

TECHNICAL MANUAL

QuantiFluor® RNA System

Instructions for Use of Product
E3310



QuantiFluor[®] RNA System

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

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1. Description

The QuantiFluor[®] RNA System^(a) contains a fluorescent RNA-binding dye (492nm_{EX}/540nm_{EM}) that enables sensitive quantitation of small amounts of RNA in purified samples. The assay is linear over a range of 0.1–500ng of RNA input (0.1–500ng/μl from 1μl of original sample).

The dye-based system provides concentrated QuantiFluor[®] RNA dye, dilution buffer and RNA standard. Simply dilute the dye, add standards and unknown samples, and read on a fluorometer. The QuantiFluor[®] RNA System can be used with any single-tube fluorometer (e.g., Quantus[™] Fluorometer [Section 3]) or may be scaled up for use in instruments that can read multiwell plates (e.g., GloMax[®] Discover System [Section 4]) at the appropriate excitation and emission wavelengths.



2. Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|-------------------------|------|-------|
| QuantiFluor® RNA System | 1ml | E3310 |

This system contains sufficient reagents for 2,000 assays at a 200µl scale. Includes:

- 25ml 20X TE Buffer (pH 7.5)
- 1ml QuantiFluor® RNA Dye
- 100µg RNA Standard, 100µg/ml

Storage Conditions: Store the QuantiFluor® RNA Dye at –30°C to –10°C, protected from light. Store the RNA Standard, 100µg/ml, below –10°C. Store the 20X TE Buffer at –30°C to +30°C.



Instructions for handling and disposal of QuantiFluor® RNA Dye are located in Section 7.E.

3. Protocol for Quantitating RNA in a Single Tube Using the Quantus™ Fluorometer

Materials to Be Supplied by the User

- nuclease-free water
- thin-walled 0.5ml PCR tubes (Cat.# E4941 or Axygen Cat.# PCR-05-C)
- **optional:** RQ1 RNase-Free DNase (Cat.# M6101) to remove contaminating DNA; Section 7.C

Warm all assay components to room temperature before use. The QuantiFluor® RNA Dye is dissolved in 100% DMSO and frozen at or below 4°C. Prior to dilution, thaw dye at room temperature, protected from light.

The QuantiFluor® RNA System enables accurate detection of RNA over a broad dynamic range. Quantitating unknown samples requires instrument calibration using a blank and a single standard sample. Prepare a standard sample using the 1.2kb RNA Standard. This standard should be appropriate for the expected range of nucleic acid concentrations for your unknown samples. For samples in the range of 10–500ng/µl, prepare working solution and standard sample by following instructions for the “High Standard Calibration.” For lower level samples in the range 0.1–10ng/µl, prepare working solution and a standard sample according to the “Low Standard Calibration” instructions. Instructions for use of the Quantus™ Fluorometer can be found in the *Quantus™ Fluorometer Operating Manual* #TM396 available at:

www.promega.com/protocols

We recommend preparing a standard curve that extends above and below the likely concentration range for your unknown samples. In addition, a blank sample containing 1X TE buffer should be used to assess the background level of the assay.

Note: Other single-tube fluorometers can be used with the QuantiFluor® RNA System if capable of measuring the following wavelengths (492nm_{ex}/540nm_{em}) and calibrated using manufacturer’s instructions.

