# High Throughput, Low Volume DNA Quantitation Using the QuantiFluor® dsDNA System

*Promega Corporation*

**Materials Required**

- QuantiFluor® dsDNA System (Cat.# E2670)
- Multiwell detection instrument capable of measuring fluorescence (e.g., GloMax® Discover System [Cat.# GM3000])
- Nuclease-Free Water (Cat.# P1195)
- Black, flat-bottom 384-well plates (Corning; Cat.#3573)
- 100 ml Polypropylene Reagent Troughs (TECAN, Cat.#10613048 or similar)
- Optional: K562 Genomic DNA (Cat.# E4931)

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

**Protocols:** *QuantiFluor® dsDNA System Technical Manual*, TM346, is available at: **www.promega.com/protocols/**

## 1. Introduction

DNA quantitation and normalization are critical steps for a wide range of molecular biology applications such as cloning, quantitative PCR (qPCR) and next-generation sequencing (NGS). Fluorescent dye-based DNA quantitation uses specially designed DNA binding compounds that intercalate only with double-stranded DNA (dsDNA) molecules. When excited by a specific wavelength of light, only dye in the DNA-bound state will fluoresce. These aspects of the technique contribute to low background signal, high accuracy and specificity—making it a suitable measure of DNA concentration prior to advanced downstream applications.

Another desirable attribute of fluorescence quantitation over other methods is ease of use and scalability for laboratories conducting all levels of throughput. In this application note, we demonstrate the flexibility of the QuantiFluor® dsDNA System for use in 40µl reaction volumes compatible with 384-well plates. We also provide guidance for optimization of the assay according to variables such as dynamic range, sensitivity, accuracy, cost per reaction and other features of high-throughput workflows.

## 2. Protocol

The following protocol assumes a 40µl total assay volume per well, utilizing 36µl of working solution plus 4µl of standard/blank/unknown.

1. **Prepare 1X TE Buffer**: Dilute the 20X TE Buffer 20-fold with Nuclease-Free Water.
2. **Prepare QuantiFluor® dsDNA Dye working solution:** Reference Section 4 to determine the most appropriate dye dilution for your particular application.
   **Note:** Working solution is stable at room temperature under normal ambient lighting conditions for at least 2 hours (see Section 3 for details concerning dye working solution stability).

3. **Prepare a Standard Curve:** Perform a seven-point titration with dsDNA standard of known concentration that extends above and below the concentration range of any unknown sample. Remember to adjust concentration of standards for the 4µl assay input.
   **Note:** Lambda DNA Standard is included in the QuantiFluor® dsDNA System; however, we recommend choosing the most appropriate standard for your particular sample type. See Section 5 for considerations in choosing a DNA standard.
4. Transfer 36µl of QuantiFluor® dsDNA Dye working solution into each well that is intended for an unknown, blank or standard sample.
5. Dispense 4µl of the dsDNA standards to wells (see Figure 1 for example plate layout). We recommend pipetting duplicates or triplicates of the standards.
6. For the blank, dispense 4µl of 1X TE buffer.
7. Dispense 4µl of unknown sample to the remaining wells.

