

QuantiFluor® RNA Select System

Instructions for Use of Products **E6640 and E6780**

Single-Tube Fluorometer Protocol

Materials Required

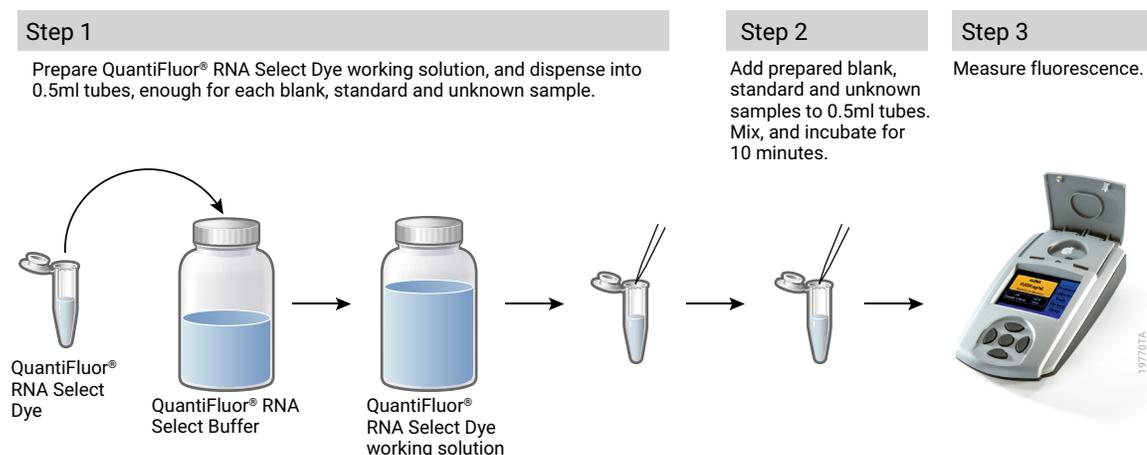
- QuantiFluor® RNA Select System (Cat.# E6640, E6780)
- Quantus™ Fluorometer (Cat.# E6150) or equivalent single-tube fluorometer
- thin-walled 0.5ml PCR tubes (Cat.# E4941)

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 Select System Technical Manual #TM773* are available at: www.promega.com/protocols

Protocol



- 1. Prepare Working Solution:** Dilute the QuantiFluor® RNA Select Dye 1:200 in QuantiFluor® RNA Select Buffer (e.g., add 10µl of QuantiFluor® RNA Select Dye to 2,000µl of QuantiFluor® RNA Select Buffer), and mix by inverting 3–4 times or vortex 5–10 seconds. Protect from light (e.g., cover with foil).

Note: The working solution is stable for 8 hours at room temperature or 1 week at 2–10°C (protected from light).

- 2. Prepare Calibration Standards:**

Blank: Prepare by adding 10µl of Nuclease-Free Water to 200µl of QuantiFluor® RNA Select Dye working solution in an empty 0.5ml PCR tube.

RNA Standard: Prepare a 1,000ng standard by adding 10µl of RNA Standard, 100ng/µl, to 200µl of QuantiFluor® RNA Select Dye working solution in a 0.5ml PCR tube.

Mix tube contents by vortexing for 1 second, pause 1 second and repeat three times. Centrifuge tubes briefly at 2,000 × g to remove bubbles.

- 3. Prepare Unknown Sample(s):** Add 1–20µl of unknown samples to 200µl of QuantiFluor® RNA Select Dye working solution in 0.5ml PCR tubes. Mix tube contents by vortexing for 1 second, pause 1 second and repeat three times. Centrifuge tubes briefly at 2,000 × g to remove bubbles.
- 4.** Incubate all tubes for 5–10 minutes at room temperature, protected from light.
- 5.** To measure fluorescence, choose the **RNA Select** protocol on the Quantus™ Fluorometer. If needed, calibrate the Quantus™ Fluorometer by reading the blank and standard (Step 2) samples in the 'Calibration' screen, then select **Save**.

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6. Enter the unknown sample volume used (1–20µl used in Step 3) and desired concentration units.
7. Measure fluorescence of the unknown sample. The number displayed is the original sample concentration.

