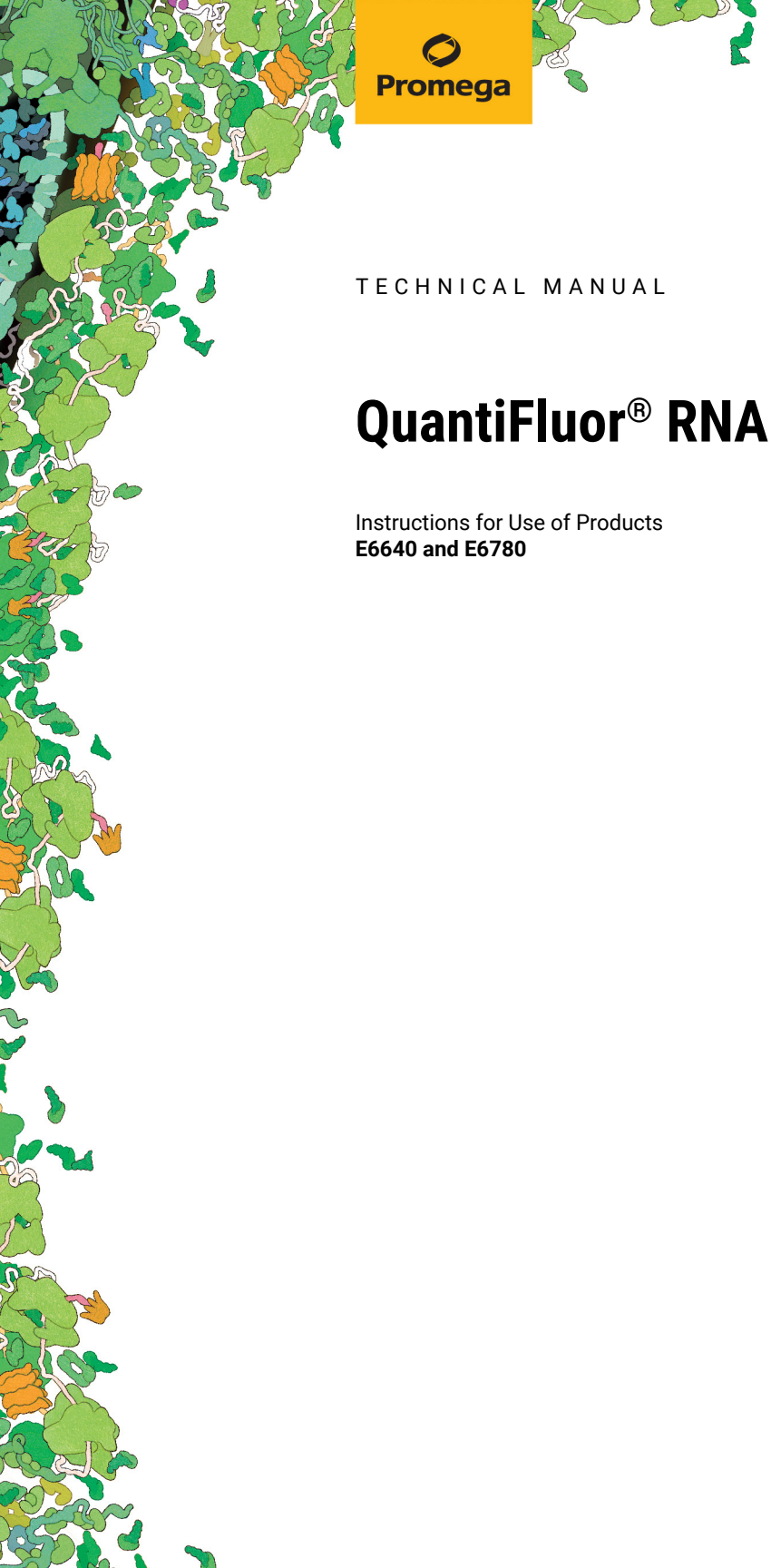

TECHNICAL MANUAL

QuantiFluor® RNA Select System

Instructions for Use of Products
E6640 and E6780

2/26
TM773

Promega

QuantiFluor® RNA Select System

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Manual.
Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The QuantiFluor® RNA Select System provides a sensitive, red-fluorescent dye-based method (648nm$_{Ex}$/665nm$_{Em}$) for quantitating purified RNA over a broad linear range of 16–1,000ng per assay well (equivalent to 1.6–100ng/µl when using a 10µl sample). The dye exhibits high specificity for RNA with minimal background, is compatible with common laboratory buffers and the working solution is stable for >8 hours at 20–25°C when protected from light. Quantitation can be performed on single-tube fluorometers (e.g., Quantus™ Fluorometer) or can be scaled up for use on instruments that read multiwell plates (e.g., GloMax® Discover System, or any reader equipped with the appropriate excitation and emission wavelengths). Resulting data are suitable to prepare samples for downstream applications such as RT-qPCR, RNA-seq and library preparation.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
QuantiFluor® RNA Select System	**100 reactions**	**E6640**

Contains sufficient reagents for 100 reactions. Includes:

- 1 × 22ml QuantiFluor® RNA Select Buffer
- 1 × 0.11ml QuantiFluor® RNA Select Dye
- 2 × 10µg RNA Standard, 100µg/ml
- 1 × 1.25ml Nuclease Free Water

PRODUCT	SIZE	CAT.#
QuantiFluor® RNA Select System	**1,000 reactions**	**E6780**

Contains sufficient reagents for 1,000 reactions. Includes:

- 1 × 200ml QuantiFluor® RNA Select Buffer
- 1 × 1.0ml QuantiFluor® RNA Select Dye
- 1 × 100µg RNA Standard, 100µg/ml
- 1 × 25ml Nuclease-Free Water

Storage Conditions: Upon receipt, store the QuantiFluor® RNA Select Dye and RNA Standard, 100µg/ml, at –30°C to –10°C, protected from light. Store the QuantiFluor® RNA Select Buffer at –30°C to +10°C, protected from light.

Stability Information:

System or Component	–30°C to –10°C Storage	+2°C to +10°C Storage	Notes
