Promega

Application Note

Calibrating the Qubit® 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 2 µg/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® Fluorometer to be used for measuring the QuantiFluor™ dsDNA system using the preprogrammed High Sensitivity settings.

MATERIALS REQUIRED

- QuantiFluor™ dsDNA System (Cat.# E2670)
- 0.5 ml PCR tubes
- Qubit® Fluorometer

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

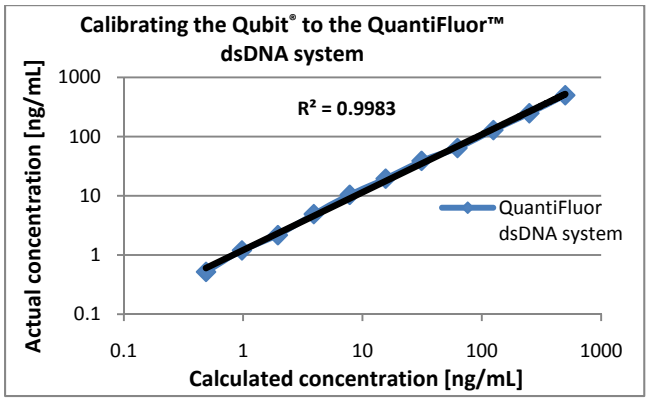

Figure 1. Calibrating the Qubit® Fluorometer to the QuantiFluor™ dsDNA system. *The Qubit® fluorometer has a limit of detection of 0.5 ng/ml and an upper limit of 600 ng/ml (0.6 µg/ml) when the High Sensitivity protocol has been selected. More concentrated samples will require dilution to fit within this working range. For diluted samples, the Qubit® fluorometer allows selection of a dilution factor.*

EXPERIMENTAL PROTOCOL

1. Dilute the QuantiFluor™ DNA dye 1:200 in 1XTE buffer to make dye working solution. Protect from light.
2. Add 100 µl of each standard and unknown to the appropriate tube and mix well.
   - Use 1 µg/ml (0.5 µg/ml final after mixing 1:1 with dye working solution) for the standard (dilute 100 µg/ml lambda DNA 1:100).
   - Use 1X TE buffer for the blank.
   - Dilute unknown samples in 1X TE to a final volume of 100 µl and add to PCR tubes.

   Note: Label the **lids** of all PCR tubes, not the sides.

3. Add 100 µl of QuantiFluor™ dye working solution to each tube used for standards and unknowns.
4. Incubate at room temperature for 5 minutes protected from light.
5. Calib rate the Qubit® Fluorometer using the High Sensitivity preprogrammed protocol according to the instructions provided in the Qubit® technical manual.
   - Blank will be inserted first followed by the 0.5 µg/ml standard
   - After instrument calibration, all unknown sample concentrations will be displayed in direct concentration on the screen.

CONTACT INFORMATION

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