Measuring Single-Stranded DNA Concentration Using the QuantiFluor® ssDNA System with the GloMax® Discover System

Promega Corporation

Materials Required

- QuantiFluor® ssDNA System (Cat.# E3190)
- GloMax® Discover System (Cat.# GM3000)
- Nuclease-Free Water (Cat.# P1195)
- black, flat-bottom 96-well plates
- optional: shrimp DNase (USB Cat.# 78314); see Section 8.D of the *QuantiFluor® ssDNA System Technical Manual* #TM376

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® ssDNA System Technical Manual* #TM376 are available at: **www.promega.com/protocols/**

Detecting and quantitating small amounts of single-stranded (ssDNA) are important steps in many molecular biology techniques, including DNA sequencing, site-directed mutagenesis, DNA amplification and gene expression. Traditional spectrophotometric assays cannot determine DNA concentrations below 2µg/ml; however, many isolated DNA samples have concentrations well below that level.

The QuantiFluor® ssDNA System contains a fluorescent DNA-binding dye that sensitively and specifically quantitates small amounts of ssDNA in solution. When used with the GloMax® Discover System, the QuantiFluor® ssDNA System provides a fast, easy and sensitive method for determining DNA concentrations over a large linear range. For ssDNA samples that may contain contaminating double-stranded DNA (dsDNA), we recommend a brief shrimp DNase treatment to degrade any dsDNA present for accurate ssDNA quantitation.

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

For detailed instructions and assay notes, see the *QuantiFluor® ssDNA System Technical Manual* #TM376. The following procedure can be used for calculating ssDNA concentration in a 200µl assay format.

Preparing the QuantiFluor® ssDNA Dye Working Solution

1. Warm all assay components to room temperature before use. The QuantiFluor® ssDNA 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® ssDNA Dye with 1X TE buffer. For a high-concentration ssDNA standard curve, perform a 1:200 dilution. For a low-concentration ssDNA standard curve, perform a 1:1,000 dilution. Prepare enough QuantiFluor® ssDNA Dye working solution to quantitate both standards and unknown samples.

Generating a Standard Curve

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

High-Concentration ssDNA Standard Curve

1. For a high-concentration ssDNA standard curve (31.3–2,000ng/ml), dilute the ssDNA Standard (100µg/ml) 1:25 in 1X TE buffer to a concentration of 4µg/ml. For example, add 40µl of ssDNA Standard to 960µl of 1X TE buffer.
2. Prepare the standard samples shown in Table 1 for a high-concentration ssDNA standard curve.

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

Standard	Volume of ssDNA Standard	Volume of 1X TE Buffer (µl)	ssDNA Amount Per 100µl (ng)	ssDNA Concentration Before Adding Dye (ng/ml)	Final ssDNA Concentration After Adding Dye (ng/ml)
Blank	0	1,000	0	0	0
A	1,000µl[1]	0	400	4,000	2,000
B	500µl of Standard A	500	200	2,000	1,000
C	500µl of Standard B	500	100	1,000	500
D	500µl of Standard C	500	50.0	500	250
E	500µl of Standard D	500	25.0	250	125
F	500µl of Standard E	500	12.5	125	62.5
G	500µl of Standard F	500	6.25	62.5	31.3

[1]Use 1,000µl of the 4µg/ml ssDNA Standard prepared in Step 1.

Low-Concentration ssDNA Standard Curve

1. For a low-concentration ssDNA standard curve (0.8–50ng/ml), dilute the ssDNA Standard (100µg/ml) 1:25 in 1X TE buffer to a concentration of 4µg/ml. For example, add 40µl of ssDNA Standard to 960µl of 1X TE buffer.
2. Dilute the 4µg/ml ssDNA Standard (prepared in Step 1) 1:40 in 1X TE buffer to a concentration of 0.1µg/ml (100ng/ml). For example, add 25µl of 4µg/ml ssDNA Standard to 975µl of 1X TE buffer.
3. Prepare the standard samples shown in Table 2 for a low-concentration ssDNA standard curve.

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

Standard	Volume of ssDNA Standard	Volume of 1X TE Buffer (µl)	ssDNA Amount Per 100µl (ng)	ssDNA Concentration Before Adding Dye (ng/ml)	Final ssDNA 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 ssDNA Standard prepared in Step 2.

Protocol

1. Dilute unknown samples to 100µl total volume with 1X TE buffer.
2. Add 100µl of QuantiFluor® ssDNA Dye working solution to each well containing 100µl of unknown, blank or standard sample, and mix briefly.
   Note: For samples containing a mixture of ssDNA and dsDNA, we recommend a shrimp DNase treatment to degrade contaminating dsDNA and quantitate ssDNA more specifically. See Section 8.D of the *QuantiFluor® ssDNA System Technical Manual* #TM376.
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 ssDNA 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

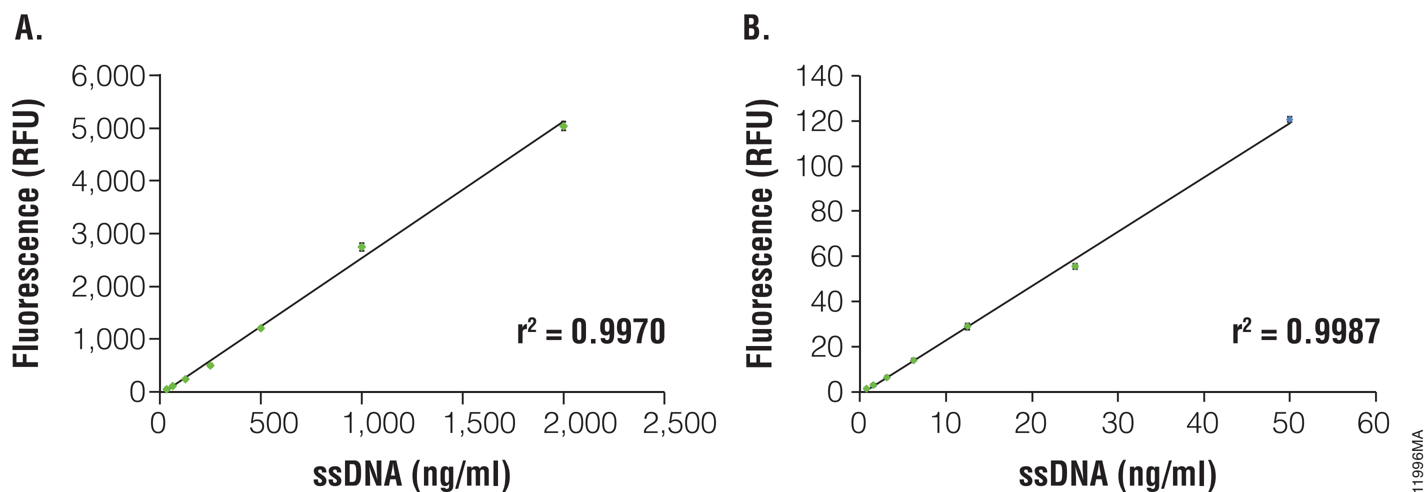

Figure 1. Representative ssDNA high- and low-concentration standard curves in a 96-well-plate format. The final amounts of the ssDNA Standard in the 96-well, 200µl assay format. Points represent the average of three replicates with standard deviation shown.

Conclusion

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