Promega

Application Note

Calibrating the Qubit® 2.0 Fluorometer for use with the QuantiFluor™ dsDNA System

INTRODUCTION

Accurate quantitation of DNA concentration is critical for many applications. Traditional spectrophotometric assays cannot determine DNA concentrations below 2µg/ml; however, many isolated DNA samples have concentrations well below this level. The QuantiFluor™ dsDNA System (Cat.# E2670) is a fast, easy and sensitive method for determining low DNA concentrations.

The QuantiFluor™ dsDNA System contains a fluorescent DNA-binding dye that enables sensitive quantitation of small amounts of double-stranded DNA (dsDNA) in solution. The quantitation of dsDNA is an important step in many biological applications, particularly in standard molecular biology techniques. This dye shows minimal binding to single-stranded DNA (ssDNA) and RNA, allowing specific quantitation of dsDNA.

This application note describes the protocol for calibrating the Qubit® 2.0 Fluorometer to measure the QuantiFluor™ dsDNA System using the preprogrammed High Sensitivity settings.

MATERIALS REQUIRED

- QuantiFluor™ dsDNA System (Cat.# E2670)
- 0.5ml PCR tubes (Axygen Cat.# PCR-05-C, available through Fisher or VWR)
- Qubit® 2.0 Fluorometer (Life Technologies)

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

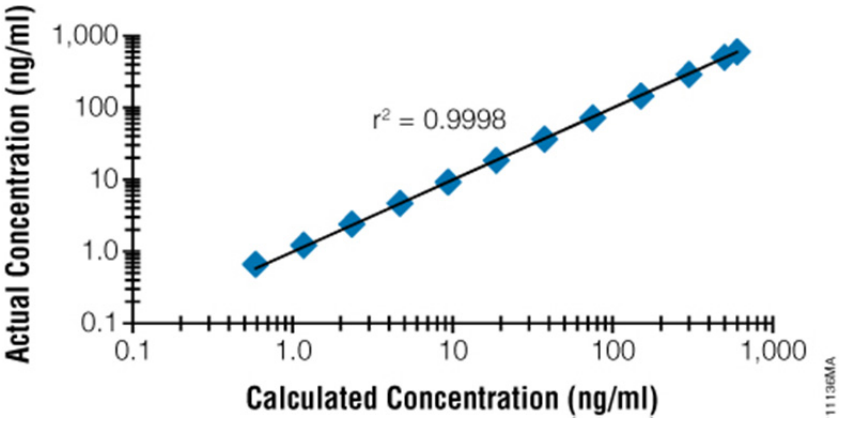

Figure 1. Measuring dsDNA concentration using the QuantiFluor™ dsDNA System and the Qubit® 2.0 Fluorometer. *Using the QuantiFluor™ dsDNA Dye with the Qubit® 2.0 Fluorometer provided a linear quantitation of dsDNA of 0.59–600µg/ml.*

EXPERIMENTAL PROTOCOL

Note: Unless indicated otherwise, all concentrations listed in this protocol are those after adding the QuantiFluor™ dsDNA Dye working solution.

A. Preparing Solutions and Standards

1. Prepare 1X TE buffer by diluting the 20X TE Buffer 1:20 in Nuclease-Free Water (Cat.# P1195).
2. Dilute the QuantiFluor™ dsDNA Dye 1:200 in 1X TE buffer to make the QuantiFluor™ dsDNA Dye working solution. Protect from light.
3. Add 100µl of 1X TE buffer and 100µl of QuantiFluor™ dsDNA Dye working solution to a 0.5ml PCR tube, and mix. Protect from light. This is the blank used in Section B, Step 3.
4. Dilute the Lambda DNA Standard 1:100 in 1X TE buffer to a concentration of 1,000ng/ml (concentration before adding dye; for example, add 10µl of DNA Standard to 990µl of 1X TE buffer, and mix).
5. Add 100µl of diluted Lambda DNA and 100µl of QuantiFluor™ dsDNA Dye working solution to a 0.5ml PCR tube, and mix. This is the 500ng/ml DNA Standard used in Section B, Step 4.
6. Add 100µl of the unknown DNA sample and 100µl of QuantiFluor™ dsDNA Dye working solution to a 0.5ml PCR tube, and mix.

   Note: If the volume of the unknown DNA sample is less than 100µl, add 1X TE buffer to a final volume of 100µl. Record the volume added; this dilution factor will be used later to calculate the final DNA concentration of the sample.

B. Setting Up the Qubit® 2.0 Fluorometer

1. From the home screen, select DNA protocol, then select the dsDNA High Sensitivity assay type. The Broad Range protocol cannot be used with QuantiFluor™ dsDNA Dye systems.
2. On the standard screen, select “Yes” to read new standards.
3. Insert the blank (Standard 1), and press “Read”.
4. Insert the Lambda DNA standard (Standard 2), and press “Read”. The instrument is now calibrated.
5. Insert DNA sample, and press “Read”. The concentration displayed is the concentration of DNA in the tube in ng/ml (200µl volume). To have the Dilution Calculator on the Qubit® 2.0 Fluorometer calculate the concentration of your original sample:
   a. Press the Calculate Stock Conc. button on the instrument.
   b. Select the volume of the original sample that was added to the tube.
   c. The instrument displays the initial sample concentration. Select units as desired (e.g., ng/ml, ng/µl, pg/µl, etc.).
6. To save results, press “Data”, insert a USB flash drive into the port, select the desired sample data points, and press the USB icon with a green light to transfer data.

Refer to the Qubit® 2.0 Fluorometer technical manual for more details.

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