Measuring Double-Stranded DNA Concentration Using the QuantiFluor® dsDNA System with the GloMax® Discover System

Promega Corporation

Materials Required

- QuantiFluor® dsDNA System (Cat.# E2670)
- 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® dsDNA System Technical Manual* #TM346 are available at: **www.promega.com/protocols/**

Accurate quantitation of DNA is critical for many biological applications. Traditional spectrophotometric assays cannot determine DNA concentration below 2µg/ml; however, many isolated DNA samples have concentrations well below that level. Using the GloMax® Discover System with the QuantiFluor® dsDNA System provides a fast, easy and sensitive method for determining DNA concentrations over a large linear range in concentration.

The QuantiFluor® dsDNA System contains a fluorescent DNA-binding dye that sensitively and specifically quantitates small amounts of double-stranded DNA (dsDNA) in solution. The dye shows minimal binding to single-stranded DNA (ssDNA) and RNA for specific quantitation of dsDNA.

Using the QuantiFluor® dsDNA 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® dsDNA System with the GloMax® Discover System.

For detailed instructions and assay notes, see the *QuantiFluor® dsDNA System Technical Manual* #TM346. The following procedure can be used for calculating dsDNA concentration in a 200µl assay format.

Preparing the QuantiFluor® dsDNA Dye Working Solution

1. Warm all assay components to room temperature before use. The QuantiFluor® dsDNA 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® dsDNA Dye with 1X TE buffer. For the standard curve, perform a 1:200 dilution. Prepare enough QuantiFluor® dsDNA 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 dsDNA. Generate a standard curve appropriate for the expected dsDNA concentration range of your unknown samples and your sample analysis setup. We recommend preparing a standard curve using dsDNA of a similar size as the dsDNA you wish to quantitate. For example, if you are quantitating genomic DNA, you should prepare a standard curve using a genomic DNA sample of known concentration. The Lambda DNA Standard included is 48.5kb.

1. For the dsDNA standard curve (0.2–1,000ng/ml), dilute the Lambda DNA Standard 1:50 in 1X TE buffer to a concentration of 2ng/µl. For example, add 20µl of Lambda DNA Standard to 980µl of 1X TE buffer.
2. Prepare the standard samples shown in Table 1 for a dsDNA standard curve.

Table 1. Preparing a dsDNA Standard Curve.

Standard	Volume of dsDNA Standard	Volume of 1X TE Buffer (µl)	dsDNA Amount Per 100µl (ng)	dsDNA Concentration Before Adding Dye (ng/ml)	Final dsDNA Concentration After Adding Dye (ng/ml)
Blank	0	1,000	0	0	0
A	1,000µl[1]	0	200	2,000	1,000
B	250µl of Standard A	750	50	500	250
C	250µl of Standard B	750	12.5	125	62.5
D	250µl of Standard C	750	3.1	31	16
E	250µl of Standard D	750	0.78	7.8	3.9
F	250µl of Standard E	750	0.2	2.0	1.0
G	250µl of Standard F	750	0.05	0.5	0.2

[1]Use 1,000µl of the 2ng/µl Lambda DNA Standard prepared in Step 1.

Protocol

1. Dilute unknown samples to 100µl total volume with 1X TE buffer.
2. Add 100µl of QuantiFluor® dsDNA 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 dsDNA concentration as follows: Subtract the fluorescence of the blank sample from that of each standard and sample. Use the corrected data from the DNA standards to generate a standard curve of fluorescence versus DNA concentration. Determine the DNA 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**

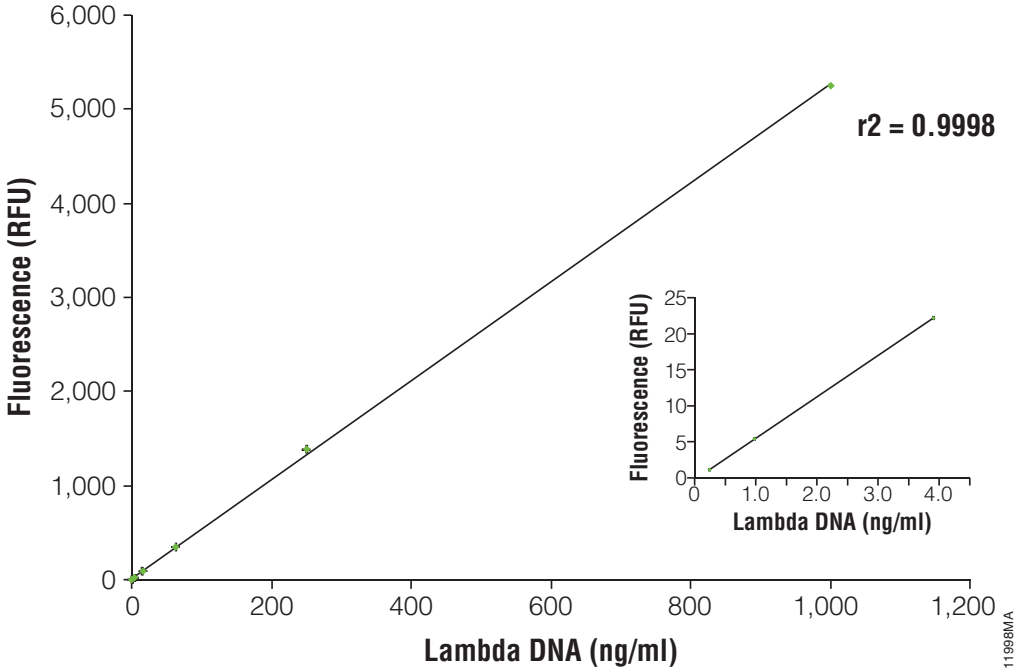

Figure 1. Representative dsDNA standard curve in a 96-well-plate format. The final amounts of the Lambda DNA Standard in the 96-well, 200µl assay format. Points represent the average of three replicates with standard deviation shown. **Inset:** Expanded view of the low end of the standard curve.

Conclusion

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