Promega

Application Note

Measuring the QuantiFluor™ ssDNA System Using the QuantiFluor™-ST 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 3.125ng/ml (or 0.625ng/tube). The dye is capable of detecting lower amounts, but 3.125ng/ml is the lowest detected using the Quantifluor™-ST instrument. The QuantiFluor™ ssDNA System contains a fluorescent DNA-binding dye that enables sensitive quantitation of small amounts of ssDNA in solution. For 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 QuantiFluor™-ST Fluorometer and the PCR Tube Adapter. Representative data are shown in Figure 1.

MATERIALS REQUIRED

- QuantiFluor™ ssDNA System (Cat.# E3190)
- QuantiFluor™-ST Fluorometer (Cat.# (Cat.# E6090 or E6105)
- PCR Tube Adapter, QuantiFluor™ (Cat.# E6101)
- 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.

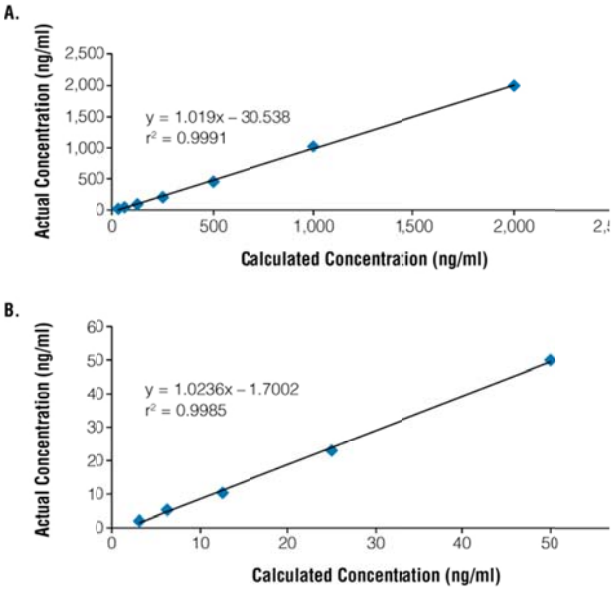

Figure 1. Determining ssDNA concentration using the QuantiFluor™ ssDNA System and the QuantiFluor™- ST Fluorometer with PCR Tube Adapter. Panel A. High Standard Curve. Panel B. Low Standard Curve. The QuantiFluor™ PCR tube Adapter allows for sample volumes as little as 100µl without sacrificing instrument sensitivity.

EXPERIMENTAL PROTOCOLS

A. High-Concentration Samples (31.25–2,000ng/ml, or 6.25–400ng per tube)

1. Dilute the QuantiFluor™ ssDNA Dye 1:200 in 1X TE buffer to make a working solution. For example, add 10µl QuantiFluor™ ssDNA Dye to 1,990µl 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 Section C, Step 7, below. Protect from light.
3. Dilute the ssDNA standard 1:25 in 1X TE buffer to a concentration of 4ng/µl. For example, add 40µl ssDNA Standard to 960µl 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 Section C, Step 9, 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 volume of unknown sample added per tube. This dilution factor will be used later to calculate the final DNA concentration in ng/ml. For example, add 1µl of unknown ssDNA sample and 99µl of 1X TE to prepare the 100µl sample addition.
6. Incubate the standard and unknowns at room temperature for 5 minutes, protected from light.

B. Low-Concentration Samples (3.125–50ng/ml or 0.625–10ng/tube)

1. Dilute the QuantiFluor™ ssDNA Dye 1:1,000 in 1X TE buffer to make a working solution. For example, add 2µl QuantiFluor ssDNA Dye to 1,998µl 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 Section C, Step 7 below. Protect from light.
3. Dilute the DNA Standard 1:1,000 in 1X TE buffer to a concentration of 0.1ng/µl. For example, add 2µl DNA Standard to 1,998µl of 1X TE and mix.
4. Add 100µl of the diluted ssDNA Standard and 100µl of the QuantiFluor™ ssDNA Dye working solution to a 0.5ml PCR tube and mix. This will be your Standard used in Section C, Step 9 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 volume of unknown DNA sample added per tube. This dilution factor will be used later to calculate the final DNA concentration in ng/ml. For example, add 1µl of unknown ssDNA sample and 99µl of 1X TE to prepare the 100µl sample addition.
6. Incubate the Standard and unknowns at room temperature for 5 minutes, protected from light.

C. Setting Up the QuantiFluor™-ST Fluorometer

1. Insert the PCR Tube Adapter into the QuantiFluor™-ST Fluorometer.
   Note: The PCR Tube Adapter is multidirectional and can be inserted in any orientation.
2. Press the **ON/OFF** button to turn the instrument on.
3. Set the instrument to the Blue channel by pressing the **A/B** button. The display should read "BLUE".
4. Set the standard value by pressing the **STD VAL** button.
   a. If using the **High Standard Dilution**, set the Instrument standard to **400 (ng per tube)**.
   b. If using the **Low Standard Dilution**, set the Instrument standard to **10 (ng per tube)**.
5. Press the **CAL** button; the screen will display "Calib BLUE <ENT> to start".
6. Press the **ENTER** button to move to the next screen, which will display "Insert Blank then press <ENT>".
7. Insert the blank sample, and press the **ENTER** button. The QuantiFluor™-ST Fluorometer will calculate the average reading over 10 seconds and set the zero (blank) point. During this time the screen will display "Reading Blank".
8. After the instrument has set the zero point, the screen will display "Insert Cal Soln then press <ENT>".
9. Insert your ssDNA standard (from Section A, Step 4, or Section B, Step 4), and press the **ENTER** button. The instrument is now calibrated.
10. Insert an unknown ssDNA sample. Press the **READ** button. The instrument will display the concentration of DNA in ng/tube. If 1µl of sample was added in Section A, Step 5, or Section B, Step 5, then the value displayed is equal to the concentration in ng/µl. If 2µl were added, then divide the displayed value by 2 to calculate the concentration in ng/µl of the original undiluted unknown. If 5µl were added, divide the displayed value by 5 to calculate the concentration in ng/µl of the original undiluted unknown. Multiply by 5 to convert the concentration to ng/ml in the 200µl assay volume as desired.

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