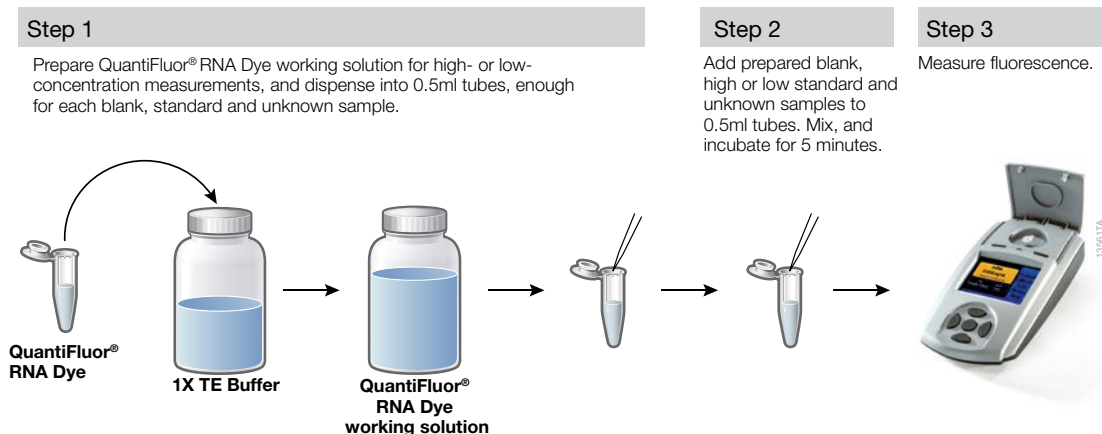


Figure 1. Overview of the single-tube format quantitation using the QuantiFluor® RNA System.

Note: If the Quantus™ Fluorometer was previously calibrated, you may not need to calibrate it again. Therefore, do not prepare blank and standard samples, and skip Steps 3, 4 and 8.

- 1. Prepare 1X TE buffer:** Dilute the 20X TE Buffer 20-fold with nuclease-free water (not provided). For example, add 1ml of 20X TE Buffer to 19ml of Nuclease-Free Water (Cat.# P1195), and mix.

- 2. Prepare Working Solution:**

High Standard Calibration: Dilute the QuantiFluor® RNA Dye 1:400 in 1X TE buffer to make the QuantiFluor® RNA Dye working solution. For example, add 10µl of QuantiFluor® RNA Dye to 3,990µl of 1X TE buffer, and mix thoroughly.

Low Standard Calibration: Dilute the QuantiFluor® RNA Dye 1:2,000 in 1X TE buffer to make the QuantiFluor® RNA Dye working solution. For example, add 2µl of QuantiFluor® RNA Dye to 3,998µl of 1X TE buffer, and mix thoroughly.

- !** Protect the working solution from light by covering with foil or placing in the dark.

Note: The QuantiFluor® Dye working solution is stable for 2–3 hours at 25°C.

- 3. Prepare Blank Sample:** Add 200µl of QuantiFluor® RNA Dye working solution to an empty 0.5ml PCR tube. This will be the blank sample used in Step 8. Protect tube from light.

- 4. Prepare RNA Standard Sample:**

High Standard Calibration: Prepare a 500ng standard by adding 5µl of the provided RNA Standard (100ng/µl) 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 first diluting the RNA Standard (100ng/µl) 1:100 in 1X TE buffer (10µl of RNA Standard + 990µl of 1X TE buffer). Next, add 10µl of diluted standard to 200µl of QuantiFluor® RNA Dye working solution in a 0.5ml PCR tube.

3. Protocol for Quantitating RNA in a Single Tube Using the Quantus™ Fluorometer (continued)

5. **Prepare Unknown Sample:** Add 1–20µl of unknown sample to 200µl of QuantiFluor® RNA Dye working solution in a 0.5ml PCR tubes. For example, add 1µl sample to 200µl QuantiFluor® RNA Dye working solution. Vortex well, and protect tube from light.

Note: See Section 7.D, Tips for Pipetting Small Volumes, if you are pipetting 1µl samples. Alternatively, minimize pipetting error by using >1µl of sample.

6. Incubate the prepared samples at room temperature for 5 minutes, protected from light.
7. Select the RNA protocol on the Quantus™ Fluorometer. Depending on which standard calibration you prepared, select “High” or “Low.”
8. If needed, calibrate the Quantus™ Fluorometer by reading the blank (prepared in Step 3) and standard (prepared in Step 4) samples in the Calibration screen, and then select “Save”.
9. Enter the volume of the unknown sample and desired concentration units.
Note: This volume is the amount of sample that is added for the quantitation. For example, if 2µl of sample was mixed with 200µl of QuantiFluor® RNA Dye working solution, then the volume entered on this screen should be 2µl.
10. Measure fluorescence of the unknown sample using the Quantus™ Fluorometer. The number displayed represents concentration of the original sample.

4. 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])
- Nuclease-Free Water (Cat.# P1195)
- black flat-bottom 96-well plates
- **optional:** RQ1 RNase-Free DNase (Cat.# M6101) to remove contaminating DNA; Section 7.C

Warm all assay components to room temperature before use. The QuantiFluor® RNA Dye is dissolved in 100% DMSO and frozen at or below 4°C. Prior to dilution, thaw dye at room temperature, protected from light.

