Promega

Application Note

Measuring the QuantiFluor™ ssDNA System Using the Qubit® 2.0 Fluorometer

INTRODUCTION

Detecting and quantitating small amounts of single-stranded (ssDNA) is an important step in many molecular biology techniques, including DNA sequencing, site directed mutagenesis, DNA amplification, and gene expression. Traditional spectrophotometric assays cannot determine DNA concentrations below 2µg/ml; however, many isolated DNA samples have concentrations well below that level.

The QuantiFluor™ ssDNA System (Cat.# E3190) provides a fast, easy, and sensitive method for determining ssDNA concentrations as low as 10ng/ml (or 2ng/tube) when used with the Qubit® 2.0 Fluorometer. The system contains a fluorescent ssDNA-binding dye that enables sensitive quantitation of small amounts of ssDNA in solution. For those ssDNA samples that may contain contaminating double-stranded DNA (dsDNA) we recommend a brief Shrimp DNase treatment to degrade any dsDNA present and ensure the most accurate ssDNA quantitation.

This Application Note describes the protocol for using the QuantiFluor™ ssDNA system with the Qubit® 2.0 Fluorometer. Representative data are shown in Figure 1.

MATERIALS REQUIRED

- QuantiFluor™ ssDNA System (Cat.# E3190)
- Qubit® 2.0 Fluorometer (Life Technologies)
- 0.5 ml PCR tubes (Axygen #PCR-05-C, available through Fisher or VWR)
- Shrimp DNase (USB Cat.# 78314)

Caution: We recommend use of gloves, lab coats and eye protection when working with these or any chemical reagents.

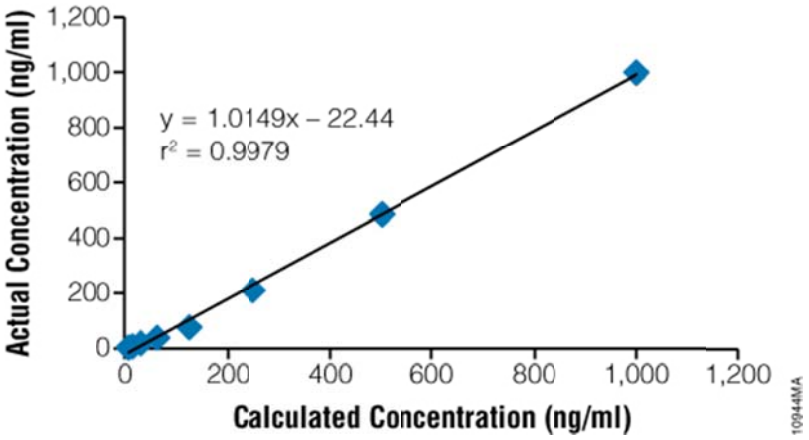

Figure 1. Determining ssDNA concentration using the QuantiFluor™ ssDNA System and the Qubit® 2.0 Fluorometer (data were generated using the protocols described below).The figure shows assay linearity. The ssDNA concentrations shown were generated after addition of the QuantiFluor™ ssDNA Dye working solution.

EXPERIMENTAL PROTOCOLS

A. ssDNA samples between 10–1,000ng/ml (2–200ng per tube):

1. Dilute the QuantiFluor™ ssDNA Dye 1:400 in 1X TE buffer to make a working solution. For example, add 5µl QuantiFluor™ ssDNA Dye to 1,995µl of 1X TE, and mix.
2. Add 100µl of 1X TE and 100µl of QuantiFluor™ ssDNA Dye working solution to an empty 0.5ml PCR Tube. This will be the Blank used in Step 4 of Section B, below. Protect from light.
3. Dilute the ssDNA standard 1:50 in 1X TE buffer to a concentration of 2ng/µl. For example, add 20µl ssDNA Standard to 980µl of 1X TE and mix.
4. Add 100µl of diluted ssDNA Standard and 100µl of QuantiFluor™ ssDNA Dye working solution to a 0.5ml PCR tube and mix. This will be the Standard used in Step 5 of Section B, below.
5. Add 100µl of the unknown sample and 100µl of the QuantiFluor™ ssDNA Dye Working Solution to a 0.5ml PCR tube and mix.
   Note: Record the amount of unknown sample added per tube. This will be used later to determine the final sample concentration in ng/ml. For example, add 1µl of unknown ssDNA sample to 99µl of 1X TE to comprise the 100µl sample addition.
6. Incubate the standard and unknowns at room temperature for 5 minutes, protected from light.

B. Setting up the Qubit® 2.0

1. From the Home Screen select the ssDNA protocol. **Note:** The ssDNA protocol on the Qubit® 2.0 uses the appropriate excitation / emission wavelengths for the QuantiFluor™ ssDNA Dye.
2. Press the **Standards** tab at the bottom of the screen.
3. Press **Yes** to read new standards.
4. Insert the blank sample ("Standard #1"), and press the **READ** button.
5. Insert the ssDNA Standard ("Standard #2") (from Section A, Step 4 above) and press the **READ** button. The instrument is now calibrated.
6. Press the **Sample** tab at the bottom of the screen.
7. Insert an unknown ssDNA sample tube. Press the **READ** button. The instrument will display the concentration of sample in ng/ml.

   The value displayed on the Qubit® 2.0 is equal to ng/ml (in the 200µl volume) regardless of the volume of unknown sample added in Section A, Step 5. To correct for dilution of the unknown sample:
   a. Press the "**Calculate Stock Conc**." Button on the instrument.
   b. Select the volume of the original sample that was added to the assay tube. For example if 2µl was added into the assay tube, select "2". If 5 µl was added, select "5".
   c. The instrument will then display the initial sample concentration. Select different units as desired.
8. Please refer to the Qubit® 2.0 technical manual for more details.

CONTACT INFORMATION

Toll-Free: (800) 356-9526
Fax: (800) 356-1970

www.promega.com

Email: custserv@promega.com for ordering inquiries
Email: techserv@promega.com for technical inquiries

Mailing Address:

Promega Corporation
2800 Woods Hollow Rd.
Madison, WI 53711 USA