Promega

Application Note

Measuring the QuantiFluor™ RNA System Using the QuantiFluor™-ST Fluorometer

INTRODUCTION

Detecting and quantitating small amounts of RNA is a very important step that is used in many molecular biology techniques. These include measuring yields of in vitro transcribed RNA and measuring 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 0.5ng/ml (or 100pg/well). 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 QuantiFluor™-ST Fluorometer and the PCR Tube Adapter.

MATERIALS REQUIRED

- QuantiFluor™ RNA System (Cat.# E3310)
- QuantiFluor™-ST Fluorometer (Cat.# E6090)
- PCR Tube Adapter, QuantiFluor™ Fluorometers (Cat.# E6101)
- 0.5ml PCR tubes (Axygen Cat.# 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.

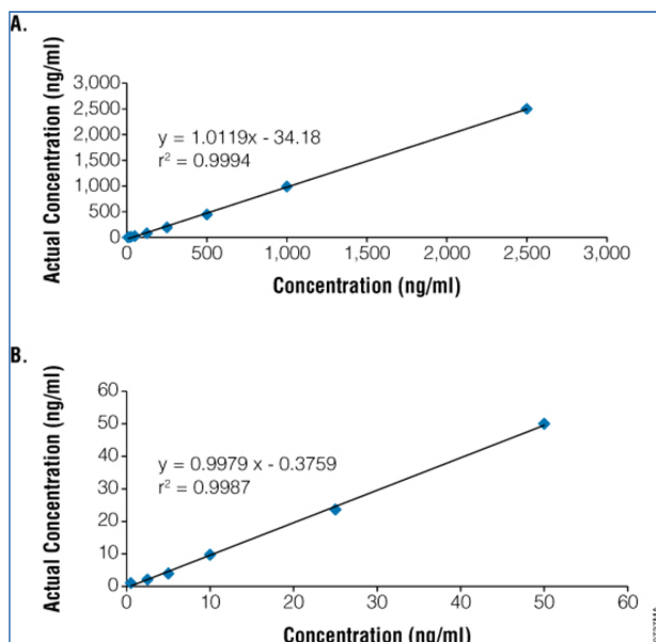

***Figure 1. RNA concentration using the QuantiFluor™ RNA System and the QuantiFluor™- ST Fluorometer with PCR Tube Adapter* (data generated using protocols below). Panel A** shows assay linearity when using the high-concentration standard curve. **Panel B** shows assay linearity when using the low-concentration standard curve. The PCR Tube Adapter allows sample volumes as little as 100µl without sacrificing instrument sensitivity. 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. For High-Concentration RNA Samples (10–2,500ng/ml or 2–500ng per tube):

1. Dilute the QuantiFluor™ RNA Dye 1:200 in 1X TE buffer to make a working solution. (For example, add 10µl of QuantiFluor™ RNA Dye with 1,990µl of 1X TE buffer, and mix.) Protect from light.
2. Add 100µl of 1X TE buffer and 100µl of the QuantiFluor™ RNA Dye working solution to an empty 0.5ml PCR tube. This is the blank used in Section C, Step 7. Protect from light.
3. Dilute the RNA Standard 1:20 in 1X TE buffer to a concentration of 5ng/µl (concentration before adding dye; for example, add 50µl of RNA Standard to 950µl of 1X TE buffer, and mix).
4. Add 100µl of the 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 QuantiFluor™-ST Fluorometer for quantitation of high-concentration samples in Section C, Step 9.
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. For Low-Concentration RNA Samples (0.5–50ng/ml or 0.1–10ng per tube):

1. Dilute the QuantiFluor™ RNA Dye 1:1,000 in 1X TE buffer to make a working solution. (For example, add 2µl of QuantiFluor™ RNA Dye with 1,998µl of 1X TE buffer, and mix.) Protect from light.
2. Add 100µl of 1X TE buffer and 100µl of the QuantiFluor™ RNA Dye working solution to an empty 0.5ml PCR tube. This is the blank used in Section C, Step 7. Protect from light.
3. Dilute the RNA Standard 1:1,000 in 1X TE buffer to a concentration of 0.1ng/µl (concentration before adding dye; for example, add 2µl of RNA Standard to 1,998µl of 1X TE buffer, and mix).
4. Add 100µl of the 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 QuantiFluor™-ST Fluorometer for quantitation of low-concentration samples in Section C, Step 9.
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.

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 **500 (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 RNA 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 RNA sample. Press the **READ** button. The instrument will display the concentration of RNA 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. To convert from ng/µl to ng/ml, multiply by 1,000.

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