QuantiFluor® RNA Select System	≥2 years	N.A.	
RNA Standard, 100µg/ml	≥2 years	≥6 months	
QuantiFluor® RNA Select Dye	≥2 years	≥6 months	
QuantiFluor® RNA Select Buffer	≥2 years	≥6 months	Stable for ten freeze-thaw cycles from –20°C to +20°C; we recommend dispensing into several aliquots.

N.A. = not applicable

Prepared Reagents Storage Conditions:

- The QuantiFluor® RNA Select Dye working solution (mixture of diluted dye and buffer) is stable for 8 hours at 20–25°C or for 1 week at 4°C, protected from light.
- RNA standards and sample unknowns mixed with working solution are stable for 8 hours at 20–25°C.

3. Before You Begin

Assay Considerations

- Prepare an RNase-free environment in a controlled laboratory setting designed to prevent RNA degradation by eliminating ribonuclease (RNase) contamination through the use of dedicated equipment, RNase-free consumables and strict cleaning and handling procedures.
- Protect the working solution from light throughout the procedure.
- Set fluorometer at $648nm_{Ex}/665nm_{Em}$ or in the "Red" Ex/Em range.
- Prepare a standard tube or multipoint standard curve as listed below.
- Pipet carefully and accurately. Accurate pipetting depends on proper calibration, correct technique and consistent environmental conditions. Always use a pipettor where the desired volume falls within the middle 35–100% of its range (e.g., use a P200 for 50–200µl, not a P1000). The QuantiFluor® RNA Select Buffer and concentrated QuantiFluor® RNA Select Dye are slightly viscous and have a higher surface tension than other QuantiFluor® products. The QuantiFluor® RNA Select Buffer contains detergent. To minimize bubbles and pipet accurately, slower aspiration and dispense cycles are important for reproducible and accurate results when handling these components.

4. Protocol for Quantitating RNA with a Single-Tube Quantus™ Fluorometer

Materials to Be Supplied By the User

- Quantus™ Fluorometer (Cat.# E6150) or equivalent handheld fluorometer
- thin-walled 0.5ml PCR tubes (Cat.# E4941 or Axygen Cat.# PCR-05-C), or equivalent

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.

