A Modulus™ Microplate Fluorometer Method for Quantitation of ssDNA Using Quant-iT™ OliGreen® ssDNA Assay Kit

1. INTRODUCTION

Quant-iT™ OliGreen® oligonucleotide reagent is an ultra-sensitive fluorescent nucleic acid stain. Use of OliGreen allows performance of simple and rapid procedures for quantitating oligonucleotides and single-stranded DNA (ssDNA). Short, synthetic oligonucleotides are used in a number of molecular biology techniques, including: DNA sequencing, site-directed mutagenesis, DNA amplification, and *in situ* hybridization.

The most commonly used technique for measuring oligonucleotide and ssDNA concentration is by determining absorbance at 260 nm. Major disadvantages of this method are poor sensitivity and interference in signal levels from contaminating components such as nucleotides, proteins, and salts in the RNA solution. The use of an ultra-sensitive fluorescent nucleic acid stain such as OliGreen® alleviates these problems.

The Turner BioSystems Modulus™ Microplate Fluorometer used in conjunction with the Molecular Probes OliGreen® ssDNA Reagent Kit allows for rapid and accurate measurement of ssDNA concentrations in small-volume microplates (200 µL per well).

As little as 100 pg of ssDNA can be quantitated using the Blue Fluorescent Optical Kit configuration of the Modulus™ Microplate Fluorometer. The linear dynamic range extends over 4 orders of magnitude from 100 pg/mL - 1 µg/mL ssDNA (Figure 1).

2. MATERIALS REQUIRED

2.1. Included Materials

- Modulus™ Microplate Multimode Reader
- Fluorescence Optical Kit - Blue, 490/515-580 nm
- Quant-iT™ OliGreen® ssDNA Assay Kit (O-11492) Molecular Probes, Inc. including:
  - **Quant-iT™ OliGreen® ssDNA reagent** (1 mL solution in DMSO)
  - **20 x TE**, 25 mL of 200 mM Tris-HCL, 20 mM EDTA, pH 7.5
  - **Oligonucleotide standard** 1 mL of 100 µg/mL in TE.

Note: Handling, storage, and usage of reagents should be performed in accordance with the product information sheet supplied by Molecular Probes, Inc.

2.2. Additional Materials Needed

- Nuclease-free water (Ambion, AM9930)
- Black 96-well microplates FluoTrac 200 (E&K Scientific, EK-25076)

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 OliGreen® reagent and samples of oligonucleotide and ssDNA. TE assay buffer must be free of all contaminating nucleic acids. The 20 x TE buffer included in the Quant-iT™ OliGreen® ssDNA Quantitation Kit is free of any nucleaseor nucleic acid. Prepare the 1 x TE working solution by diluting concentrated buffer 20-fold with nuclease-free water.

3.2. Reagent Preparation

The OliGreen® oligonucleotide quantitation reagent is supplied as a 1-mL solution of concentrated dye in anhydrous dimethylsulfoxide (DMSO).

Prepare the assay reagent by making a 1:200 dilution of concentrated OliGreen® reagent into previously-prepared TE assay buffer. To prepare enough working solution to assay one 96-well assay plate, add 50 µL of OliGreen® reagent into 9.95 mL of TE assay buffer.

Prepare this solution in a plastic container, as the reagent may adsorb to glass surfaces. The working solution of OliGreen® reagent must be protected from light with foil or by placing it in the dark to prevent photo degradation. For best results, use the solution within a few hours of preparation.

3.3. Oligonucleotide Standard Curve

3.3.1. To make a 2-µg/mL working solution, use the 100-µg/mL oligonucleotide standard as provided in the OliGreen® ssDNA Quantitation Kit and dilute it 50-fold in TE assay buffer. For example, 40 µL of the oligonucleotide standard mixed with 1.96 mL of TE assay buffer will be sufficient for the standard curve described in Table 1.

