subtract the reagent's blank fluorescence reading from each sample. Plot a standard curve of dsDNA concentration against fluorescence (RFU).

5. Using the standard curve, determine the dsDNA concentration of each unknown sample.

Vol. (mL) 2 mg/mL DNA stock	Vol. (mL) of TE	Final dsDNA concentration in PicoGreen® assay
1000	0	1000 (ng/mL)
100	900	100 (ng/mL)
10	990	10 (ng/mL)
1	999	1 (ng/mL)
0	1000	blank

Table 1. Protocol for Preparing High-Range Standard Curve

Vol. (mL) 50 mg/mL DNA stock	Vol. (mL) of TE	Final dsDNA concentration in PicoGreen® assay
1000	0	25 (ng/mL)
100	900	2.5 (ng/mL)
10	990	250 (pg/mL)
1	999	25 (pg/mL)
0	1000	blank

Table 2. Protocol for Preparing Low-Range Standard Curve

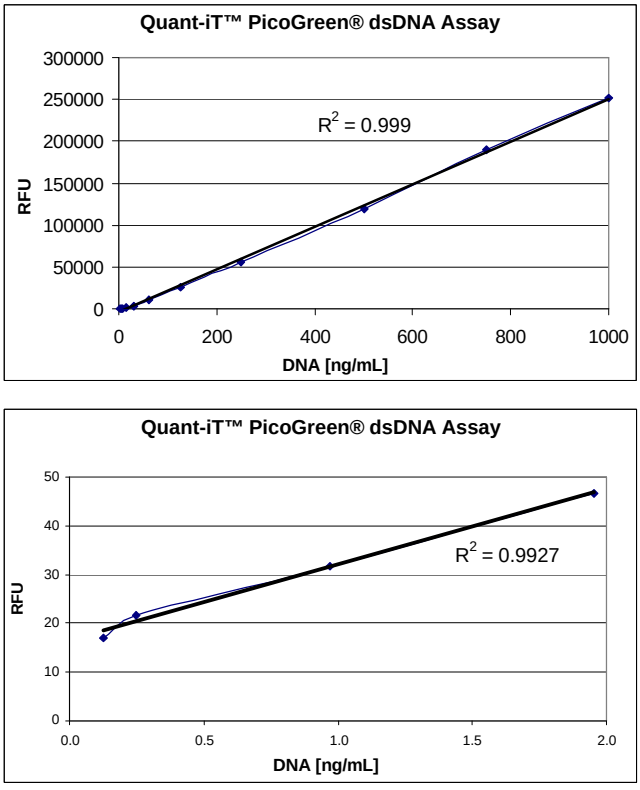

Figure 1. High-range (A) and low-range (B) dsDNA standard curves. The lambda DNA standard assays performed using the Quant-iT™ PicoGreen® dsDNA Reagent and the Modulus™ Microplate Fluorometer with the Blue Fluorescence Optical Kit.

4. RESULTS

Sensitivity:

- < 250 pg/mL

Instrument Dynamic Range:

- Up to six orders of magnitude within dynamic range

Minimum Detection Limit:

- 21 pg/mL dsDNA
- Calculated using 3 x standard deviation of the assay background, n = 24

5. CONCLUSION

The Quant-iT™ PicoGreen® dsDNA Assay Kit makes for an easy, reliable, and accurate method to quantify dsDNA samples when used in conjunction with the Modulus™ Microplate Fluorometer from Turner BioSystems, Inc. The superior sensitivity and performance of the Modulus™ Microplate Fluorometer allows for dsDNA detection as low as 250ng/mL. The Modulus™ Microplate Fluorometer offers both superior sensitivity and dynamic range.

The Modulus™ Microplate Fluorometer achieves superior performance by use of a dedicated fluorescence detector. The detector is not shared with any other detection modes. The individual Fluorescent Optical Kit of the Modulus™ Microplate Fluorometer uses solid-state optics and a powerful wavelength-matched LED to deliver excellent sensitivity and dynamic range.

The modular approach of the Modulus™ Microplate Fluorometer allows for instrument capability expansion as needs in the lab change. Luminescence and/or Absorbance Detection Modules as well as other accessories can be added after initial purchase.

Superior performance, ease of use, and utmost flexibility make the Modulus™ Microplate

Reader an ideal choice for today's life science laboratory.

6. WARNINGS AND PRECAUTIONS

Caution: No data are available addressing the mutagenicity or toxicity of Quant-iT™ PicoGreen® dsDNA Reagent.

Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care.Handle the DMSO stock solution with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling the DMSO stock solution. As with all nucleic acid reagents, solutions of Quant-iT™ PicoGreen® dsDNA Reagent should be poured through activated charcoal before disposal. Incinerate contaminated charcoal to destroy the dye.

REFERENCES

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6. Sambrook, J., Fritsch, E.F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual, Second Edition.*Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 1989.

PATENTS AND TRADEMARKS

The Quant-iT™ PicoGreen® dsDNA 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. Quant-iT™ PicoGreen® is a registered trademark of Molecular Probes, Inc.

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