Multiwell Plate Protocol

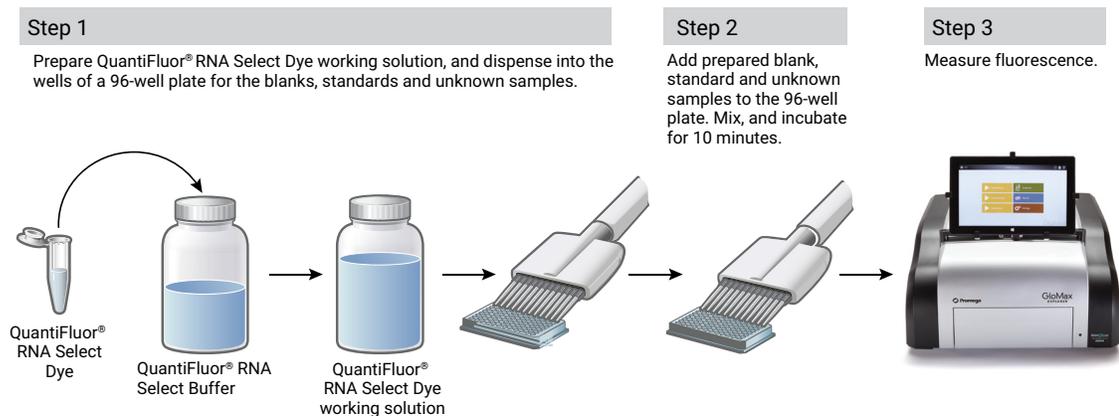
Materials Required

- multiwell detection instrument capable of measuring fluorescence (e.g., GloMax® Discover System [Cat.# GM3000])
- QuantiFluor® RNA Select System (Cat.# E6640, E6780)
- Nuclease-Free Water (Cat.# P1193)
- black, flat-bottom 96-well plates (Corning® Cat.# 3650 or equivalent)
- 1.5ml tubes

Warm all assay components to room temperature before use.

The *QuantiFluor® RNA Select System Technical Manual #TM773* are available at: www.promega.com/protocols

Protocol



1. Prepare Working Solution:

Calculate the volume needed: Number of wells × 200µl (+ 20% extra). Dilute the QuantiFluor® RNA Select Dye 1:200 in QuantiFluor® RNA Select Buffer (e.g., for 24 wells, use 30µl of QuantiFluor® RNA Select Dye + 6,000µl of QuantiFluor® RNA Select Buffer). Mix by inverting 3–4 times or vortex 5–10 seconds, and protect from light (e.g., cover with foil).

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2. Prepare RNA Standard Curve:

Prepare RNA standard curve using the RNA Standard, 10µg/ml, as described in the table below:

Standard	RNA Standard Volume	Nuclease-Free Water Volume	Final RNA Concentration (ng/µl)
A	80µl of RNA Standard, 100µg/ml	0µl	100
B	40µl of Standard A	40µl	50
C	40µl of Standard B	40µl	25
D	40µl of Standard C	40µl	12.5
E	40µl of Standard D	40µl	6.25
F	40µl of Standard E	40µl	3.13
G	40µl of Standard F	40µl	1.56
H	0µl	40µl	0

3. Pipet 200µl of QuantiFluor® RNA Select Dye working solution into each well for standards, blanks and unknown samples.

4. Dispense 10µl of RNA standards prepared in Step 2 (labeled Standards A–G) to rows A–G of the 96-well plate (see Figure 1). We recommend pipetting triplicates of the standards.

5. Add 1–20µl of unknown sample to the remaining wells, recording the volume for each sample.

6. Mix the plate using one of the two following methods:

Method 1 Orbital shaker (preferred): Shake at 200–300 rpm for 5–10 minutes visually confirming the liquid moves in a gentle swirling motion (not splashing).

Method 2 Pipette mixing: Set a P200 pipette to 100µl and mix each well three to five times with slow aspirating-dispensing cycles to minimize bubble formation.

7. Incubate for 5–10 minutes at room temperature, protected from light.

8. Measure fluorescence (648nm_{Ex}/665nm_{Em}). For the GloMax® Discover System, select **QuantiFluor RNA Select System**.

9. Calculate the RNA concentration as follows:

- Subtract blank fluorescence from all samples.
- Generate standard curve (fluorescence vs. ng/well).
- Determine sample concentration from curve.
- Apply dilution factor: Sample concentration (ng/µl) = nanogram from curve ÷ microliters sample added

Example: If curve gives 177ng and you added 5µl:

$$\text{Concentration} = 177\text{ng} \div 5\mu\text{l} = 35.4\text{ng}/\mu\text{l}$$

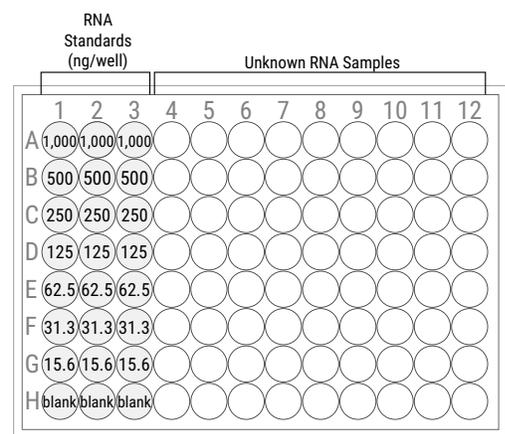


Figure 1. RNA standard curve layout on a 96-well plate for the QuantiFluor® RNA Select System.