Measuring RNA Concentration Using the QuantiFluor® RNA System with the GloMax® Discover System

Promega Corporation

Materials Required

- QuantiFluor® RNA System (Cat.# E3310)
- GloMax® Discover System (Cat.# GM3000)
- Nuclease-Free Water (Cat.# P1195)
- black, flat-bottom 96-well plates

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

Protocols: *GloMax® Discover System Technical Manual* #TM397 and *QuantiFluor® RNA System Technical Manual* #TM377 are available at: **www.promega.com/protocols/**

Detecting and quantitating small amounts of RNA are important steps in many molecular biology techniques. These include measuring yields of in vitro transcribed RNA and determining RNA concentration before performing Northern blot analysis, S1 nuclease assays, RNase protection assays, cDNA library preparation, reverse transcription PCR and differential display PCR. Traditional spectrophotometric assays cannot determine RNA concentrations below 2µg/ml; however, many isolated RNA samples have concentrations well below that level. Using the GloMax® Discover System with QuantiFluor® RNA System provides a fast, easy and sensitive method for determining RNA concentrations over a large linear range.

The QuantiFluor® RNA System contains a fluorescent dye that sensitively quantitates small amounts of RNA in solution. For those RNA samples that may contain contaminating genomic DNA, we recommend a brief DNase treatment to degrade any genomic DNA present in the sample for accurate RNA quantitation.

The QuantiFluor® RNA System is made easy with the GloMax® Discover System. The instrument is operated by an integrated tablet PC, which provides quick and easy navigation through the control options. The extended dynamic range and minimal well-to-well cross talk of the GloMax® Discover System can easily measure various sample signal intensities on the same plate. Fluorescence readings are collected using a standard black 96-well plate and the GloMax® Discover System (Figure 1). This Application Note describes the protocol for using the QuantiFluor® RNA System with the GloMax® Discover System.

For detailed instructions and assay notes, see the *QuantiFluor® RNA System Technical Manual* #TM377. The following procedure can be used for calculating RNA concentration in a 200µl assay format.

Preparing the QuantiFluor® RNA Dye Working Solution

1. 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.
2. Prepare 1X TE buffer by diluting the 20X TE Buffer 20-fold with Nuclease-Free Water.
3. Dilute the QuantiFluor® RNA Dye with 1X TE buffer. For a high-concentration RNA standard curve (39.1–2,500ng/ml or 7.8–500ng per well), perform a 1:200 dilution. For a low-concentration RNA standard curve (0.8–50ng/ml or 0.16–10ng per well), perform a 1:1,000 dilution. Prepare enough QuantiFluor® RNA Dye working solution to quantitate both standards and unknown samples.

Generating a Standard Curve

Quantitation of unknown samples requires comparison of the unknown samples to a standard curve of RNA. Generate a standard curve appropriate for the expected RNA concentration range of your unknown samples and your sample analysis setup (i.e., high-concentration or low-concentration standard curve).

High-Concentration RNA Standard Curve

1. For a high-concentration RNA standard curve (39.1–2,500ng/ml), dilute the RNA Standard (100µg/ml) 1:20 in 1X TE buffer to a concentration of 5µg/ml. For example, add 50µl of RNA Standard to 950µl of 1X TE buffer.
2. Prepare the standard samples shown in Table 1 for a high-concentration RNA standard curve.

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

Standard	Volume of RNA Standard	Volume of 1X TE Buffer (µl)	RNA Amount Per 100µl (ng)	RNA Concentration Before Adding Dye (ng/ml)	Final RNA Concentration After Adding Dye (ng/ml)
Blank	0	1,000	0	0	0
A	1,000µl[1]	0	500	5,000	2,500
B	500µl of Standard A	500	250	2,500	1,250
C	500µl of Standard B	500	125	1,250	625
D	500µl of Standard C	500	62.5	625	313
E	500µl of Standard D	500	31.3	313	156
F	500µl of Standard E	500	15.6	156	78.1
G	500µl of Standard F	500	7.8	78	39.1

[1]Use 1,000µl of the 5µg/ml RNA Standard prepared in Step 1.

Low-Concentration RNA Standard Curve

1. For a low-concentration RNA standard curve (0.8–50ng/ml), dilute the RNA Standard (100µg/ml) 1:20 in 1X TE buffer to a concentration of 5µg/ml. For example, add 50µl of RNA Standard to 950µl of 1X TE buffer.
2. Dilute the 5µg/ml RNA Standard (prepared in Step 1) 1:50 in 1X TE buffer to a concentration of 0.1µg/ml (100ng/ml). For example, add 20µl of 5µg/ml RNA Standard to 980µl of 1X TE buffer.
3. Prepare the standard samples shown in Table 2 for a low-concentration RNA standard curve.