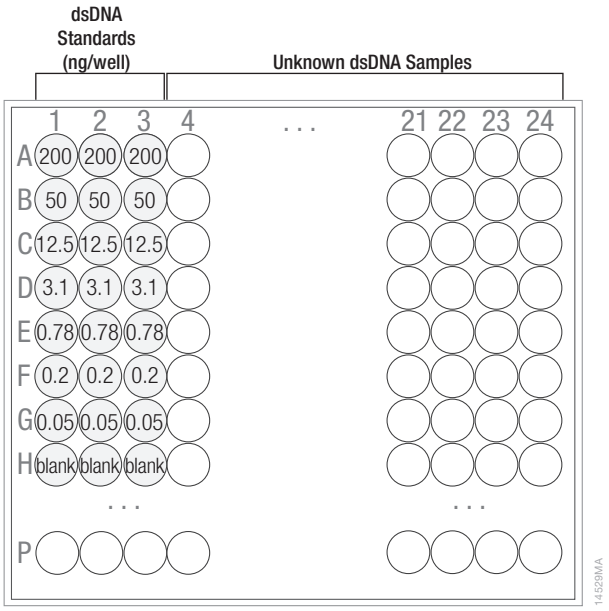


**Figure 1. Example 384-well plate map with standards, blanks and unknowns labeled.**

8. Mix thoroughly using a plate shaker or by pipetting the contents of each well up and down. Failure to mix well will lead to variable Relative Fluorescence Units (RFU) readings between wells. Use caution to avoid introducing air bubbles, which will interfere with fluorescence.
9. Incubate for 5 minutes at room temperature.
10. Measure fluorescence ($504nm_{Ex}/531nm_{Em}$).
11. Calculate the dsDNA concentration as follows: Subtract the fluorescence of the blank sample (1X TE Buffer) 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 by either a linear regression or power regression (see Section 6 on considerations for regression models).

## 3. Diluted QuantiFluor® Dye Stability

The QuantiFluor® dsDNA Dye, diluted in 1X TE Buffer as a working solution, will perform well if left in a trough on the deck of a liquid handler, exposed to light at room temperature for at least 2 hours (Figure 2). This stability is important for high-throughput labs that desire to make enough working solution to quantitate many plates in succession over the course of several hours or longer. While it is always best practice to protect the dye from light where possible, the robust nature of the QuantiFluor® dsDNA Dye allows for added flexibility and convenience in reagent handling.

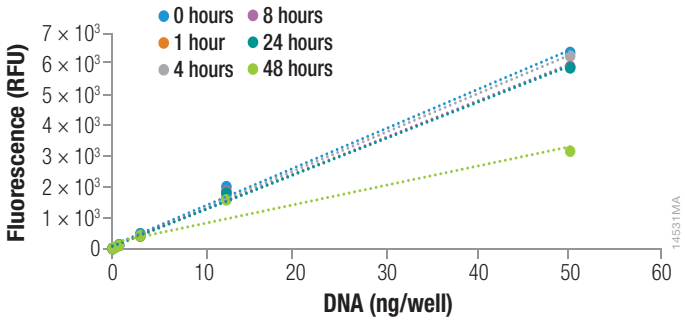


**Figure 2. QuantiFluor® dsDNA Dye working solution.** A 1:200 dilution working solution was left in a trough on the deck of a liquid handler exposed to ambient laboratory temperature and light. Dye was used at several time points to measure DNA in 384-well plate format over the range 0.05–50ng/well. A decrease in RFU signal was observed only after 24–48 hours.

## 4. Optimizing dilution of QuantiFluor® dsDNA Dye working solution for 384-well format

Purified sample concentration will vary dramatically depending on sample type and method of purification. Saliva, for example, will often yield a high concentration of DNA while formalin-fixed, paraffin-embedded (FFPE) tissue or circulating, cell-free DNA (ccfDNA) from plasma are usually much less concentrated. Depending on the number of sample types and the expected range in sample concentration, a given laboratory may need to optimize their quantitation around several parameters including: dynamic range, sensitivity, accuracy and cost per reaction.

The QuantiFluor® dsDNA System provides accurate quantitation over a range of 0.05–1000ng DNA (Figure 3). In Table 1 we give a general starting point for optimizating dilution of QuantiFluor® Dye working solution for a 384-well format.

**Table 1. Guidance on recommended dilution of QuantiFluor® Dye working solution with corresponding dynamic range.**

| Dye Dilution | | |
|---|---|---|
| Volume of Dye per Well | Total Dye Dillution (40µl assay) | Assay Dynamic Range[1,2] |
| 0.1µl | 1:400 | 1–50ng |
| 0.2µl | 1:200 | 0.05–50ng |
| 0.5µl | 1:80 | 1–200ng |
| 1µl | 1:40 | 0.2–400ng |
| 2µl | 1:20 | 1–1000ng |

[1]The above dynamic ranges refer to DNA concentration per well in the final assay. Concentration of initial sample is dependent on volume and any dilutions of sample used for the assay. For example, if 4µl of sample is used without dilution, the initial sample concentration for a 1ng/well read will be 1ng/well ÷ 4µl =0.25ng/µl.

[2]Dynamic range and accuracy of any given point within that range is dependent on type of analytical regression curve used. We recommend using a power regression to ensure best accuracy across all points of the above ranges.