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

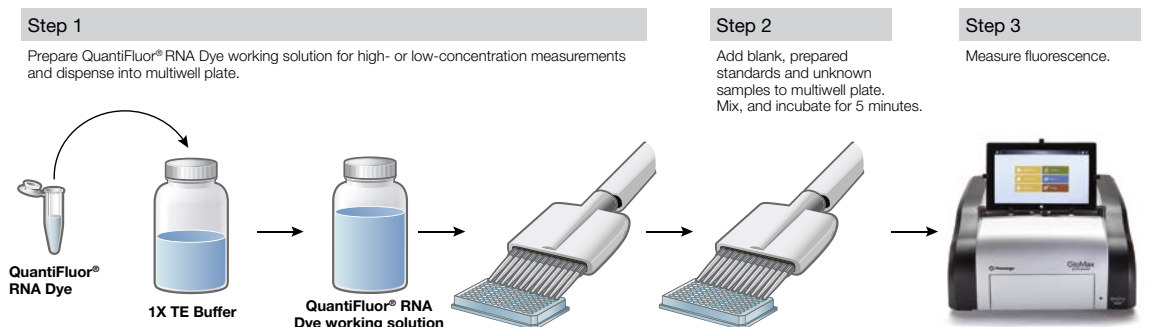


Figure 2. Overview of multiwell plate quantitation protocol using the QuantiFluor® RNA System.

The QuantiFluor® RNA System can accurately assess RNA concentration over a broad dynamic range. For samples 10–500ng/μl, prepare working solution and a standard curve by following instructions for the “High-Concentration Standard Curve.” For lower concentration samples (0.1–10ng/μl), prepare working solution and a standard curve according to the “Low-Concentration Standard Curve” instructions.

The following protocols are examples of how to prepare standard curves of two different concentration ranges. We recommend preparing a standard curve that extends above and below the likely concentration range for your unknown samples. In addition, a blank sample containing 1X TE Buffer should be used to assess the background level of the assay.

1. **Prepare 1X TE buffer:** Dilute the 20X TE Buffer 20-fold with nuclease-free water (not provided). For example, add 1ml of 20X TE Buffer to 19ml of Nuclease-Free Water (Cat.# P1195), and mix.
2. **Prepare Working Solution:** Prepare enough QuantiFluor® RNA Dye working solution to quantitate blank, standard and unknown samples. Protect the working solution from light by covering with foil or placing in the dark.

High Standard Curve (for samples 10–500ng/μl): Dilute the QuantiFluor® RNA Dye 1:400 in 1X TE buffer to make the QuantiFluor® RNA Dye working solution. For example, add 10μl of QuantiFluor® RNA Dye to 3,990μl of 1X TE buffer, and mix thoroughly.

Low Standard Curve (for samples 0.1–10ng/μl): Dilute the QuantiFluor® RNA Dye 1:2,000 in 1X TE buffer to make the QuantiFluor® RNA Dye working solution. For example, add 2μl of QuantiFluor® RNA Dye to 3,998μl of 1X TE buffer, and mix thoroughly.



Note: The QuantiFluor® RNA Dye working solution is stable for 2–3 hours at 25°C.

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

3. Prepare RNA Standard Curve:

High-Concentration Standard Curve: The following recommended standards result in 7.8–500ng/well, and are designed for optimal pipetting accuracy, transferring 10µl of standard to each well.

- Prepare seven 1.5ml tubes labeled: 500, 250, 125, 62.5, 31.3, 15.6, 7.8.
- Prepare RNA standards by serially diluting QuantiFluor® RNA Standard (100ng/µl) as shown in Table 1. Take care to not introduce air bubbles.

Table 1. Preparing High-Concentration RNA Standard Curve Samples.

| Standard | Volume of RNA Standard | Volume of 1X TE Buffer | Final RNA Concentration (ng/µl) |
|----------|------------------------|------------------------|---------------------------------|
| A | 50µl | 50µl | 50 |
| B | 50µl of Standard A | 50µl | 25 |
| C | 50µl of Standard B | 50µl | 12.5 |
| D | 50µl of Standard C | 50µl | 6.25 |
| E | 50µl of Standard D | 50µl | 3.13 |
| F | 50µl of Standard E | 50µl | 1.56 |
| G | 50µl of Standard F | 50µl | 0.78 |

Low-Concentration Standard Curve: The following recommended standards result in 0.16–10ng/well and are designed for optimal pipetting accuracy, transferring 10µl of standard to each well.

