QuantiFluor® RNA System

Instructions for Use of Product E3310.

Quick Protocol

Materials Required

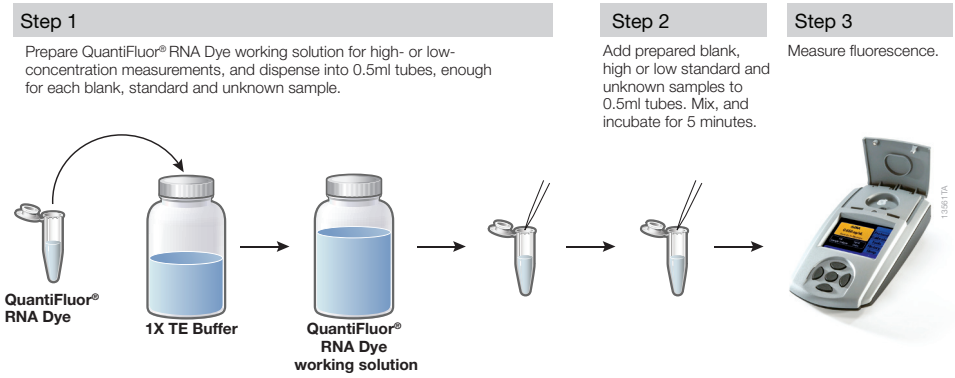
- QuantiFluor® RNA System (Cat.# E3310)
- Quantus™ Fluorometer (Cat.# E6150)
- thin-walled 0.5ml PCR tubes (Cat.# E4941 or Axygen Cat.# PCR-05-C)
- nuclease-free water

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**

Single-Tube Format 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.
2. **Prepare Working Solution:**
   High Standard Calibration: Dilute the QuantiFluor® RNA Dye 1:400 in 1X TE buffer, and mix thoroughly.
   Low Standard Calibration: Dilute the QuantiFluor® RNA Dye 1:2,000 in 1X TE buffer, and mix.
3. **Prepare Blank:** Add 200µl of QuantiFluor® RNA Dye working solution in an empty 0.5ml PCR tube. Protect tube from light.
4. **Prepare 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. 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.
5. **Prepare Unknown(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.
6. Incubate the prepared samples at room temperature for 5 minutes, protected from light.
7. Select the RNA protocol on the Quantus™ Fluorometer. If needed, calibrate the Quantus™ Fluorometer by reading the blank (Step 3) and standard (Step 4) samples in the Calibration screen, then select “Save”.
8. Enter the volume of the unknown sample (1–20µl used in Step 5) and desired concentration units.
9. Measure fluorescence of the unknown sample and record the final sample concentration results.

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

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Instructions for Use of Product E3310.

Quick Protocol

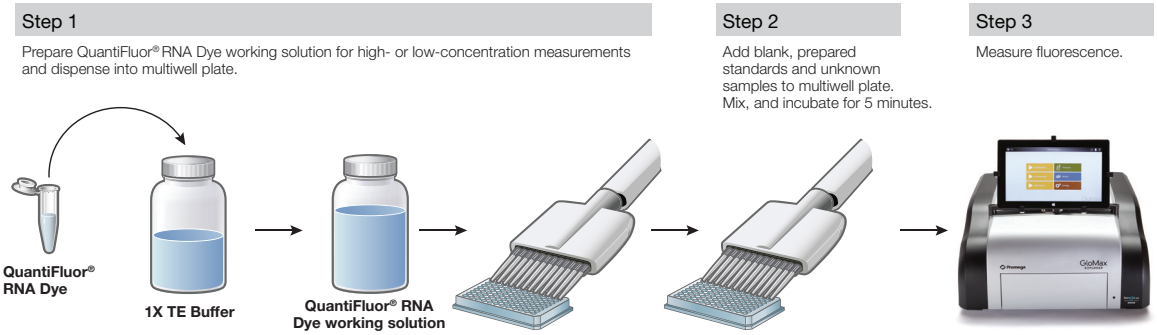
Materials Required

- 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 is available at: **www.promega.com/protocols**

Multiwell Plate Protocol



1. **Prepare 1X TE Buffer:** Dilute the 20X TE Buffer 20-fold with nuclease-free water.
2. **Prepare Working Solution:**
   High Standard Curve: Dilute the QuantiFluor® RNA Dye 1:400 in 1X TE buffer, and mix thoroughly.
   Low Standard Curve: Dilute the QuantiFluor® RNA Dye 1:2,000 in 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.
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.

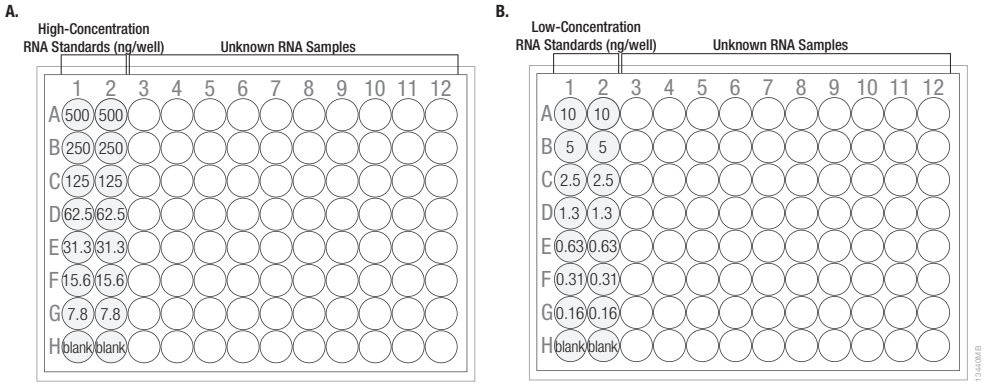


Figure 1. Dispense standard dilutions and blank samples in duplicate into Columns 1 and 2 of a multiwell plate. Panel A. High-concentration RNA standard and blank samples. **Panel B.** Low-concentration RNA standard and blank samples.

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.
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/**

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