

Using the QuantiFluor™ dsDNA System and the GloMax® Multi+ Fluorometer with Instinct™ Software

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Accurate quantitation of DNA concentration is critical for many applications. Traditional spectrophotometric assays are not practical for measuring DNA concentrations below 1ng/μl; however, many clinical and forensic samples have concentrations well below this level. The QuantiFluor™ dsDNA System is a fast, easy and sensitive method for determining low DNA concentrations. In this article, we have coupled the QuantiFluor™ dsDNA system with the GloMax® Multi+ fluorometer equipped with Instinct software allowing for instant DNA quantification by fitting the unknown sample's signal to a calibration curve. This article will help guide the user through sample and calibration curve setup, GloMax® Multi+ Instrument setup and Instinct data analysis.

The QuantiFluor™ dsDNA System contains a new fluorescent DNA-binding dye for quantitating small amounts of dsDNA.

Introduction

The QuantiFluor™ dsDNA System^(a) contains a new fluorescent DNA-binding dye that enables sensitive quantitation of small amounts of double-stranded DNA (dsDNA). This dye shows minimal binding to single-stranded DNA (ssDNA) and RNA, allowing specific quantitation of double-stranded DNA. This assay requires a fluorometer with a Blue optical channel (504nm_{Ex}/531nm_{Em}). The dye itself has little fluorescence when excited at 504nm. In this article we focus on the GloMax® Multi+ fluorometer equipped with Instinct™ software; however, many other fluorometers are compatible with this assay.

Basic Protocol

Mix each unknown DNA sample and standard curve sample with the QuantiFluor™ dsDNA Dye (diluted from 200X to 1X in TE buffer) in equal volumes. In this paper, all experiments used 200μl total volume (100μl sample/standard and 100μl Dye) and were performed in black, flat-bottomed, 96-well plates. After mixing gently, the samples were incubated at room temperature for 5 minutes while being protected from light (covered in foil). Fluorescence was measured with the GloMax® Multi+ fluorometer and analysis performed by the Instinct software.

Dye Preparation and Standard Curve Setup

After allowing the QuantiFluor™ DNA dye, Lambda DNA standard, and 20X TE buffer to equilibrate to room temperature, a 1X TE working stock was produced. Using 1X TE, the QuantiFluor™ DNA dye (200X) was diluted to 1X and covered in foil to protect from light. A five point standard curve was created using serial dilution of the Lambda DNA standard (100μg/ml). The initial concentrations were 2, 0.2, 0.02, 0.002, and 0.0002μg/ml. The final concentrations will be twofold more because equal amount of standard will be added to an equal amount of QuantiFluor™ dye. It is important to use a DNA standard with a similar size range to the DNA being quantitated. For example, if one is analyzing genomic

DNA, a genomic DNA standard should be used (e.g., Cat.# E3041). Results may significantly vary with an incorrect standard.

Sample/Standard Setup

One hundred microliters of the blank (1X TE buffer), genomic DNA standard dilutions and the unknown samples were added to a black 96-well plate in triplicates according to Figure 1. The QuantiFluor™ DNA Dye (100µl) was added to each sample well and gently mixed by tapping the plate. The plate was covered in foil and incubated for five minutes at room temperature. During this time, the GloMax® Multi+ was set up for data analysis.

GloMax® Multi+ with Instinct Setup

The GloMax®-Multi Optical Kit Blue (Cat.# E8921) is required for this protocol. Consult the instrument manual (TM340) for instructions on changing filters.

From the home menu, select “Instrument Control” (Figure 2, Panel A). Drag and drop the “FLUOR” protocol from the Protocol Options to the Protocol Composer (Figure 2, Panels B and C). Click on the “FLUOR” button and change the optical kit to “Blue” (Figure 2, Panel D). Next, select the picture of the plate to begin plate setup (Figure 2, Panel E). Select the fit field and change to “linear fit” (Figure 2, Panel F). After clicking

“OK” twice, highlight the three wells corresponding to the blanks and select the “B” button to create the blank wells (Figure 2, Panel G). To create the standards, select the three wells corresponding to the first standard, and press the “S” button (Figure 2, Panel H). Select the “Conc.” field and type in the first standard concentration (1.0 in this situation) (Figure 2, Panel H). Also select the “Units” field and type in the units ($\mu\text{g ml}^{-1}$). Repeat for the rest of the standards. To create sample wells, highlight the replicate wells and press the “U” (unknown) button (Figure 2, Panel I). With the unknown sample highlighted, select the “Dilution” field, and change to correct concentration (Figure 2, Panel I). The dilution will be two times the initial dilution. This extra twofold dilution is from the dilution created when the QuantiFluor™ DNA dye is added (100µl sample + 100µl dye). Repeat for the rest of the unknown samples. After entering all of the standards and unknown samples, click “OK”. Press the “DOOR” button, and load the plate according to the label on the instrument (Figure 2, Panel J). Press the “DOOR” button again. Press the green “START” button, and enter a name for the experimental run. After selecting “OK”, the instrument will begin its run.