Instructions for use of the Quantus™ Fluorometer can be found in the *Quantus™ Fluorometer Operating Manual* #TM396 available at: **www.promega.com/protocols**

Notes:

a. Other single-tube fluorometers can be used with the QuantiFluor® RNA Select System if capable of measuring 648nm$_{Ex}$/665nm$_{Em}$ wavelengths and calibrated using the manufacturer's instructions.

b. If the Quantus™ Fluorometer was previously calibrated, you may not need to calibrate it again. If your fluorometer has been already calibrated, do not prepare blank and standard samples, and skip Steps 2, 3 and 6.

4. Protocol for Quantitating RNA with a Single-Tube Fluorometer (Quantus™ Fluorometer)

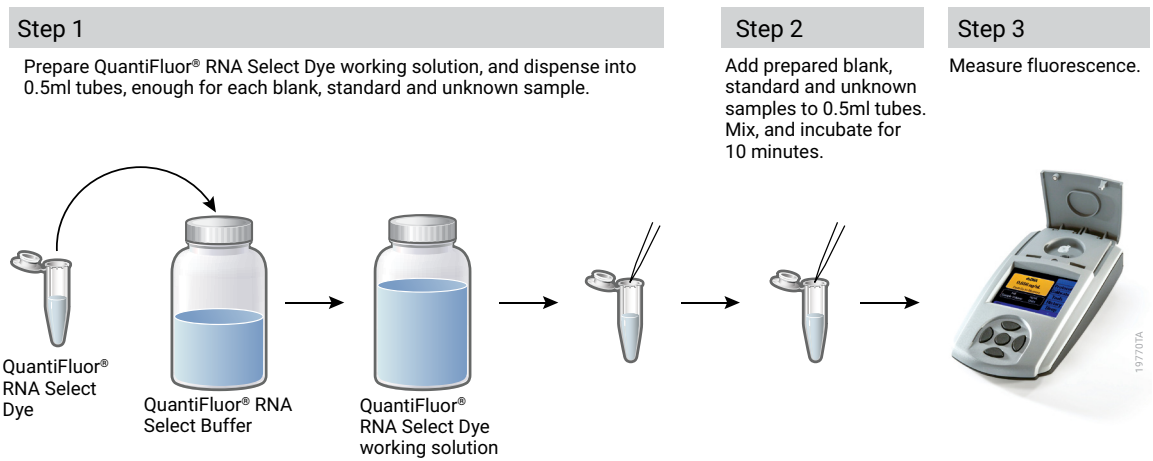

Figure 1. Overview of the single-tube format quantitation using the QuantiFluor® RNA Select System.

1. **Prepare Working Solution:** Dilute the QuantiFluor® RNA Select Dye 1:200 in QuantiFluor® RNA Select Buffer. For example, add 10µl of QuantiFluor® RNA Select Dye to 2,000µl of QuantiFluor® RNA Select Buffer. Mix by inverting the tube three to four times, or vortex at high speed for 5–10 seconds. Protect the working solution from light by covering it with foil or placing in the dark.

   Note: The QuantiFluor® RNA Select Dye working solution is stable for 8 hours at 20–25°C. It can also be stored for 1 week at 2–10°C protected from light.

2. **Prepare Blank Sample:** Prepare a blank standard by adding 10µl of Nuclease-Free Water provided to 200µl of QuantiFluor® RNA Select Dye working solution in an empty 0.5ml PCR tube. Vortex tube at high speed for 1 second, pause for 1 second, and repeat this cycle three times. Centrifuge briefly at 2,000 × *g* in a benchtop centrifuge to remove bubbles from the bottom of the tube.

3. **Prepare RNA Standard Sample:** Prepare a 1,000ng standard by adding 10µl of the provided RNA Standard (100ng/µl) to 200µl of QuantiFluor® RNA Select Dye working solution in an empty 0.5ml PCR tube. Vortex tube at high speed for 1 second, pause for 1 second and repeat this cycle three times. Centrifuge briefly at 2,000 × *g* in a benchtop centrifuge to remove bubbles from the bottom of the tube.