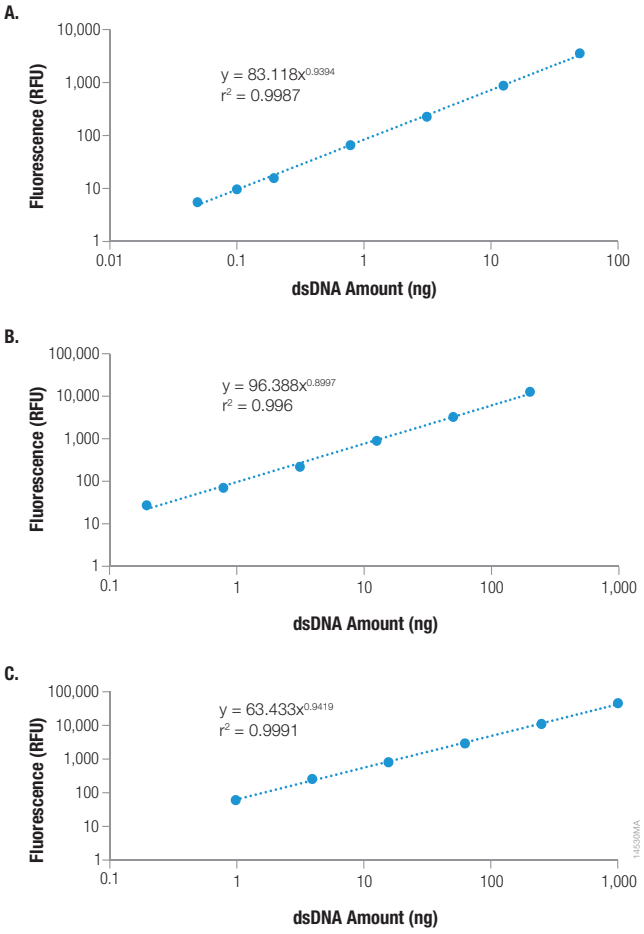


**Figure 3. QuantiFluor® dsDNA Dye at three different concentrations accurately quantitates DNA over a range of 0.05–1,000ng in 384-well format.** Panel A. 0.2µl dye/well; Panel B. 1µl dye/well; Panel C. 3µl dye/well.

## 5. DNA Standards

Quantitation of unknown samples requires comparison to a dsDNA standard; the Lambda DNA Standard is provided for this purpose. While lambda DNA is sufficient for many applications, not every DNA type will perform consistently as a standard (Figure 4). We recommend preparing a standard using dsDNA of a size similar to the dsDNA you wish to quantitate. K562 Genomic DNA (human chronic myelogenous leukemia cell line; Cat.# E4931) is available for users interested in processing blood or samples of higher molecular weight.

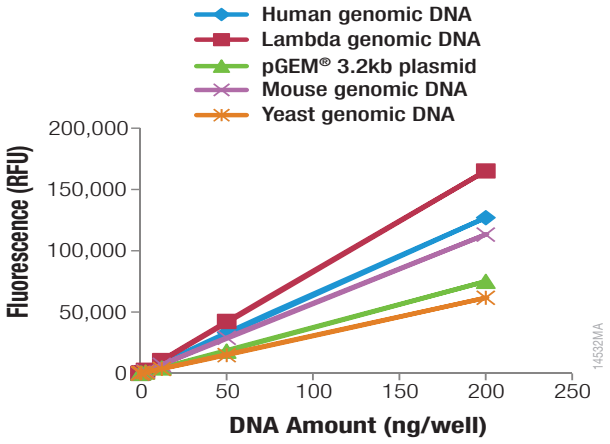


**Figure 4. Five different DNA standards measured using the QuantiFluor® dsDNA System.** Relative fluorescence intensity varies for equal concentrations of different DNA types.

## 6. Regression Models

Almeida, Castel-Branco and Falcão (2002) highlight a common pitfall for analytical methods where a large range (greater than an order of magnitude) of a variable is used. They state that homoscedasticity is often not met for analytical data over large ranges in concentration. Because larger variations at higher concentrations tend to influence the regression line more than smaller deviations associated with smaller concentrations, the accuracy in the lower end of the curve is impaired. To avoid this effect they suggest using a weighted least squares linear regression (WLSLR). WLSLR weights each sample differently in the regression and therefore compensates for the inaccuracy of a normal linear regression.