- Prepare seven 1.5ml tubes labeled: 10, 5, 2.5, 1.3, 0.63, 0.31, 0.16.
- Prepare RNA standards by serially diluting QuantiFluor® RNA Standard (100ng/µl) as shown in Table 2. Take care to not introduce air bubbles.

Table 2. Preparing a Low-Concentration RNA Standard Curve.

| Standard | Volume of RNA Standard | Volume of 1X TE Buffer | Final RNA Concentration (ng/µl) |
|----------|------------------------|------------------------|---------------------------------|
| A | 10µl | 990µl | 1.0 |
| B | 50µl of Standard A | 50µl | 0.5 |
| C | 50µl of Standard B | 50µl | 0.25 |
| D | 50µl of Standard C | 50µl | 0.13 |
| E | 50µl of Standard D | 50µl | 0.063 |
| F | 50µl of Standard E | 50µl | 0.031 |
| G | 50µl of Standard F | 50µl | 0.016 |

4. Pipet 200µl of QuantiFluor® RNA Dye working solution into each well that is intended for an unknown, blank or standard sample.
5. Dispense 10µl of the high-concentration RNA standards prepared in Table 1 (labeled Standards A–G) to rows A–G of the 96-well plate (Figure 3, Panel A). Alternatively, dispense 10µl of the low-concentration RNA standards prepared in Table 2 (labeled Standards A–G) to rows A–G of the 96-well plate (Figure 3, Panel B). We recommend pipetting duplicates or triplicates of the standards.
6. For the blank, pipet 10µl of 1X TE buffer into row H.
7. Add 1–20µl of unknown sample to the remaining wells.



Record the dilution factor that was used for each unknown sample. The dilution factor will be used when calculating the concentration of the unknown sample (Section 5, Step 4).

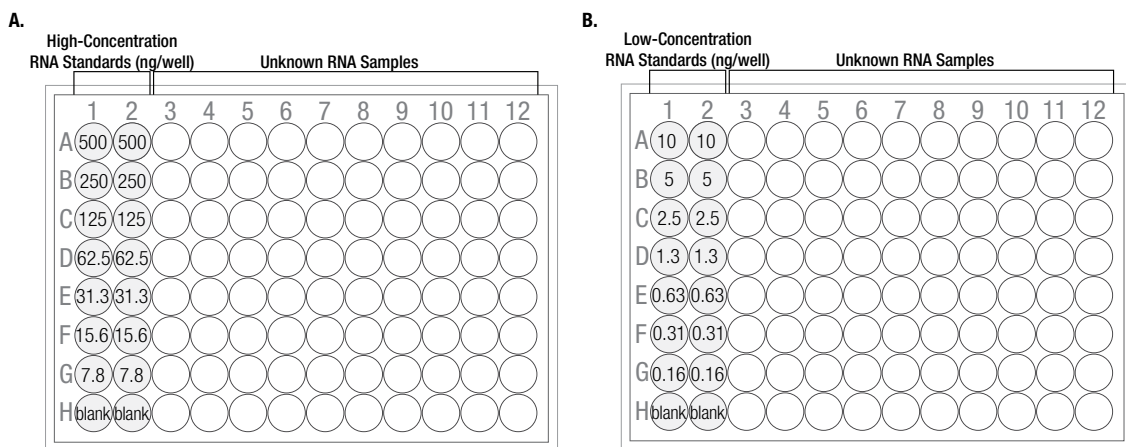


Figure 3. Dispense standard dilutions and blank samples in duplicate into Columns 1 and 2 of a multiwell plate. Panel A. High-concentration RNA standard and blank samples. **Panel B.** Low-concentration RNA standard and blank samples.

8. Mix the plate thoroughly using a plate shaker or by pipetting the contents of each well.
9. Incubate assays for 5 minutes at room temperature, protected from light.
10. Measure fluorescence (492nm_{Ex}/540nm_{Em}) using your plate reader. If using the GloMax® Discover System, select the preloaded protocol: “QuantiFluor RNA System.”
11. Calculate the RNA concentration as follows: Subtract the fluorescence of the blank sample (1X TE buffer) 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. Alternatively, copy and paste your raw fluorescence data into our online tool:
www.promega.com/resources/tools/quantifluor-dye-systems-data-analysis-workbook.