4. **Prepare Unknown(s):** Add 1–20µl of unknown sample(s) to 200µl of QuantiFluor® RNA Select working solution in 0.5ml PCR tubes. Vortex each unknown tube at high speed for 1 second, pause for 1 second and repeat this cycle for a total of three times. Centrifuge briefly at 2,000 × *g* in a benchtop centrifuge to remove bubbles from the bottom of the tube.

5. Incubate the prepared samples at room temperature for 5–10 minutes, protected from light.

6. Select the **RNA Select** protocol on the Quantus™ Fluorometer. If needed, calibrate the Quantus™ Fluorometer by reading the blank (prepared in Step 2) and standard (prepared in Step 3) samples in the Calibration screen, then select **Save.**

Note: Quantus™ instruments running firmware version 2.27 or later will have the RNA Select protocol preloaded. Quantus™ instruments with earlier firmware versions will not have the RNA Select protocol, but you can easily create a user-defined protocol to run the assay. See the *Quantus™ Fluorometer Operating Manual* #TM396, Section 5.B, for detailed information on creating a user-defined protocol. To modify a user-defined protocol for the QuantiFluor® RNA Select System, change the numeric value to '5.0' and the dye channel to 'Red'. Save the new protocol under an appropriate name.

7. Enter the volume of the unknown sample that was added (1–20µl) in Step 4 and desired concentration units.

8. Measure fluorescence of the unknown sample. The number displayed represents the concentration of the original sample.

5. Protocol for Quantitating RNA in Multiwell Plates

Materials to Be Supplied by the User

- multiwell plate detection instrument capable of measuring fluorescence (e.g., GloMax® Discover System [Cat.# GM3000] or GloMax® Explorer System [Cat.# GM3500])
- black flat-bottom 96-well plates (e.g. Corning® Cat.# 3650 or equivalent)
- Nuclease-Free Water (Cat.# P1193)

Instructions for use of the GloMax® Discover System can be found in the *GloMax® Discover System Operating Manual* #TM397, available at: **www.promega.com/protocols**

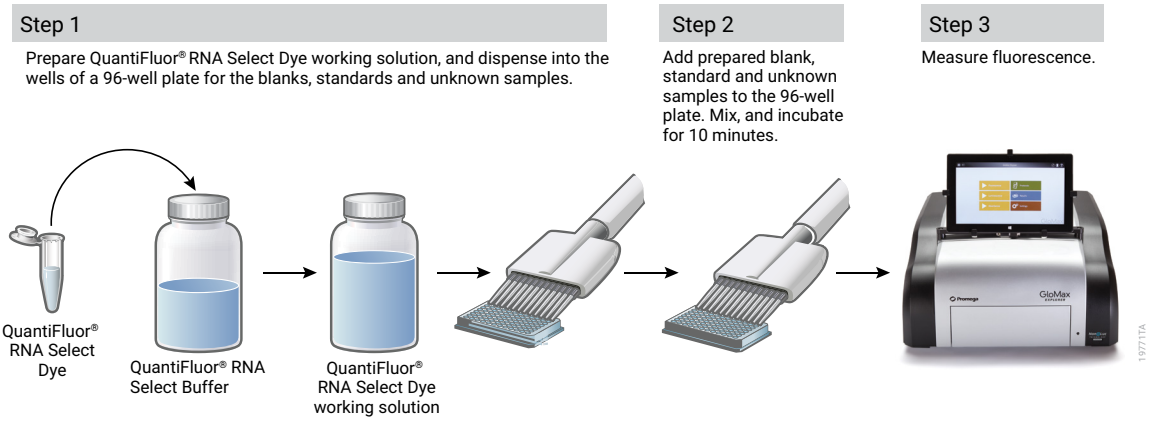

Figure 2. Overview of multiwell plate quantitation protocol using the QuantiFluor® RNA Select System.

Warm all assay components to room temperature before use.

Caution: Wear gloves, a lab coat and eye protection when working with these or any chemical reagents.

The QuantiFluor® RNA Select System can accurately assess RNA concentration over a broad dynamic range. For samples 15.6–1,000ng/well, prepare working solution and a standard curve by following instructions below. In addition, use a blank sample containing Nuclease-Free Water to assess the background level of the assay.

