Quick Protocol

QuantiFluor® RNA System

Instructions for Use of Product **E3310**

Single-Tube-Format Protocol

Materials Required

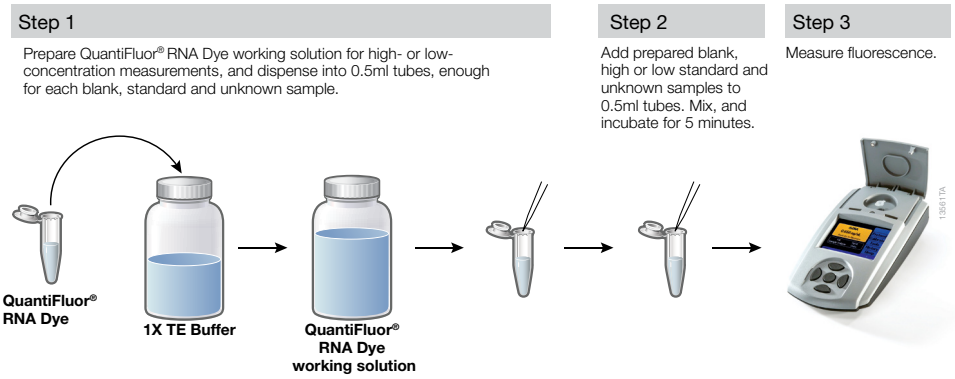
- QuantiFluor® RNA System (Cat.# E3310)
- Quantus™ Fluorometer (Cat.# E6150)
- thin-walled 0.5ml PCR tubes (Cat.# E4941 or Axygen Cat.# PCR-05-C)
- nuclease-free water

Warm all assay components to room temperature before use.

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

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

Protocol



Note: If the Quantus™ Fluorometer was previously calibrated, you may not need to calibrate it again. Therefore, do not prepare blank and standard samples.

1. **Prepare 1X TE Buffer:** Dilute the 20X TE Buffer 20-fold with nuclease-free water. For 20 samples, make 4ml of 1X TE Buffer using 200µl of 20X TE Buffer and 3.8ml of nuclease-free water.

   Note: Make enough for all your samples (at least 200µl per sample plus blank and standard if needed).

2. **Prepare Working Solution:**

   Low Standard Calibration: Dilute the QuantiFluor® RNA Dye 1:400 in 1X TE buffer (e.g.,add 10µl of QuantiFluor® RNA Dye to 3,990µl of TE buffer), and mix thoroughly.

   Low Standard Calibration: Dilute the QuantiFluor® RNA Dye 1:2,000 in 1X TE buffer (e.g., add 2µl of QuantiFluor® RNA Dye to 3,998µl of 1X TE buffer), and mix.

3. Mix working solution by inverting 3–4 times or vortex 5–10 seconds. Protect from light (e.g., cover with foil). The working solution is stable for 8 hours at room temperature or 1 week at 2–10°C (protected from light).

4. **Prepare Blank:** Add 200µl of QuantiFluor® RNA Dye working solution in an empty 0.5ml PCR tube. Protect tube from light.

5. **Choose your standard calibration range:**

   High Standard Calibration: Use for samples with 10–500ng/µl RNA.

   Low Standard Calibration: Use for samples with 0.2–10ng/µl RNA.

   If the RNA sample concentration is unknown, start with high standard calibration. If your samples read too low, switch to low standard calibration.

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6. **Prepare Chosen Standard:**

   High Standard Calibration: Prepare a 500ng standard by adding 5µl of the provided RNA Standard to 200µl of QuantiFluor® RNA Dye working solution in an empty 0.5ml PCR tube. Mix, and protect tube from light.

   Low Standard Calibration: Prepare a 10ng standard by diluting the provided RNA Standard 1:100 in 1X TE buffer(2ul standard to 198ul 1x TE buffer). Next, add 10µl of diluted standard to 200µl of QuantiFluor® RNA Dye working solution in a 0.5ml PCR tube. Mix, and protect tube from light.

7. **Prepare Unknown Sample(s):** Add 1–20µl of unknown samples to 200µl of QuantiFluor® RNA Dye working solution in 0.5ml PCR tubes. Vortex well, and protect tube from light.
8. Incubate all tubes for 5 minutes at room temperature, protected from light.
9. Select the RNA protocol on the Quantus™ Fluorometer. If needed, calibrate the Quantus™ Fluorometer by reading the blank (Step 4) and standard (Step 6) samples in the 'Calibration' screen, then select **Save**.
10. Enter the volume of the unknown sample (1–20µl used in Step 7) and desired concentration units.
11. Measure fluorescence of the unknown sample and record the final sample concentration results.

Multiwell Plate Protocol

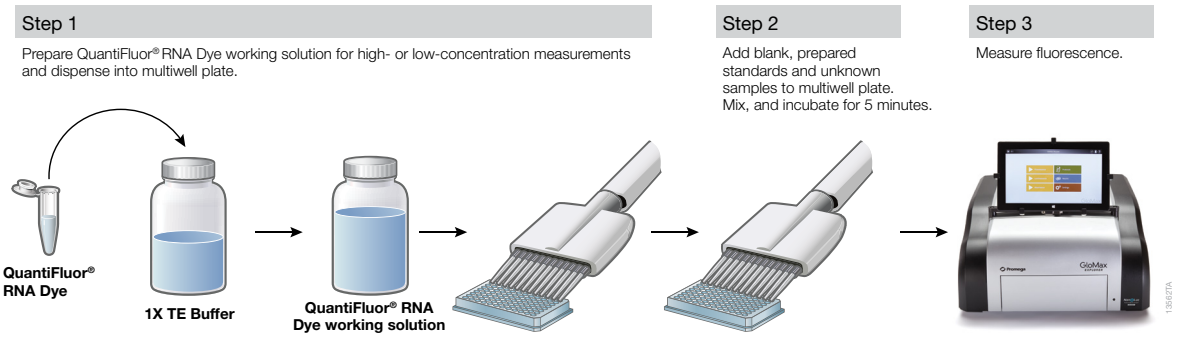
Materials Required

- multiwell detection instrument capable of measuring fluorescence (e.g., GloMax® Discover System [Cat.# GM3000])
- Nuclease-Free Water (Cat.# P1195)
- black, flat-bottom 96-well plates
- 1.5ml tubes