5. Representative Data

Representative data for the QuantiFluor® RNA Dye are shown in Table 3 and Figure 4.

Table 3. Representative Data for the High-Concentration RNA Standard Curve and QuantiFluor® RNA Dye in 96-Well Plate Format.

| RNA Standard Mass (ng/well) | Average Fluorescence (RFU) ¹ |
|-----------------------------|---|
| 0 | 0 |
| 7.8 | 1,150 |
| 15.6 | 2,852 |
| 31.3 | 6,851 |
| 62.5 | 15,957 |
| 125 | 35,091 |
| 250 | 79,382 |
| 500 | 151,761 |

¹Background fluorescence has been subtracted. n = 3

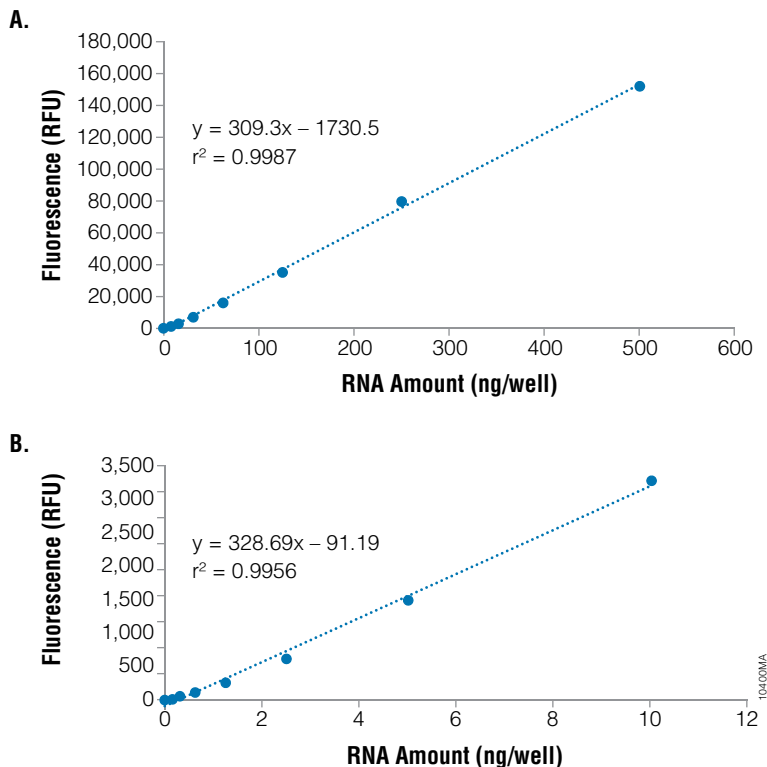


Figure 4. Representative RNA standard curves in a 96-well-plate format. Panel A. The high-concentration RNA standard curve. **Panel B.** The low-concentration RNA standard curve. The final concentrations of RNA standard in 96-well plate, 200µl assay format are listed in Table 3.



5. Representative Data (continued)

Example Calculation:

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

| | Unknown Sample | Standard Samples | | | | | | | |
|--------------|----------------|------------------|-------|--------|--------|--------|--------|--------|---------|
| | | 0ng | 7.8ng | 15.6ng | 31.3ng | 62.5ng | 125ng | 250ng | 500ng |
| Fluorescence | 17,775 | 944 | 2,094 | 3,796 | 7,795 | 16,901 | 36,035 | 80,326 | 152,705 |

1. Subtract the 1X TE buffer blank (average of blank standards) from all samples:

| | Unknown Sample | Standard Samples | | | | | | | |
|--------------|----------------|------------------|-------|--------|--------|--------|--------|--------|---------|
| | | 0ng | 7.8ng | 15.6ng | 31.3ng | 62.5ng | 125ng | 250ng | 500ng |
| Fluorescence | 16,831 | 0 | 1,150 | 2,852 | 6,851 | 15,957 | 35,091 | 79,382 | 151,761 |

2. Determine the linear regression from the standard curve (Figure 4).
 $y = 309.3x - 1730.5$
3. Calculate the RNA concentration of the unknown sample in the 200 μ l assay volume by solving for x in the linear regression equation, where
 $y = 16,831$
 $x = (y + 1730.5)/309.3 = 60\text{ng}$
4. Account for any dilution of the unknown sample. For example, if 1 μ l of sample was added per well, the sample concentration is 60ng/ μ l. If 5 μ l of sample was added per well, the sample concentration is 60ng \div 5 μ l = 12ng/ μ l.