1. **Prepare Working Solution:** Prepare enough QuantiFluor® RNA Select Dye working solution to quantitate blank, standard and unknown samples. Protect the working solution from light by covering it with foil or placing in the dark. For example, 24 wells of a standard curve would require 6,000µl total [(24 × 200µl)+ 20% extra = 6,000µl].

   Dilute the QuantiFluor ® RNA Select Dye 1:200 in QuantiFluor® RNA Select Buffer to make the working solution. For example, add 30µl of QuantiFluor® RNA Select Dye to 6,000µl of QuantiFluor® RNA Select Buffer, and mix by inverting the tube three to four times or vortex at high speed for 5–10 seconds.

   Note: The QuantiFluor® RNA Select Dye working solution is stable for 8 hours at 20–25°C. It can also be stored for 1 week at 2–10°C protected from light, though some loss of signal should be expected during extended storage.

2. **Prepare RNA Standard Curve:** The following recommendation results in triplicate standards of 15.6–1,000ng/well, and are designed for optimal pipetting accuracy, transferring 10µl of standard to each well.

   a. Prepare eight 1.5ml tubes (or individual wells in a 96-well plate) labeled: 1,000, 500, 250, 125, 62.5, 31.3, 15.6 and 0.

   b. Prepare RNA standards by serially diluting RNA Standard,100µg/ml, in Nuclease-Free Water as shown in Table 1.

Table 1. Preparing RNA Standard Curve Samples.

Standard	Volume of RNA Standard	Volume of Water	Final RNA Concentration (ng/µl)
A	80µl	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 that is intended for an unknown, blank or standard sample.

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

5. For the blank, pipet 10µl of Nuclease-Free Water into row H.

6. Add 1–20µl of unknown sample to the remaining wells.

5. Protocol for Quantitating RNA in Multiwell Plates (continued)

7. Record the volume that was used for each unknown sample. The volume will be used when calculating the unknown sample concentration.

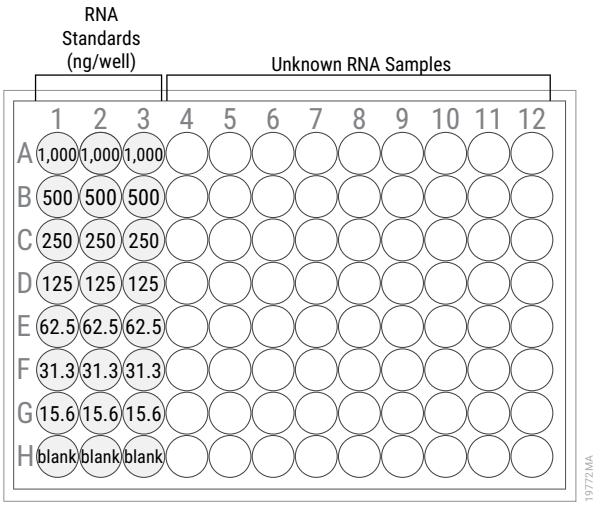

Figure 3. RNA standard curve layout on a 96-well plate for the QuantiFluor® RNA Select System.

8. After sample addition mix contents by orbital shaking at 200–300rpm for 5–10 minutes to ensure uniform distribution of reagents and consistent fluorescence signal across all wells. Confirm the orbital shaker rpm settings by visual observation. Observe the well contents during operation to ensure that liquid movement produces a gentle, uniform swirling motion or slight vortex in each well, indicating adequate mixing. Avoid excessive shaking or vortexing, as this may introduce air bubbles or cause splashing that interferes with fluorescence measurements or leads to cross contamination between adjacent wells If unable to mix by plate shaking pipette mixing, is also acceptable. We recommend setting a P200 pipette to 100µl and mixing each well 3–5 times with slow aspiration and dispense cycles to minimize bubbles.
9. Incubate assays for 5–10 minutes at room temperature, protected from light.
10. Measure fluorescence ($648nm_{Ex}/665nm_{Em}$) using your plate reader. If using the GloMax® Discover or Explorer Systems, select the preloaded protocol: QuantiFluor® RNA Select System.
11. Calculate the RNA concentration as follows: Subtract the fluorescence of the blank sample (water) from all of the standard and unknown samples. Use the corrected data from the RNA standards to generate a standard curve of fluorescence versus RNA concentration. Determine the RNA concentration of the sample from the standard curve and multiply the resulting number by the dilution factor, if applicable.