3.3.2. For the high-range standard curve, dilute the 100-µg/mL oligonucleotide standard 50-fold in TE assay buffer to make a 2-µg/mL working solution. Dilute this solution according to the examples shown in Table 1. For the low-range standard curve, first dilute the 2-µg/mL oligonucleotide solution 20-fold with TE assay buffer to make a 100 ng/mL oligonucleotide stock solution. Then use this to prepare the dilutions shown in Table 2. Add 100 µL of each standard to separate wells of a 96-well assay plate. It is recommended to obtain duplicates or triplicates of each standard for best results in determining the accuracy of the standard curve.

3.3.3. Add to each well 100 µL of the appropriate aqueous working solution of OliGreen® reagent, as prepared in Section 3.2.

3.3.4. Set up the Modulus™ Microplate Fluorometer as per instructions in the *Operating Manual* and read the assay plate.

3.3.5. Subtract the fluorescence value of the reagent blank from that of each sample. Use corrected data to generate a standard curve of fluorescence verses oligonucleotide concentrations.

Vol. (uL) 2-ug/mL RNA stock	Vol. (uL) of TE	Final oligonucleotide concentration in OliGreen® assay
1000	0	1000 (ng/mL)
500	500	500 (ng/mL)
100	900	100 (ng/mL)
10	990	10 (ng/mL)
0	1000	Blank

Table 1. Protocol for Preparing High-Range Standard Curve

Vol. (uL) 100-ng/mL of Oligomer stock	Vol. (uL) of TE	Final oligonucleotide concentration in OliGreen® assay
1000	0	50 (ng/mL)
100	900	5 (ng/mL)
10	990	500 (pg/mL)
2	998	100 (pg/mL)
0	1000	Blank

Table 2. Protocol for Preparing Low-Range Standard Curve

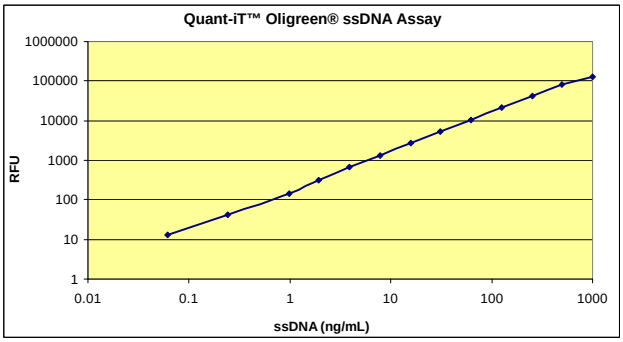

Figure 1. Linear range of standard assay performed using OliGreen® ssDNA quantitation reagent and the Modulus™ Microplate Fluorometer.

3.4. Sample Analysis

3.4.1. Dilute each experimental oligonucleotide solution in TE assay buffer to a final volume of 100 uL and add to microtiter plate. You may wish to prepare more than one dilution of each experimental sample.

3.4.2. Add to each sample 100 uL of the OliGreen® reagent as prepared in Section 3.2. Incubate for two - five minutes at room temperature, protected from light.

3.4.3. Measure the fluorescence of the samples. Use the same instrument conditions that were used to generate the standard curve (see Section 3.3.).

3.4.4. If the standard curve has been constructed from background-subtracted data (see Section 3.3.), subtract the reagent blank fluorescence reading from that of each sample.

3.4.5. Determine the oligonucleotide concentration of each sample from the standard curve as generated in Section 3.3.

4. REFERENCE

1. Molecular Probes Data Sheet MP 7582 07/01/2005: Quant-iT™ Oligreen® ssDNA Reagent Kit.

5. RESULTS

Sensitivity:

- < 100 pg/mL

Dynamic Range:

- Up to 4 orders of magnitude within dynamic range

Minimum Detection Limit:

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

6. CONCLUSION

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 makes the Modulus™ Microplate Reader the ideal choice for today's life science laboratory.

7. WARNINGS AND PRECAUTIONS

Caution: No data are currently available addressing the mutagenicity or toxicity of Quant-iT™ OliGreen® reagent.

This reagent binds to nucleic acids, and therefore should be treated accordingly as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. It is strongly recommended to use double gloves when handling the DMSO stock solution. As with all nucleic acid reagents, solutions of Quant-iT™ OliGreen® reagent should be poured through activated charcoal before disposal. Incinerate the charcoal to destroy the dye.

TRADEMARK

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