6. Troubleshooting

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

| Symptoms | Causes and Comments |
|---------------------------------|---|
| Low or no fluorescence detected | <p>Check that the correct filter set was used for the QuantiFluor® RNA Dye, and read the fluorescence at 492nm_{Ex}/540nm_{Em} (see Figure 3 for excitation and emission spectra).</p> <p>The QuantiFluor® RNA Dye is light-sensitive. Exposure to light will reduce the sensitivity of the assay. Store the QuantiFluor® RNA Dye and working solution protected from light.</p> <p>Confirm that dye was added. Add an equal volume of QuantiFluor® RNA Dye working solution to each sample.</p> <p>Check that unknown and standard samples were diluted appropriately. Increase the concentration of the unknown and standard samples, if necessary.</p> <p>Confirm that the unknown sample calculations were performed correctly and, if applicable, the concentrations calculated from the linear regression were multiplied by the dilution factor (Section 5, Step 4).</p> <p>Check that the unknown sample was within the dynamic range of the assay and standard curve. Determine the average fluorescence and standard deviation of the blank standards. Subtract the average fluorescence of the blank standard from the average fluorescence of the unknown and standard samples. These blank-subtracted values should be more than three standard deviations (as determined for the blank standards) above the average fluorescence for the blank standards.</p> <p>The high end of the standard curve was not within the dynamic range for the QuantiFluor® RNA Dye. Evaluate the blank-subtracted fluorescence of the standard curve. The values should be proportional to the dilution factors used to create the standard curve. If the increase in fluorescence is not proportional to the increase in RNA amount, the QuantiFluor® RNA Dye may be saturated. Recreate the standard curve, and decrease the concentration of the highest point of the standard curve.</p> |

6. Troubleshooting (continued)

| Symptoms | Causes and Comments |
|---|---|
| Low or no fluorescence detected in the standard samples | 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 the QuantiFluor® RNA Dye. The QuantiFluor® RNA Dye is light-sensitive. Exposure to light will reduce the sensitivity of the assay. Store the QuantiFluor® RNA Dye and working solution protected from light. Check that the standard samples were diluted appropriately. |
| 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. |
| RNA concentration determined using the QuantiFluor® RNA Dye differed from concentration determined using an alternative quantitation method | RNA concentrations determined using the QuantiFluor® RNA Dye and optical density readings at 260nm will be different due to inherent differences between methodologies. An optical density reading at A_{260} reflects the amount of light that is neither transmitted nor reflected and is proportional to the amount of all nucleic acid (dsDNA, ssDNA, RNA and nucleotides) in the sample. If comparing concentrations determined using another dye-based quantitation method, carefully examine the blank-subtracted fluorescence of the two standard curves. The values should be proportional to the dilution factors used to create the standard curve. If the increase in fluorescence is not proportional to the increase in RNA amount, the fluorescent dye(s) may be saturated. Recreate the standard curve, and decrease the concentration of the highest point of the standard curve. Contaminating DNA may be present. Treat sample with DNase (Section 7.C). |

Symptoms

RNA concentration determined using the QuantiFluor® RNA Dye differed from concentration determined using an alternative quantitation method (continued)

Causes and Comments

Determine the average fluorescence and standard deviation of the blank standards. Subtract the average fluorescence of the blank standards from the average fluorescence of the unknown and standard samples. These blank-subtracted values should be more than three standard deviations (as determined for the blank standards) above the average fluorescence for the blank standards.

Nonlinear standard curve

Check that standard samples were diluted appropriately. If the high or low end of the curve is nonlinear, then adjust the standard sample dilutions such that the standard curve is linear.

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.

Check that the lower-concentration standards are within the linear range for the assay and assay format. Determine the average fluorescence and standard deviation of the blank standards. Subtract the average fluorescence of the blank standards from the average fluorescence of the unknown and standard samples. These blank-subtracted values should be greater than three standard deviations (as determined for the blank standards) above the average fluorescence for the blank standards.