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

Standard	Volume of RNA Standard	Volume of 1X TE Buffer (µl)	RNA Amount Per 100µl (ng)	RNA Concentration Before Adding Dye (ng/ml)	Final RNA Concentration After Adding Dye (ng/ml)
Blank	0	1,000	0	0	0
A	1,000µl[1]	0	10	100	50
B	500µl of Standard A	500	5	50	25
C	500µl of Standard B	500	2.5	25	12.5
D	500µl of Standard C	500	1.3	13	6.3
E	500µl of Standard D	500	0.63	6.3	3.1
F	500µl of Standard E	500	0.31	3.1	1.6
G	500µl of Standard F	500	0.16	1.6	0.8

[1]Use 1,000µl of the 100ng/ml RNA Standard prepared in Step 2.

Protocol

1. Dilute unknown samples to 100µl total volume with 1X TE buffer.
2. Add 100µl of QuantiFluor® RNA Dye working solution to each well containing 100µl of unknown, blank or standard sample, and mix briefly.
3. 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.
4. Incubate assays for 5 minutes at room temperature, protected from light.
5. Measure fluorescence on the GloMax® Discover System (475nm$_{Ex}$/500–550nm$_{Em}$).
6. Calculate the RNA concentration as follows: Subtract the fluorescence of the blank sample from that of each standard and sample. 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. Alternatively, copy and paste your raw fluorescence data into the Promega online tool: **www.promega.com/resources/tools/quantifluor-dye-systems-data-analysis-workbook**

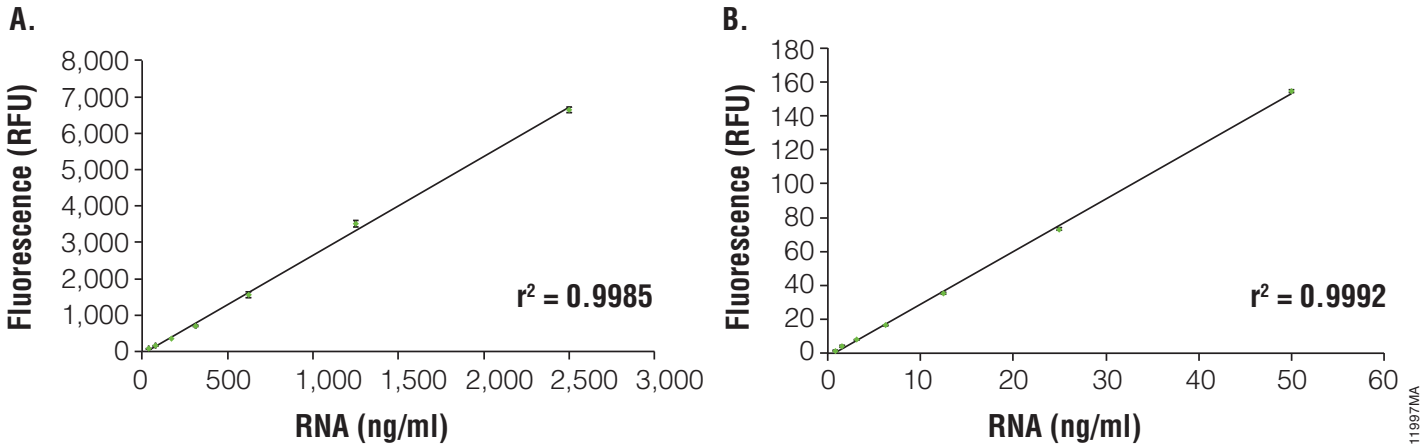

Figure 1. Representative RNA high- and low-concentration standard curves in a 96-well-plate format. The final amounts of the RNA Standard in the 96-well, 200µl assay format. Points represent the average of three replicates with standard deviation shown.

Conclusion

The GloMax® Discover System offers excellent performance quantitating RNA using the QuantiFluor® RNA System.

Summary

The GloMax® Discover System offers superior sensitivity, dynamic range and limited well-to-well cross talk. The instrument was developed and optimized with Promega cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax® Discover System provides flexible use of filters for fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance measurements for a wide variety of laboratory applications. Exporting your results is made seamless with a variety of options, including exporting data to your local network.

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