

# Measuring the Quant-iT™ RiboGreen® Assay Using the QuantiFluor™-ST 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. Quant-iT™ RiboGreen® RNA Reagent provides a sensitive method for measuring low RNA concentrations.

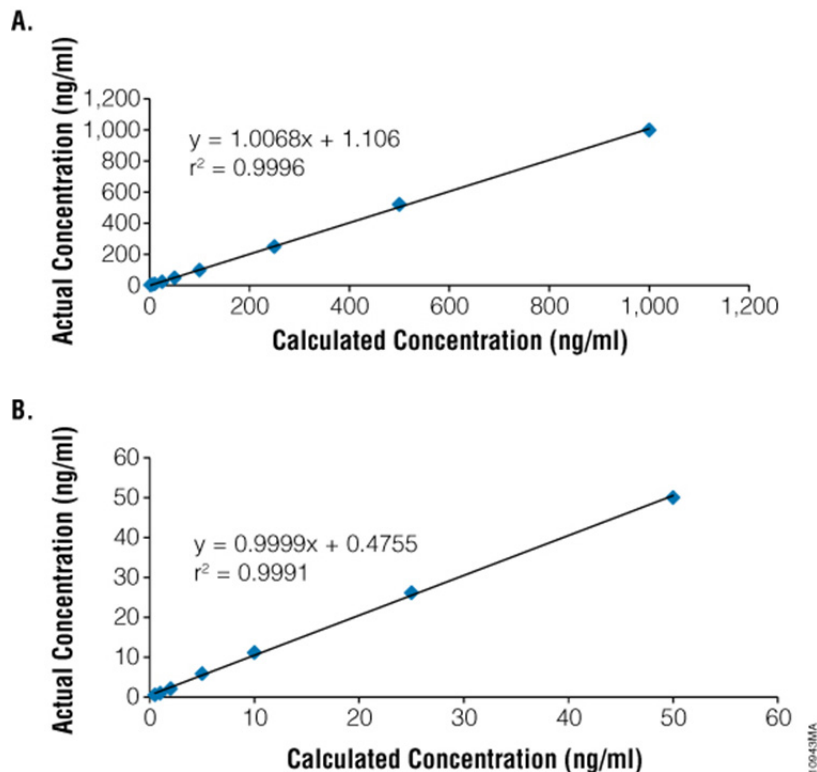
Quant-iT™ RiboGreen® RNA Reagent contains a fluorescent RNA-binding dye that enables sensitive quantitation of small amounts of RNA in solution. Since the Quant-iT™ RiboGreen® RNA Reagent is not specific for RNA, a brief DNase treatment to degrade any genomic DNA present in the sample will help ensure the most accurate RNA quantitation.

This Application Note describes the protocol for using Quant-iT™ RiboGreen® RNA Reagent with the QuantiFluor™-ST Fluorometer or QuantiFluor™-P Fluorometer. Representative data are shown in Figure 1.

## MATERIALS REQUIRED

- Quant-iT™ RiboGreen® RNA Reagent (Invitrogen Cat.# R-11490)
- QuantiFluor™-ST Fluorometer (Cat.# E6090) or QuantiFluor™-P Fluorometer (Cat.# E6105)
- PCR Tube Adapter, QuantiFluor™ Fluorometers (Cat.# E6101)
- 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. Measuring RNA concentration using Quant-iT™ RiboGreen® RNA Reagent and the QuantiFluor™-ST Fluorometer with PCR Tube Adapter.** The PCR Tube Adapter accommodates sample volumes as low as 100µl without sacrificing instrument sensitivity. **Panel A.** A high-concentration RNA standard curve. **Panel B.** A low-concentration RNA standard curve.

## EXPERIMENTAL PROTOCOLS

### A. High-Concentration RNA Samples (2–1,000ng/ml or 0.4–200ng per tube)

1. Dilute the Quant-iT™ RiboGreen® RNA Reagent 1:200 in 1X TE buffer to make a working solution. For example, add 10µl of Quant-iT™ RiboGreen® RNA Reagent to 1,990µl of 1X TE buffer, and mix.
2. Add 100µl of Quant-iT™ RiboGreen® RNA Reagent working solution and 100µl of 1X TE buffer to an empty 0.5ml PCR tube. Protect from light. This is the blank used in Section C, Step 8.
3. Dilute the ribosomal RNA standard 1:50 in 1X TE buffer to a concentration of 2ng/µl. For example, add 20µl of ribosomal RNA standard to 980µl of 1X TE buffer, and mix.
4. Add 100µl of diluted ribosomal RNA standard (2ng/µl) and 100µl of Quant-iT™ RiboGreen® RNA Reagent working solution to a 0.5ml PCR tube, and mix. The final RNA concentration is 1ng/µl. This is the RNA standard used in Section C, Step 10.
5. Add 100µl of unknown sample and 100µl of Quant-iT™ RiboGreen® RNA Reagent working solution to a 0.5ml PCR tube, and mix.

