Promega

Product Application

Calibrating the Qubit™ 3.0 Fluorometer for use with the QuantiFluor® RNA System

Introduction

Accurate quantitation of RNA concentration is critical for many applications. Traditional spectrophotometric assays cannot determine RNA concentrations below 2ng/µl; however, many isolated RNA samples have concentrations well below this level. The QuantiFluor® RNA System (Cat.# E3310) is a fast, easy, and sensitive method for determining low RNA concentrations.

The QuantiFluor® RNA System contains a fluorescent RNA-binding dye that enables sensitive quantitation of small amounts of RNA in solution. Detecting and quantitating small amounts of RNA is important for many biological applications, including determining yield of in vitro transcribed RNA, and measuring RNA concentration before Northern blot analysis, S1 nuclease assays, RNase protection assays, cDNA library preparation, RT-PCR, and differential display PCR.

This application note describes the protocol for calibrating the Qubit™ 3.0 Fluorometer to measure the QuantiFluor® RNA System using the preprogrammed High Sensitivity settings.

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM377, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Materials Required

- QuantiFluor® RNA System (Cat.# E3310)
- 0.5ml PCR tubes (Cat.# E4941)
- Qubit™ 3.0 Fluorometer (Life Technologies)

Protocol:

A. Preparing Solutions and Standards

1. Prepare 1X TE buffer by diluting the 20X TE Buffer 1:20 with nuclease-free water.
2. Thaw the dye at room temperature protected from light. Dilute the QuantiFluor® RNA Dye 1:400 in 1X TE buffer to make a working solution. Protect from light.
3. Prepare RNA standards. These standards will be used to calibrate the Qubit™ 3.0 in Section B, Steps 2-4.

a. Standard #1: 1X TE (blank)

b. Standard #2: Dilute the RNA Standard (100ng/µl) 1:50 in 1X TE. For example, dilute 10µl of standard in 490µl of 1X TE for a final concentration of 2ng/µl.

4. Add 100µl of both standards to 0.5ml PCR tubes, label accordingly.
5. Dilute unknown samples to a volume of 100µl with 1X TE. 1-20µl of sample may be used. For example, dilute 5µl of sample with 95µl 1X TE. Record the volume of sample used.

6. Transfer 100µl of QuantiFluor® RNA Dye working solution into each tube of sample and standard. The final volume in each tube should be 200µl.
7. Mix briefly by vortexing or pipetting and incubate for 5 minutes, protected from light.

B. Setting up and using the Qubit™ 3.0 Fluorometer

1. From the Home Screen, select “Oligo” and the “ssDNA”. The ssDNA protocol on the Qubit™ 3.0 Fluorometer uses the appropriate excitation/emission wavelengths for the QuantiFluor® RNA Dye.
2. Select “Read standards”. Ensure that Standard #1 is the 1X TE blank and Standard #2 contains 200ng of RNA.
3. Insert Standard #1 and press the “Read” button.
4. Insert Standard #2 and press the “Read” button. The instrument is now calibrated.
5. Press “Run Samples” and input the amount of sample added to each tube to ensure the proper dilution factor is applied. For example: if 5µl of RNA sample was mixed with 95µl 1X TE and added to 100ul QuantiFluor® RNA Dye working solution for a total assay volume of 200µl, the volume of sample added is 5µl.
6. Insert each RNA sample tube. Press the “Read” button. The number displayed is the concentration of the original sample. All samples can be tested in succession, data can be exported at the experiment’s conclusion.

Results:

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

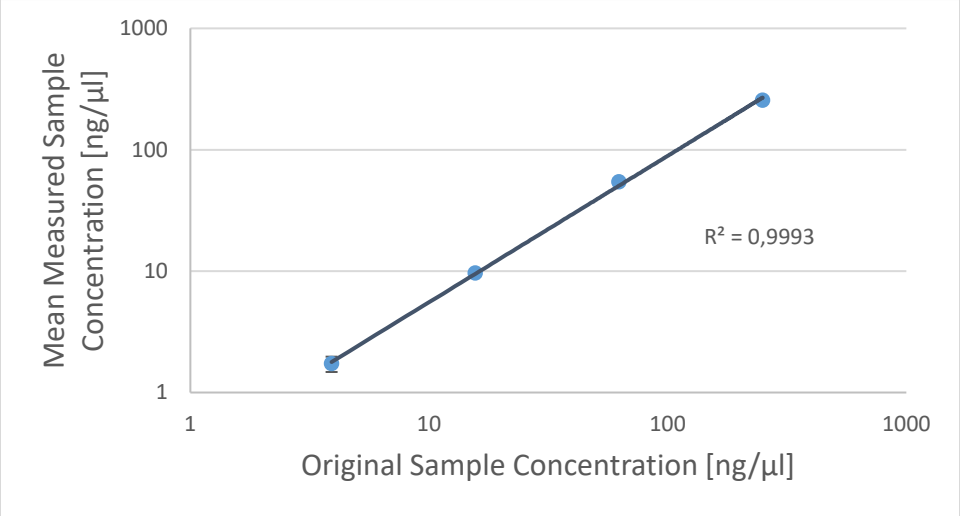

Figure 1. Measuring RNA concentration using the QuantiFluor® RNA System on the Qubit™ 3.0 Fluorometer. The QuantiFluor® RNA Dye enables linear quantitation of RNA on the Qubit™ 3.0 Fluorometer (data generated using the protocol described above). Shown is the concentration of the sample, assuming that 1µl was used for quantitation.