The standard curve for the QuantiFluor® dsDNA System is greater than one order of magnitude, thus larger deviations present at higher concentrations will negatively impact the accuracy at the lower end of the dynamic range. For this reason we recommend replacing a linear regression (in the form y=mx+b) to a power regression ($y=ax^b$). A power regression is a log-log transformation and, similar to the WLSLR, will more evenly account for the sample variances across the standard curve. Although not as rigorous as a WLSLR, we have shown that the power regression provides improved accuracy over linear regression for the QuantiFluor® dsDNA System (Table 2).

## 7. Plate Reader Considerations

The GloMax® Discover and Explorer Systems are designed for optimal integration with QuantiFluor® dsDNA Systems. If using a plate reader other than a GloMax® System, we recommend the following to reduce performance variability caused by differences across plate reader makes and models:

- Choose the fluorescence wavelength settings on your plate reader that most closely match the QuantiFluor® dsDNA System ($504nm_{Ex}/531nm_{Em}$).
- Avoid edge/clipping effects, especially for 384-well formats, by ensuring the plate has been defined correctly either manually or by using the instrument plate definition wizard.
- To improve accuracy, adjust to measure 3–5 reads per well at the center of each well.

## 8. References

A.M. Almeida, M.M. Castel-Branco and A.C. Falcão (2002) Linear regression for calibration lines revisited: weighting schemes for bioanalytical methods. *J Chromatogr B Analyt Technol Biomed Life Sci.* **774(2)**, 215–22.

**Table 2. Power regression improves quantitation accuracy for low level samples when using a standard curve spanning several orders of magnitude.** The below example illustrates quantitation of dsDNA standards of known concentrations (0.2–400ng/well) measured in triplicate with the QuantiFluor® dsDNA System using a 1:40 working solution dye dilution. Concentration was calculated with linear and power regression [$x=(y/a)^{(1/b)}$] and accuracy was measured as a percentage of the expected concentration.

| | | Linear Regression (y=mx+b) | | Power Regression ($y=ax^b$) | |
|---|---|---|---|---|---|
| Standard DNA Concentration (ng/well) | Average RFU | Calculated Concentration (ng/well) | % Expected | Calculated Concentration (ng/well) | % Expected |
| 0.1953125 | $1.82\times10^1$ | 0.038898342 | 19.91595119 | 0.226747162 | 116.0945469 |
| 0.78125 | $5.96\times10^1$ | 0.645521074 | 82.62669745 | 0.769718424 | 98.52395831 |
| 3.125 | $1.95\times10^2$ | 2.629711645 | 84.15077262 | 2.611341321 | 83.56292227 |
| 12.5 | $8.53\times10^2$ | 12.2812911 | 98.25032884 | 11.95956559 | 95.6765247 |
| 50 | $3.32\times10^3$ | 48.38257382 | 96.76514764 | 48.44620442 | 96.89240885 |
| 200 | $1.40\times10^4$ | 204.8212246 | 102.4106123 | 213.5611893 | 106.7805947 |
| 400 | $2.71\times10^4$ | 397.8033678 | 99.45084194 | 423.0569355 | 105.7642339 |

## 9. Ordering information

| Product | Cat. # |
|---|---|
| GloMax® Discover System | GM3000 |
| GloMax® Explorer System | GM3500 |
| QuantiFluor® dsDNA System | E2670 |
| QuantiFluor® ONE dsDNA System | E4871 |
| QuantiFluor® RNA System | E3310 |
| K562 Genomic DNA | E4931 |

- For questions about ordering or information regarding custom formulations options, please contact customer services: **custserv@promega.com**
- For technical inquiries, troubleshooting or to request assistance with automating Promega assays on your robotic liquid handling platform, please contact technical services: **techserv@promega.com**
- For more information regarding the QuantiFluor® dsDNA System, visit: **https://www.promega.com/products/dna-purification-quantitation/dna-and-rna-quantitation/quantifluor-dsdna-system/?catNum=E2670**



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