**Note:** If the volume of unknown sample is less than 100µl, add 1X TE buffer to a final volume of 100µl. Record the volume of unknown sample added per tube. This information is used later when determining the RNA concentration in ng/ml.

6. Incubate the RNA standard and unknown samples at room temperature for 5 minutes, protected from light.

**B. Low-Concentration RNA Samples (0.5–50ng/ml or 0.1–10ng per tube)**

1. Dilute the Quant-iT™ RiboGreen® RNA Reagent 1:2,000 in 1X TE buffer to make a working solution. For example, add 1µl of Quant-iT™ RiboGreen® RNA Reagent to 1,999µl of 1X TE buffer, and mix.
2. Add 100µl of Quant-iT™ RiboGreen® RNA Reagent working solution and 100µl of 1X TE buffer to an empty 0.5ml PCR tube. Protect from light. This is the blank used in Section C, Step 8.
3. Dilute the ribosomal RNA standard 1:1,000 in 1X TE buffer to a concentration of 100ng/ml. For example, add 1µl of ribosomal RNA standard to 1,999µl of 1X TE buffer, and mix.
4. Add 100µl of diluted ribosomal RNA standard (100ng/ml) and 100µl of Quant-iT™ RiboGreen® RNA Reagent working solution to a 0.5ml PCR tube, and mix. The final RNA concentration is 50ng/ml. This is the RNA standard used in Section C, Step 10.
5. Add 100µl of unknown sample and 100µl of Quant-iT™ RiboGreen® RNA Reagent working solution to a 0.5ml PCR tube, and mix.

**Note:** If the volume of unknown sample is less than 100µl, add 1X TE buffer to a final volume of 100µl. Record the volume of unknown sample added per tube. This information is used later when determining the RNA concentration in ng/ml.

6. Incubate the RNA 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 or QuantiFluor™-P 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. Press the STD VAL button.
5. Input the concentration of the RNA standard. For the high-concentration RNA standard prepared in Section A, set the STD Val to 999 (ng/ml). For the low-concentration RNA standard prepared in Section B, set the STD Val to 50 (ng/ml).
6. Press the CAL button, the screen will display “Calib BLUE <ENT> to start”.
7. Press the ENTER button to move to the next screen, which will display “Insert Blank then press <ENT>”.
8. Insert the blank sample, and press the ENTER button. The QuantiFluor™ Fluorometer will average the reading for 10 seconds, and set the zero (blank) point. During this time the screen will display “Reading Blank”.
9. After the instrument has set the zero point, the screen will display “Insert Cal Soln then press <ENT>”.
10. Insert the diluted ribosomal RNA standard prepared in Section A or B, Step 4, and press the ENTER button. The instrument is now calibrated.
11. Insert an unknown RNA sample. Press the READ button. The instrument will display the RNA concentration of the sample in ng/ml (in the final assay volume of 200µl). To determine the concentration of the original unknown samples, the RNA concentration in ng/ml must be converted to ng/µl and adjusted for the dilution factor. Use the follow equation:  
$$\left(\frac{\text{displayed value in ng/ml}}{5}\right) / (\text{volume of unknown sample added in } \mu\text{l}) = \text{Concentration of unknown sample in ng/}\mu\text{l}$$

For example, the concentration displayed by the fluorometer is 500ng/ml and 2 $\mu$ l of unknown sample was added.

$$500/5 = 100/2 = 50\text{ng}/\mu\text{l}$$

The RNA concentration of the unknown sample is 50ng/ $\mu$ l.

For example, the concentration displayed by the fluorometer is 1,000ng/ml and 5 $\mu$ l of unknown sample was added.

$$1000/5 = 200/5 = 40\text{ng}/\mu\text{l}$$

The RNA concentration of the unknown sample is 40ng/ $\mu$ l.

#### **CONTACT INFORMATION**

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