Promega

Application Note

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

INTRODUCTION

Detecting and quantitating small amounts of RNA is an important step in many molecular biology techniques to measure yields of in vitro transcribed RNA and determine RNA concentration before performing Northern blot analysis, S1 nuclease assays, RNase protection assays, cDNA library preparation, reverse transcription PCR and differential display PCR. Traditional spectrophotometric assays cannot determine RNA concentrations below 2µg/ml; however, many isolated RNA samples have concentrations well below that level.

The QuantiFluor™ RNA System (Cat.# E3310) provides a fast, easy and sensitive method for determining RNA concentrations as low as 10ng/ml (or 2ng/tube). While the dye is capable of detecting lower amounts of RNA than 10ng/ml, this level was the lowest obtained with the Qubit 2.0 Fluorometer. The QuantiFluor™ RNA System contains a fluorescent dye that enables sensitive quantitation of small amounts of RNA in solution. For those RNA samples that may contain contaminating genomic DNA, we recommend a brief DNase treatment to degrade any genomic DNA present in the sample to ensure the most accurate RNA quantitation.

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

MATERIALS REQUIRED

- QuantiFluor™ RNA System (Cat.# E3310)
- Qubit® 2.0 Fluorometer (Life Technologies)
- 0.5ml PCR tubes (Axygen #PCR-05-C, available through Fisher or VWR)

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

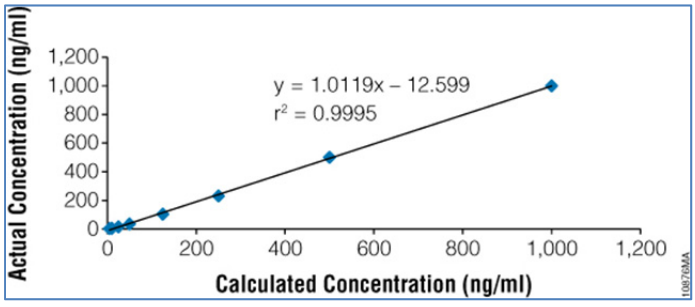

***Figure 1. Determining RNA concentration using the QuantiFluor™ RNA System and the Qubit® 2.0 Fluorometer* (data were generated using the protocols below).** The figure shows assay linearity. The RNA concentrations shown are those after addition of the QuantiFluor™ RNA Dye working solution.

EXPERIMENTAL PROTOCOLS

Note: Unless indicated otherwise, all concentrations in these protocols are those after adding the QuantiFluor™ RNA Dye.

A. RNA Samples (10–1,000ng/ml or 2–200ng per tube):

1. Dilute the QuantiFluor™ RNA Dye 1:400 in 1X TE buffer to make a working solution. (For example, add 5µl of QuantiFluor™ RNA Dye to 1,995µl of 1X TE buffer, and mix.) Protect from light.
2. Add 100µl of 1X TE buffer and 100µl of QuantiFluor™ RNA Dye working solution to an empty 0.5ml PCR tube. This is the blank used in Section B, Step 4. Protect from light.
3. Dilute the RNA Standard 1:50 in 1X TE buffer to a concentration of 2ng/µl (concentration before adding dye; for example, add 20µl of RNA Standard to 980µl of 1X TE buffer, and mix).
4. Add 100µl of diluted RNA standard and 100µl of QuantiFluor™ RNA Dye working solution to a 0.5ml PCR tube, and mix. This is the standard used to calibrate the Qubit® 2.0 Fluorometer in Section B, Step 5.
5. Add 100µl of the unknown RNA sample and 100µl of QuantiFluor™ RNA Dye working solution to a 0.5ml PCR tube, and mix.
   Note: If the volume of the unknown RNA sample is less than 100µl, add 1X TE buffer to a final volume of 100µl. Record the volume of unknown RNA sample added per tube. This dilution factor will be used later to calculate the final RNA concentration in ng/ml.
6. Incubate the standard and unknown samples at room temperature for 5 minutes, protected from light.

B. Setting Up the Qubit® 2.0 Fluorometer

1. From the Home Screen, select the ssDNA protocol. The ssDNA protocol on the Qubit® 2.0 Fluorometer uses the appropriate excitation/emission wavelengths for the QuantiFluor™ RNA 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”), which was prepared in Section A, Step 2, and press the **Read** button.
5. Insert the RNA Standard (“Standard #2”), which was prepared in Section A, Step 4, and press the **Read** button. The instrument is now calibrated.
6. Press the Sample tab at the bottom of the screen.
7. Insert the unknown RNA sample tube. Press the **Read** button. The instrument will display the concentration of RNA in the tube in ng/ml (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 to the assay tube, then select 2. If 5µl was added to the assay tube, then select 5.
   c. The instrument will then display the initial sample concentration. Select different units as desired.

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

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