Warm all assay components to room temperature before use.

The *QuantiFluor® RNA System Technical Manual* #TM377 are available at: **www.promega.com/protocols**

Protocol



1. **Prepare 1X TE Buffer:** Dilute the 20X TE Buffer 20-fold with nuclease-free water. For 20 samples, make 4ml of 1X TE Buffer using 200µl of 20X TE Buffer and 3.8ml of Nuclease-Free Water.

   Note: Make enough for all your samples (at least 200µl per sample plus blank and standard if needed).

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2. **Prepare Working Solution:**

   Low Standard Calibration: Dilute the QuantiFluor® RNA Dye 1:400 in 1X TE buffer (e.g.,add 10µl of QuantiFluor® RNA Dye to 3,990µl of TE buffer), and mix thoroughly.

   Low Standard Calibration: Dilute the QuantiFluor® RNA Dye 1:2,000 in 1X TE buffer (e.g., add 2µl of QuantiFluor® RNA Dye to 3,998µl of 1X TE buffer), and mix.

3. **Prepare RNA Standard Curve:**

   High Standard Curve: Prepare standards that result in 7.8–500ng/well when dispensing 10µl of standard to each well.

   Low Standard Calibration: Prepare standards that result in 0.16–10ng/well when dispensing 10µl of standard to each well. See Figure 1 and Table 1 for generating and dispensing the standard curve.

Table 1. Preparing the High- and Low-Concentration RNA Standard Curves.

	High-Concentration RNA Standard Curve Samples			Low-Concentration RNA Standard Curve Samples		
Standard	**RNA Standard Volume**	**1X TE Buffer Volume**	**Final RNA Concentration (ng/µl)**	**RNA Standard Volume**	**1X TE Buffer Volume**	**Final RNA Concentration (ng/µl)**
A	50µl	50µl	50	10µl	990µl	1.0
B	50µl of Standard A	50µl	25	50µl of Standard A	50µl	0.5
C	50µl of Standard B	50µl	12.5	50µl of Standard B	50µl	0.25
D	50µl of Standard C	50µl	6.25	50µl of Standard C	50µl	0.13
E	50µl of Standard D	50µl	3.13	50µl of Standard D	50µl	0.063
F	50µl of Standard E	50µl	1.56	50µl of Standard E	50µl	0.031
G	50µl of Standard F	50µl	0.78	50µl of Standard F	50µl	0.016

4. Pipet 200µl of QuantiFluor® RNA Dye working solution into each well.
5. Dispense 10µl of the prepared RNA standards as shown in Figure 1. For the blank, pipet 10µl of 1X TE Buffer.
6. Add 1–20µl of unknown sample to the remaining wells, recording the dilution factor. Mix the plate thoroughly.
7. Incubate for 5 minutes at room temperature, protected from light.

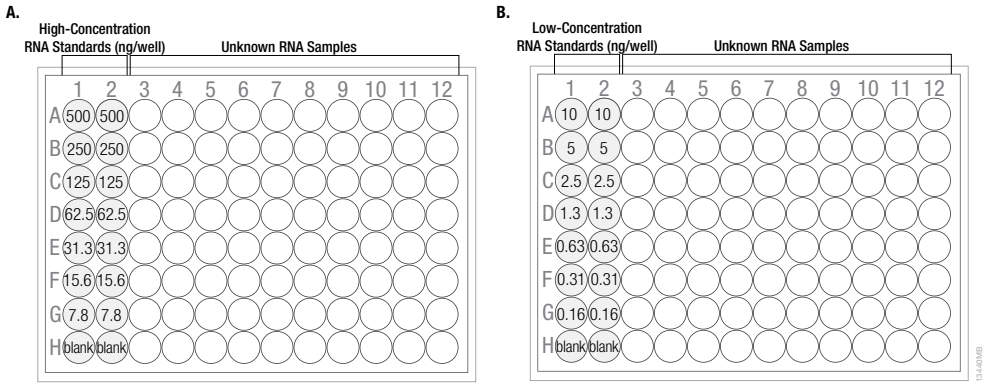

Figure 1. Dispense standard dilutions and blank samples in duplicate into Columns 1 and 2 of a multiwell plate. Panel A. High-concentration RNA standard and blank samples. **Panel B.** Low-concentration RNA standard and blank samples.

8. Measure fluorescence ($492nm_{Ex}/540nm_{Em}$). For the GloMax® Discover System, select **QuantiFluor RNA System**.
9. Calculate the RNA concentration by copying and pasting your raw fluorescence data into our online tool: **www.promega.com/resources/tools/quantifluor-dye-systems-data-analysis-workbook/**

Additional protocol information is in Technical Manual #TM377, available online at: **www.promega.com**