6. Representative Data and Analysis

Table 2. Representative Data for the RNA Standard Curve and QuantiFluor® RNA Select Dye in 96-Well Plate Format.

RNA Standard Mass (ng/well)	Average Fluorescence (RFU)[1]	Signal-to-Noise Ratio
0	0	1.0
15.6	20	1.3
31.3	41	1.6
62.5	84	2.2
125	174	3.5
250	357	6.1
500	734	11.4
1,000	1,448	21.5

[1]Background fluorescence was subtracted. n = 3.

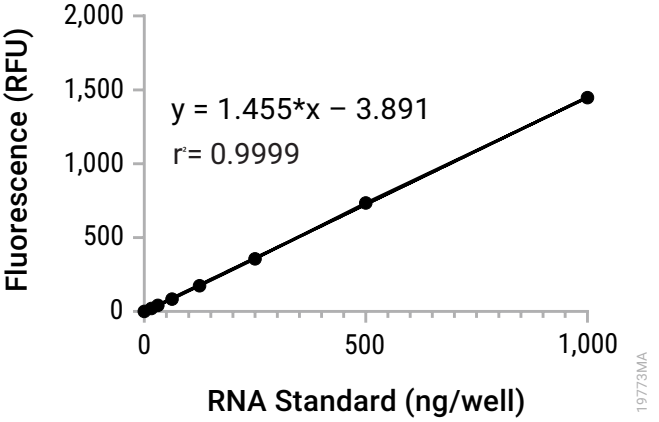

Figure 4. Typical RNA standard curve (96-well format). Linear regression: y = mx + b.

The concentration of each sample was determined using the equation of the standard curve, expressed as y = mx + b, where y is the measured response (e.g., absorbance or fluorescence), m is the slope of the line, x is the concentration, and b is the y-intercept. The sample concentration was calculated by substituting the measured response into the equation and solving for x using x = (y – b)/m. In cases where the standard curve passes through the origin (i.e., b = 0), the equation simplifies to x = y/m. This approach allows determination of the unknown sample concentration in the same units as the standards based on the linear relationship between signal and concentration.

6. Representative Data and Analysis (continued)

The standard and unknown samples have these average fluorescence values (in RFU):

	Unknown Sample	Standard Samples (ng)							
		0	15.6	31.3	62.5	125	250	500	1,000
Fluorescence	325	71	91	112	155	245	428	805	1,518

1. Subtract the water blank (average of blank standards) from all samples:

	Unknown Sample	Standard Samples (ng)							
		0	15.6	31.3	62.5	125	250	500	1,000
Fluorescence	254	0	20	41	84	174	357	734	1,448

2. Determine the linear regression from the standard curve (Figure 4): y = 1.455x – 3.891
3. Calculate the RNA concentration of the unknown sample in the 200µl assay volume by solving for x in the linear regression equation, where:

   y = 254

   x = (y + 3.891)/1.455 = 177ng
4. Account for any dilution of the unknown sample. For example, if 1µl of sample was added per well, the sample concentration is 177ng/µl. If 5µl of sample was added per well, the sample concentration is 177ng ÷ 5µl = 35.4ng/µl.

6.A. Standard Curve and Other Analysis Models

Fluorescence concentration data for the QuantiFluor® RNA Select System are analyzed using linear regression with a floating y intercept (b; if quantifying samples near the low end of the range and background signal is negligible, constrain the fit to pass through the origin, b = 0). When the true background is ~0, floating-intercept fits can introduce bias at low concentrations by estimating a small non-zero intercept. Constraining b = 0 aligns the model with the physical expectation and reduces that bias under these conditions. Depending on assay conditions and dynamic range, alternative nonlinear regression models (e.g., four-parameter logistic) may be appropriate.