Transferring Data to Computer and Analysis

After the run is complete, using a USB flash drive, transfer and open the data on a computer with Instinct Software (available via CD provided with instrument or by download from the Promega web site, details in TM340). Note, for computers without Macros enabled in Microsoft Excel®, select “Enable this content” under Options in the security warning. The Instinct Software will automatically background-subtract, calculate the standard curve, fit the unknown samples to that curve, and thus determine the concentrations of the samples. The Excel file will contain the following tab-delineated data:

- **Plate:** raw data in plate format
- **Step 1:** raw data in column format
- **Analysis:** R² value, linear fit line equation, initial plate setup, and calculated concentrations in plate format
- **Step1_average:** R² value, linear fit line equation, averaged raw data, calculated concentrations, standard deviation, percent CV, signal:blank
- **Notes:** any experiments notes can be placed here

Figure 3 shows a sample output for a standard curve created with Instinct. The equation is displayed in the top section along with the

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	1	0.1	0.01	0.001	0.0001	Sample 1	Sample 2	Sample 3	Sample 4		
B	Blank	1	0.1	0.01	0.001	0.0001	Sample 1	Sample 2	Sample 3	Sample 4		
C	Blank	1	0.1	0.01	0.001	0.0001	Sample 1	Sample 2	Sample 3	Sample 4		
D												
E												
F												
G												

Figure 1. Plate setup with five-point standard curve. The figure represents a typical plate setup for the QuantiFluor™ dsDNA System. Triplicates of a blank, five standards, and unknown samples are added to a black, flat-bottomed, 96-well plate.

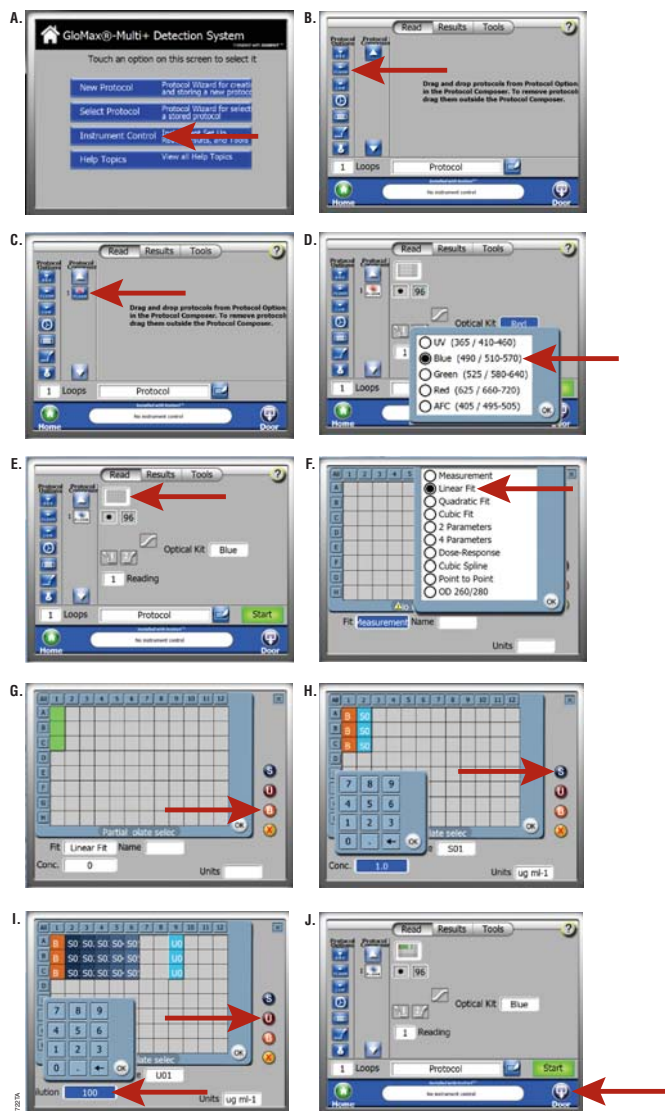


Figure 2. Walkthrough of setup on the GloMax Multi+ with Instinct.

Wells	Label	Averaged Raw Data	Standard Concentration	Calculated Concentration	Stdev of average	CV%	Signal:Blank
A1 B1 C1	blank	391.377	n/a	n/a	n/a	n/a	n/a
A2 B2 C2	S01	82966.53	1	n/a	0	0.00%	211.9607
A3 B3 C3	S02	8948.26	0.1	n/a	0	0.00%	22.8935
A4 B4 C4	S03	1258.89	0.01	n/a	0	0.00%	3.2166
A5 B5 C5	S04	482.099	0.001	n/a	0	0.00%	1.2318
A6 B6 C6	S05	396.656	0.0001	n/a	0	0.00%	1.0212
A7 B7 C7	U01	40594.344	n/a	48.6	0.477	0.98%	103.6452
A8 B8 C8	U02	4329.609	n/a	46.91	0.832	1.77%	11.0625
A9 B9 C9	U03	846.420	n/a	46.93	1.172	2.50%	2.1627
A10 B10 C10	U04	498.349	n/a	47.25	1.326	2.81%	1.2733

Figure 3. Example of output from Instinct software. The "Step1_average" tab shows the linear fit line equation and R2 value in the top section. The bottom chart gives the average raw data, calculated concentrations for unknowns, standard deviations, %CV, and Signal:Blank.