Analyze the data using either a linear regression or a power regression for accurate concentration determinations within the 10–400ng/μl portion of the standard curve. We recommend the use of a power regression for unknowns that are expected to be <10ng/μl. Alternatively, copy and paste your raw fluorescence data into the Promega online tool, which uses a power regression: **www.promega.com/resources/tools/quantifluor-dye-systems-data-analysis-workbook** or contact Technical Services for additional assistance.

The QuantiFluor® RNA Dye is light-sensitive. Exposure to light will reduce the sensitivity of the assay. Store the QuantiFluor® RNA Dye and working solution protected from light.

Check that the standard samples were diluted appropriately.

7. Appendix

7.A. Instrument Compatibility

Fluorescence measurements can be performed using any fluorescence reader capable of measuring excitation and emission at the appropriate wavelengths. For the QuantiFluor® RNA Dye, measure excitation and emission at 492nm and 540nm, respectively; emission and excitation spectra are shown in Figure 5.

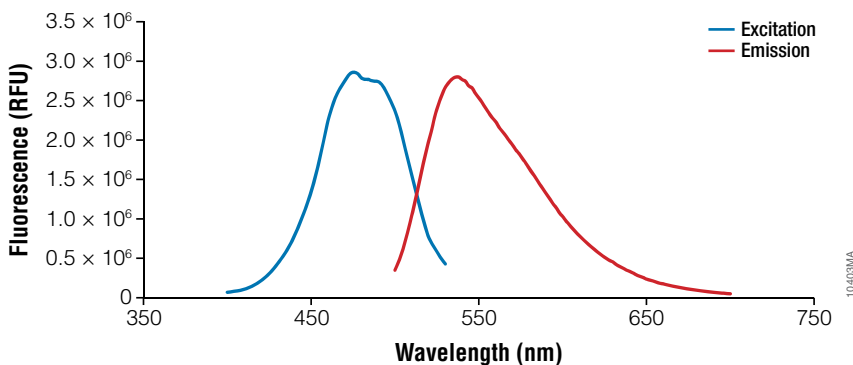


Figure 5. Excitation and emission spectra for the QuantiFluor® RNA Dye.

7.B. Interfering Compounds

Several compounds that are commonly used in nucleic acid preparation or can be found in eluates from nucleic acid purification may affect the QuantiFluor® RNA Dye. Table 4 lists compounds that have known effects on RNA quantitation using the QuantiFluor® RNA Dye and the concentrations at which they affect quantitation results.

Table 4. Compounds that Interfere with the QuantiFluor® RNA Dye.

| Chemical | Concentration Shown to Affect the QuantiFluor® RNA Dye¹ | Change in QuantiFluor® RNA Dye Signal |
|----------------------------------|---|--|
| agarose | 0.01% | 16% decrease |
| ammonium acetate | 20mM | 11% decrease |
| bovine serum albumin (BSA) | 0.003% | 9% increase |
| chloroform | 0.4% | 1% decrease |
| ethanol | 20% | 18% decrease |
| IgG | 0.02% | 1% decrease |
| boric acid | 150mM | 8% decrease |
| formamide | 5% | 3% decrease |
| 5X Transcription Reaction Buffer | 0.25% | 15% decrease |
| ImProm-II™ 5X Reaction Buffer | 2% | 4% increase |
| GoScript™ 5X Reaction Buffer | 2% | 6% decrease |
| sodium chloride | 20mM | 12% decrease |
| magnesium chloride | 1mM | 6% decrease |
| calcium chloride | 1mM | 2% decrease |
| zinc chloride | 1mM | 12% decrease |
| 1,2-propanediol | 20% | 11% increase |
| cesium chloride | 10mM | 6% decrease |
| guanidine thiocyanate | 10mM | 7% decrease |
| sucrose | >500mM | 9% increase |
| urea | 3M | 23% decrease |
| phenol | 0.5% | 13% decrease |
| polyethylene glycol (PEG 8000) | 20% | 1% decrease |
| sodium acetate | 20mM | 9% decrease |
| sodium dodecyl sulfate (SDS) | 0.005% | 3% increase |
| Triton® X-100 | 0.5% | 9% decrease |

¹Compounds were tested at the indicated concentration with 20ng per well of RNA Standard and a 1:1,000 dilution of the QuantiFluor® RNA Dye.