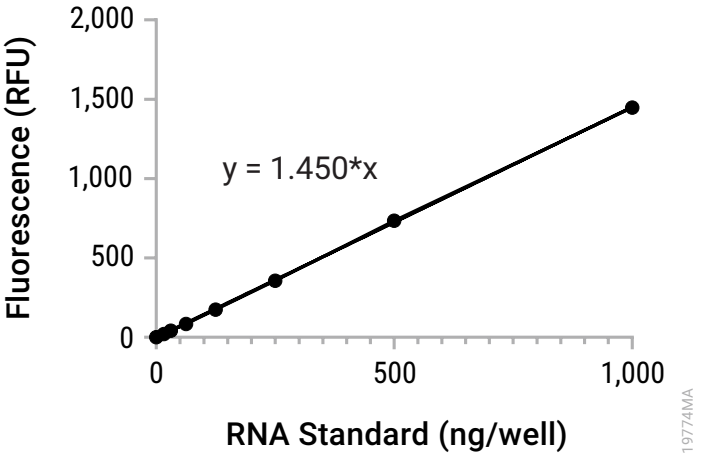

Figure 5. Alternate 96-well standard curve. Linear regression – y-intercept = 0: y = mx + 0.

7. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

Symptoms	Causes and Comments
High variance in replicate standard or unknown sample wells	Thaw all reagents, and warm to room temperature. Mix all reagents well.
	Pipet accurately, with calibrated pipettes. Aspirate and dispense liquid volumes slowly and carefully.
	Ensure complete assay volume is mixed well after RNA standard or sample is added. If using a pipette to mix, set pipette to 100µl and mix for five to seven complete aspiration/dispense cycles. Do this slowly and carefully so bubbles are not introduced to the well. If plate mixing, set mixer at a speed to produce some reasonable fluid movement such as 250–300rpm, and mix for 10 minutes. Read the plate on a plate reader. Mix by pipetting (100µl, three to five cycles), and read plate again. Similar well values will confirm that plate mixing speed and time are sufficient.
Low or no fluorescence detected in the RNA standard	Evaluate the performance of the fluorometer with an RNA sample of known concentration (e.g., RNA Standard) using the appropriate excitation and emission wavelengths for QuantiFluor® RNA Select Dye.
	Check that the standard samples were diluted appropriately.
	Reagent exposed to light. Reevaluate samples.
	Unknown sample below limit of detection. Use more concentrated sample and evaluate again.
	Unknown sample calculations performed incorrectly. Redo sample calculations.
	Working solution prepared more than 1 week before use. Prepare fresh working solution and measure RNA sample again.
Fluorescence too high	Check that the unknown and standard samples were diluted appropriately. Decrease the concentration of the unknown and standard samples if necessary.
	Adjust the gain setting on your fluorometer so that the highest point on the standard curve is approximately 90% of maximum signal. This is not necessary for the GloMax® Detection Systems because these instruments will adjust automatically. The Quantus™ Fluorometer does not require gain adjustment.

Symptoms	Causes and Comments
RNA concentration determined using QuantiFluor differed from that determined using alternative method	Use a nonlinear standard curve.

8. Appendix

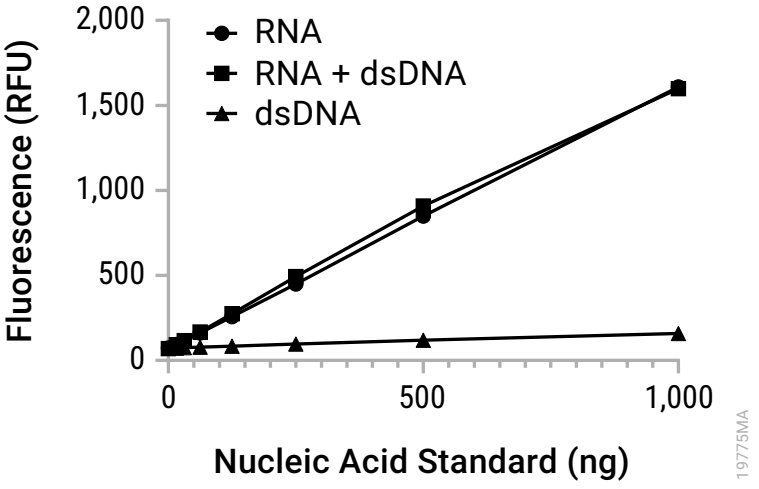

Figure 6. Selectivity and sensitivity of the QuantiFluor® RNA Select System. Triplicate 10µl samples containing either RNA Standard, Lambda dsDNA or a 1:1 RNA:dsDNA mixture were assayed using the QuantiFluor® RNA Select System. Fluorescence was recorded at 627nm (excitation) and 660–720nm (emission) and plotted against the nucleic acid mass. The assay demonstrated strong selectivity for RNA over dsDNA, with replicate measurements exhibiting ≤5% coefficient of variation (% CV), indicating high precision.

