Measuring RNA Concentration Using the Quantus™ Fluorometer with the Quant-iT™ RiboGreen® RNA Assay Kit

Promega Corporation

Materials Required

- Quantus™ Fluorometer (Cat.# E6150)
- 0.5ml PCR Tubes (Axygen Cat.# PCR-05-C, available through Fisher or VWR)
- Quant-iT™ RiboGreen® RNA Assay Kit (Life Technologies Cat.# R11490)

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

Protocol: *Quantus™ Fluorometer Operating Manual* #TM396 is available at: **www.promega.com/protocols/**

Detecting and quantitating small amounts of RNA are important steps in many molecular biology techniques to measure yields of in vitro transcribed RNA and measure RNA concentration before performing Northern blot analysis, S1 nuclease assays, RNase protection assays, cDNA library construction, reverse transcription PCR and differential display PCR.

The most commonly used technique to determine nucleic acid concentration is measuring absorbance at 260nm (A_{260}). The major disadvantages of the absorbance-based method include: the inability to distinguish among DNA (both single- and double-stranded), RNA and nucleotides, interference caused by contaminants commonly found in nucleic acid preparations, and the relative insensitivity of the assay (traditional spectrophotometric assays cannot determine nucleic acid concentrations below 2µg/ml). The use of sensitive, fluorescent nucleic acid stains alleviates many of these problems.

The Quant-iT™ RiboGreen® RNA Assay Kit can be used with the Quantus™ Fluorometer. The assay quantitates RNA in solution at a final assay concentration of 1ng/µl–1ng/ml of RNA.

This Application Note describes the protocol for using the Quant-iT™ RiboGreen® RNA Assay Kit with the Quantus™ Fluorometer.

Protocol

1. Create a custom protocol on the Quantus™ Fluorometer by selecting "New" from the menu list on the Protocol screen, and name the protocol by using the up or down buttons. For the high-concentration standard, enter the standard value of 1ng/µl. For the low-concentration standard, enter the standard value of 50ng/ml. Select the Blue channel, and save the protocol.
2. Equilibrate all reagents to room temperature.

3. Dilute the Quant-iT™ RiboGreen® reagent 1:200 for the high-concentration assay or 1:1,000 for the low-concentration assay in 1X TE buffer to make a reagent working solution. For example, add 10µl of Quant-iT™ RiboGreen® reagent to 1,990µl of 1X TE buffer, and mix for a 1:200 dilution. For a 1:1,000 dilution, add 2µl of Quant-iT™ RiboGreen® reagent to 1,998µl of 1X TE buffer, and mix.
4. Add 100µl of the Quant-iT™ RiboGreen® reagent working solution to a 0.5ml PCR tube containing 100µl of 1X TE buffer. This will be the blank sample used in Step 8. Protect from light.
5. Prepare the RNA standard. For a high-concentration standard protocol, dilute the RNA standard (Component C) to 2ng/µl by adding 40µl of Component C to 1,960µl of 1X TE buffer, and mix. For a low-concentration standard protocol, dilute the RNA standard to 100ng/ml by adding 2µl of Component C RNA to 1,998µl of 1X TE buffer, and mix. Add 100µl of the standard to 100µl of Quant-iT™ RiboGreen® reagent working solution in a 0.5ml PCR tube, and mix. This will be the standard sample used in Step 8. Protect from light.
6. Add 100µl of unknown sample and 100µl of Quant-iT™ RiboGreen® reagent 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 bring to a final volume of 100µl. For example, dilute a 1µl sample with 99µl of 1X TE buffer, then add 100µl of reagent working solution for a total volume of 200µl.
7. Incubate the blank, standard and unknown samples at room temperature for 5 minutes, protected from light.
8. Select the custom protocol created in Step 1. Go to the Calibration screen and read the blank and standard samples prepared in Steps 4 and 5. Save the calibration.
9. Enter the volume of the unknown sample and desired concentration units.

   Note: This volume is the amount of sample that is added for the quantitation. For example, if 1µl of sample was mixed with 99µl of 1X TE buffer then added to 100µl of reagent working solution for a total volume of 200µl in the tube, then the volume entered on this screen is 1µl.
10. Place the unknown sample into the tube holder, and close the lid. The instrument will automatically measure fluorescence when the lid is closed, and the calculated nucleic acid concentration will be displayed.

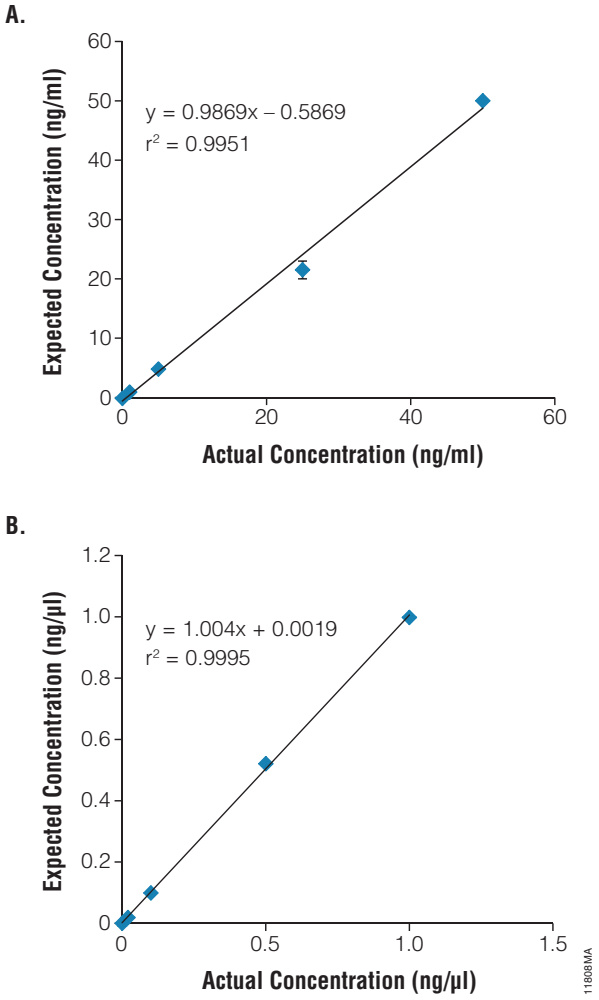

Figure 1. Measuring RNA concentration using the Quant-iT™ RiboGreen® RNA reagent and Quantus™ Fluorometer. Standard curves were generated per manufacturer's instructions to demonstrate the linearity of the Quantus™ Fluorometer. Samples were run in duplicate. **Panel A.** Assay linearity using the low-concentration standard curve. **Panel B.** Assay linearity using the high-concentration standard curve.

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