7.C. Sample Considerations

For RNA samples containing a mixture of RNA and DNA, we recommend a DNase treatment using RQ1 RNase-Free DNase (Cat.# M6101) to degrade contaminating DNA and quantitate RNA more specifically.

For samples containing significant amounts of DNA, treat the RNA sample prior to quantitation. Follow the RQ1 RNase-Free DNase Usage Information on the Cat.# M6101 Certificate of Analysis. Below is an example protocol for a final reaction volume of 10µl.

1. Add 1µl of RQ1 RNase-Free DNase 10X Reaction Buffer to each unknown RNA sample.
2. Add 1µl of RQ1 RNase-Free DNase to each sample, and mix gently. Do not vortex.
3. Incubate at 37°C for 30 minutes.
4. Add 1µl of RQ1 DNase Stop Solution to terminate the reaction.
5. Incubate at 65°C for 10 minutes to inactivate the DNase.
6. Proceed to RNA quantitation in Section 4.

7.D. Tips for Pipetting Small Volumes

1. Handheld pipettes are high-precision instruments and should be handled carefully. Pipettes also should be routinely checked for accuracy every 6–12 months and recalibrated or repaired by qualified professionals.
2. Use newer model pipettes with more modern “press fit” tips for the most accurate and reproducible results.
3. Basic pipetting guidelines:
 - When resetting a pipette to a new volume, turn the adjustment knob ½ turn higher than the target volume and then rotate back to the target volume.
 - Pipet using a slow, smooth action.
 - Hold the pipette vertically when drawing in liquid.
 - Only immerse the pipette tip slightly when aspirating liquid.
 - When dispensing the liquid, hold the pipette vertically but keep the sidewall of the receiving vessel at a 45 degree angle. Pipette against the sidewall or into liquid that is already present.
4. Use the proper pipettor for the measured volume. When pipetting 1µl or 2µl, use a P2 pipettor for accuracy and reproducibility. Using aerosol or barrier tips with a P2 is not recommended.
5. All solutions, standards and unknowns should be at room temperature prior to pipetting. See the following *Nature Methods Application Note* for more information:
www.nature.com/app_notes/nmeth/2007/071109/full/nmeth1086.html

For a comprehensive guide on pipetting, we recommend the following resource:

www.gilson.com/default/guide-to-pipetting

7.E. Handling and Disposal

QuantiFluor® RNA Dye contains DMSO, which is an irritant and facilitates the entry of organic compounds into tissues. Wear gloves, safety glasses and a lab coat, and handle dye with care. Because the QuantiFluor® RNA Dye binds to nucleic acid, it should be treated as a potential mutagen. Dispose of the QuantiFluor® RNA Dye according to local regulations.

7.F. Composition of Buffers and Solutions

20X TE Buffer (pH 7.5)

0.2M Tris buffer (pH 7.5)

20mM EDTA (pH 8.0)

Prepare this solution in nuclease-free water. Adjust pH to 7.5.

8. Related Products

| Product | Size | Cat.# |
|-------------------------------|---------------|-------|
| Quantus™ NGS Starter Package | 1 each | E5150 |
| QuantiFluor® ONE dsDNA System | 100 reactions | E4871 |
| | 500 reactions | E4870 |
| QuantiFluor® dsDNA System | 1ml | E2670 |
| QuantiFluor® ssDNA System | 1ml | E3190 |

GloMax® Instruments

| Product | Size | Cat.# |
|---|--------|--------|
| GloMax® Discover System | 1 each | GM3000 |
| GloMax® Explorer Fully Loaded Model | 1 each | GM3500 |
| GloMax® Explorer with Luminescence and Fluorescence | 1 each | GM3510 |

Handheld Fluorometer

| Product | Size | Cat.# |
|----------------------|---------|-------|
| Quantus™ Fluorometer | 1 each | E6150 |
| 0.5ml PCR Tubes | 50 pack | E4941 |

| Product | Size | Cat.# |
|--|-------|-------|
| Nuclease-Free Water | 50ml | P1193 |
| | 150ml | P1195 |
| TE Buffer, 1X, Molecular Biology Grade | 100ml | V6231 |
| | 500ml | V6232 |



9. Summary of Changes

The following changes were made to the 3/19 revision of this document:

1. Corrected dye name in Section 4, Step 2, paragraph 2.

^(a)U.S. Pat. Nos. 8,598,198 and 9,206,474 and other patents and patents pending.

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