Measuring RNA Concentration Using the Quantus™ Fluorometer with the QuantiFluor® RNA System

Promega Corporation

Materials Required

- QuantiFluor® RNA System (Cat.# E3310)
- Quantus™ Fluorometer (Cat.# E6150)
- 0.5ml PCR Tubes (Cat.# E4941)
- RQ1 RNase-Free DNase (Cat.# M6101)

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

Protocols: *Quantus™ Fluorometer Operating Manual* #TM396 and *QuantiFluor® RNA System Technical Manual* #TM377 are available at: **www.promega.com/protocols/**

Detecting and quantitating small amounts of RNA is an important step that is used for many molecular biology techniques. These include assessing yield 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. Using the Quantus™ Fluorometer (Cat# E6150) with the QuantiFluor® RNA System (Cat.# E3310) provides a fast, easy and sensitive method for determining RNA concentration. Using this system, we have detected sample RNA concentrations as low as 100pg/µl when using 1µl of sample input per assay. It is possible to quantitate more dilute samples by adding more sample per assay. Up to 100µl of sample may be measured per 200µl assay.

The QuantiFluor® RNA System contains a fluorescent dye that enables sensitive quantitation of small amounts of RNA in solution. For 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 Quantus™ Fluorometer. The Quantus™ Fluorometer measures sample volumes as little as 1µl in a 200µl assay volume without sacrificing instrument sensitivity.

For the complete protocol, see the *QuantiFluor® RNA System Technical Manual* #TM377.

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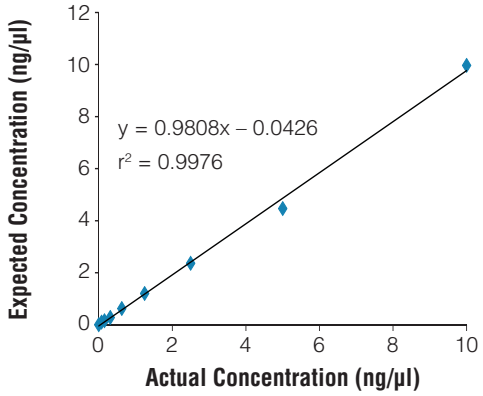
A.



B.

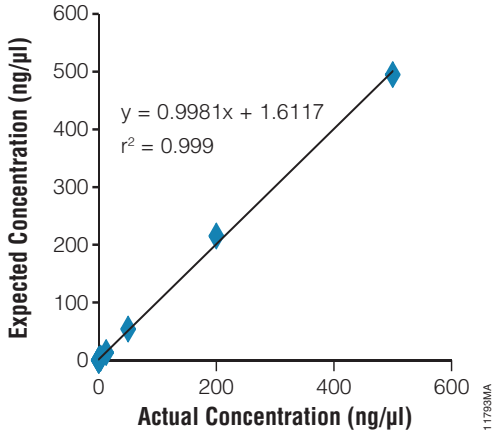


Figure 1. Measuring RNA concentration using the QuantiFluor® RNA System and the Quantus™ Fluorometer. Panel A. Assay linearity using the low-concentration standard curve. **Panel B.** Assay linearity using the high-concentration standard curve. The values presented correspond to assay tube RNA concentration and not starting sample concentration.