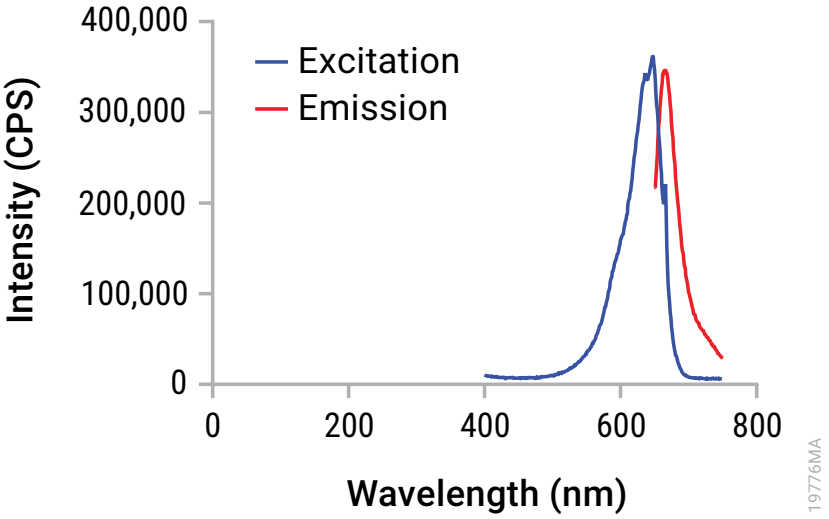

Figure 7. Excitation and emission spectra of the QuantiFluor® RNA Select Dye. The QuantiFluor® RNA Select Dye exhibits maximum excitation at approximately 648nm and maximum emission at approximately 665nm.

Instrument Compatibility: Fluorescence measurements can be performed on any fluorescence reader capable of detecting at these wavelengths. For optimal performance with the QuantiFluor® RNA Select System, set the excitation and emission filters to $648nm_{Ex}$ and $665nm_{Em}$, respectively.

8. Appendix (continued)

Table 3. Effect of Potentially Interfering Compounds on the QuantiFluor® RNA Select Assay. Each listed compound (10µl) was added to a 200µl assay reaction to assess possible interference with RNA quantitation. Results were reported as: OK = <10% quantitation error, indicating no significant interference; NR = >10% quantitation error, indicating a less reliable measurement due to compound interference.

Contaminant	Final Assay Concentration	Concentration in 10µl sample	Result[1]
Sodium chloride	10mM	200mM	OK
Magnesium chloride	2mM	40mM	OK[2]
Sodium acetate	10mM	200mM	OK[2]
Ammonium acetate	10mM	200mM	OK
Potassium phosphate (pH 7.4)	5mM	100mM	OK
Ethanol	0.3%	6%	OK
Guanidine thiocyanate	1mM	20mM	OK
SDS	0.01%	0.2%	NR
Triton® X-100	0.001%	0.02%	OK
Bovine serum albumin	5ng/µl	100ng/µl	OK
ssDNA (oligo)	1X[3]	1X[3]	OK
dNTPs (mixture of four)	1X[3]	1X[3]	OK
rNTPs (mixture of four)	1X[3]	1X[3]	OK
dsDNA (Lambda)	3X[3]	3X[3]	OK

[1]RNA standards over a range of 16–1,000ng/assay were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations of 10µl sample volumes are also listed. Results are listed as OK, less than 10% perturbation, or as NR, not recommended.

[2]An acceptable result, but with some distortion of the standard curve; for best results, add the same amount of contaminant to the standard samples.

[3]1X indicates a concentration equal to the concentration of RNA Standard.

8.A. Additional Assay Formats

Access additional experimental design considerations, assay formats and related products through the product page, available at: **www.promega.com**

8.B. Reference

1. Application Notes on QuantiFluor® Dye Systems are available at: **www.promega.com/resources/application-notes/**