R² value. The chart shows the average of each standard and the inserted standard concentration. Before calculating the linear fit equation, the software subtracts the average of the blank from the raw data for each standard; however, averaged raw data points displayed in the output do not have the background subtracted. One must take this into consideration if manually creating the standard curve using the data from Instinct.

Dynamic Range of QuantiFluor™ dsDNA System

To evaluate the dynamic range of the QuantiFluor™ dsDNA System, we created a standard curve with Lambda DNA standard included in the kit. We used 10, 1, 0.1, 0.01, 0.001, and 0.0001 µg/ml as our standards. All points were in the linear range of the assay except for the 10 µg/ml point. That point was then excluded from the analysis, and the resulting standard curve had a R² value of 1 (Figure 3). The DNA concentration was plotted versus fluorescent output (minus background) (Figure 4). This assay is linear over four orders of magnitude down to 100 pg/ml.

To demonstrate how this standard curve would be used to determine a concentration for an unknown sample, lambda DNA was diluted first to 50 µg/ml and confirmed using a spectrophotometer. The DNA was then diluted 100-, 1,000-, 10,000-, and 100,000-fold into 1X TE. These were added in triplicate on the same plate as the above standard (see Figure 1 for plate configuration) and analyzed as unknowns using the QuantiFluor™ System coupled with the GloMax® Multi+ Instrument with Instinct™ Software. The software produced the data found in Figure 3. The concentrations for each unknown sample based on the spectrophotometer should be ~50 µg/ml and were calculated to be 48.6, 46.91, 46.93, and 47.25 µg/ml with the QuantiFluor™ dsDNA System. The software also calculated standard deviations, %CV, and signal:blank for each unknown sample.

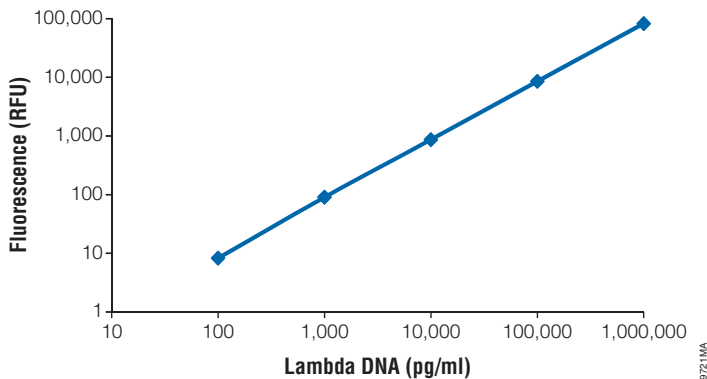


Figure 4. Standard curve created using Lambda DNA and analyzed using the QuantiFluor™ dye. The assay is linear over five logs.

Usage Tips

Samples out of standard curve range: The Instinct software will not calculate a DNA concentration for samples out of the range of the standard curve. If you have a sample that falls out of the range, change the dilution factor of the sample to fit in the standard range.

Standards out of linear range of assay: If the R^2 value of the linear fit curve is not 1 (or very close to it), one of the standards might be out of the linear range of the curve. If this happens, the Instinct software will still calculate sample concentrations; however, the values may not be accurate. Return to the GloMax® Multi+ and remove the data points for the standards that are not in the linear range by dragging those data points to the trash can symbol. An Outlier box appears and asks if you would like to “Exclude this result”. Select yes and repeat for all replicates of that standard. Next, click the “123” button. The data points are now removed. Save the data, and reopen the file on the computer. The new concentrations will be displayed. The R^2 value should now be close to 1.

Create a standard curve every time: When using the Instinct software to determine the concentration of an unknown sample, create a standard curve on every plate analyzed. The software is not capable of taking a curve from a different run and fitting data to that curve. Although the linear fit equation from a previous curve may be applied manually to determine the unknown sample concentration, the data might be less accurate.

Summary

The QuantiFluor™ dsDNA System coupled with the GloMax® Multi+ Detection System with Instinct Software offers a simple, sensitive method to accurately quantitate dsDNA. Samples may be accurately quantitated across 4 orders of magnitude and in concentrations as low as 100pg/ml, making it >20,000 times more sensitive than traditional 260nm absorbance measurements using the NanoDrop® spectrophotometer.

Ordering Information

Product	Size	Cat.#
GloMax®-Multi+ Detection System with Instinct™ Software: Base Instrument with Shaking	1 each	E8032
GloMax®-Multi+ Fluorescence Module	1 each	E8051
QuantiFluor™ dsDNA System	1ml*	E2670

* Each QuantiFluor™ dsDNA System contains sufficient volume to perform 200 assays at 2ml or 2,000 assays at 200µl.

^(a)Patent Pending.

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