

TECHNICAL MANUAL

# QuantiFluor® dsDNA System

Instructions for Use of Product  
**E2670**



# QuantiFluor® dsDNA System

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## 1. Description

The QuantiFluor® dsDNA System<sup>(a)</sup> contains a fluorescent double-stranded DNA-binding dye (504nm<sub>Ex</sub>/531nm<sub>Em</sub>) that enables sensitive quantitation of small amounts of double-stranded DNA (dsDNA).

The QuantiFluor® dsDNA System was developed using the fluorescence module of the GloMax® Multi+ Detection System and the QuantiFluor®-ST Handheld Fluorometer. The QuantiFluor® dsDNA System can be used with any fluorometer that is capable of measuring fluorescence at the appropriate excitation and emission wavelengths, including the Quantus™ Fluorometer.



## 2. Product Components and Storage Conditions

| PRODUCT                   | SIZE | CAT.# |
|---------------------------|------|-------|
| QuantiFluor® dsDNA System | 1ml  | E2670 |

This system contains sufficient reagents for 200 assays at 2ml scale or 2,000 assays at 200µl scale. Includes:

- 25ml 20X TE Buffer (pH 7.5)
- 1ml QuantiFluor® dsDNA Dye, 200X
- 100µg Lambda DNA Standard, 100µg/ml

**Storage Conditions:** Product may arrive frozen. Upon receipt, store the QuantiFluor® dsDNA Dye at 2–10°C, protected from light. Store the Lambda DNA Standard, 100µg/ml, at 2–10°C. Do not refreeze the Lambda DNA Standard, 100µg/ml. Store the 20X TE Buffer at –30°C to +30°C.



If the Lambda DNA Standard, 100µg/ml, arrived frozen, a concentration gradient may have formed and should be dispersed upon thawing. Store the Lambda DNA Standard, 100µg/ml, at 2–10°C overnight, then warm to room temperature and mix well before use.



Instructions for handling and disposal of the QuantiFluor® dsDNA Dye are located in Section 8.D.

## 3. Preparing the QuantiFluor® dsDNA Dye Working Solution

### Materials to Be Supplied by the User

- nuclease-free water
- thin-walled tubes (e.g., Axygen Cat.# PCR-05-C), cuvettes or black flat-bottom plates
- fluorometer

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.

1. Prepare 1X TE buffer by diluting the 20X TE Buffer 20-fold with nuclease-free water (not provided). For example, add 1ml of 20X TE Buffer to 19ml of Nuclease-Free Water (Cat.# P1195), and mix.
2. For the standard curve, prepare the QuantiFluor® dsDNA Dye working solution by diluting the 200X QuantiFluor® dsDNA Dye with 1X TE buffer. Prepare enough QuantiFluor® dsDNA Dye working solution to quantitate both standards and unknown samples. Use the following worksheet to determine the volume of QuantiFluor® dsDNA Dye working solution for your quantitation assay:

Number of standard samples \_\_\_\_\_

+ Number of unknown samples \_\_\_\_\_

+ 4 (for pipetting error) = \_\_\_\_\_ × 100µl = Volume of QuantiFluor® dsDNA Dye working solution needed

For example, to quantify 7 standard samples and 23 unknown samples, prepare 3,400µl  $[(7 + 23 + 4) \times 100]$  of QuantiFluor® dsDNA Dye working solution. Add 17µl of QuantiFluor® dsDNA Dye to 3,383µl of 1X TE buffer. Mix.

 Protect the working solution from light by covering it with foil or placing it in the dark.

**Note:** The QuantiFluor® Dye working solution is stable for 2–3 hours at 25°C.

#### 4. Preparing Standard Curve Samples

Quantitation of unknown samples requires comparison of the unknown samples to a dsDNA standard curve. Prepare a standard curve using the Lambda DNA Standard. Generate a standard curve appropriate for the expected range of dsDNA concentrations of your unknown samples and your sample analysis setup.

Even though the Lambda DNA Standard is provided with the QuantiFluor® dsDNA System, 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 is 48.5kb.

##### Notes:

- If the Quantus™ Fluorometer (Cat.# E6150) is used for detection, follow the instructions in the *Quantus™ Fluorometer Operating Manual #TM396* to prepare the QuantiFluor® dsDNA Dye, standard and unknown samples.
- If using the Quantus™ Fluorometer or QuantiFluor®-ST Fluorometer, only two points are needed (blank and highest dsDNA concentration). A true standard curve is not required when using the Quantus™ Fluorometer or QuantiFluor®-ST Fluorometer because these instruments generate a standard curve based on those two points and calculate the dsDNA concentrations of the unknown samples.

The following protocols are examples of how to prepare a standard curve. We recommend preparing a standard curve that extends above and below the likely concentration range for your unknown samples. In addition, a blank sample containing 1X TE buffer should be used to assess the background level of the assay.

**Note:** The minimum amount of detectable dsDNA will depend on factors such as the plasticware and reader used to measure fluorescence.

#### 4. Preparing Standard Curve Samples (continued)



If the Lambda DNA Standard, 100µg/ml, arrived frozen, a concentration gradient may have formed and should be dispersed upon thawing. Store the Lambda DNA Standard, 100µg/ml, at 2–10°C overnight, then warm to room temperature and mix well before use. Do not refreeze the Lambda DNA Standard, 100µg/ml.

- 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. Below is a table containing recommended volumes for several assay formats:

| <b>Format</b>                | <b>Volume of 1X TE Buffer (µl)</b> | <b>Volume of dsDNA Standard (µl)</b> |
|------------------------------|------------------------------------|--------------------------------------|
| 0.5ml PCR tube (200µl assay) | 980µl                              | 20µl                                 |
| 96-well plate (200µl assay)  | 980µl                              | 20µl                                 |
| 10 × 10 cuvette (2ml assay)  | 1,960µl                            | 40µl                                 |

- Prepare the standard curve samples as shown in Table 1.

**Table 1. Preparing dsDNA Standard Curve Samples.**

| <b>Standard</b> | <b>Volume of dsDNA Standard</b> | <b>Volume of 1X TE Buffer (µl)</b> | <b>dsDNA Amount Per 100µl (ng)</b> | <b>dsDNA Concentration Before Adding Dye (ng/ml)</b> | <b>Final dsDNA Concentration After Adding Dye (ng/ml)</b> |
|-----------------|---------------------------------|------------------------------------|------------------------------------|------------------------------------------------------|-----------------------------------------------------------|
| Blank           | 0                               | 1,000                              | 0                                  | 0                                                    | 0                                                         |
| A               | 1,000µl <sup>1</sup>            | 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                                                       |

<sup>1</sup>Use 1,000µl of the 2ng/µl Lambda DNA Standard prepared in Step 1.

## 5. Protocol

Quantitation of unknown samples requires comparison to a dsDNA standard curve. Prepare a standard curve using the Lambda DNA Standard as directed in Section 4.

### Notes:

- If the Quantus™ Fluorometer (Cat.# E6150) is used for detection, follow the instructions in the *Quantus™ Fluorometer Operating Manual #TM396* to prepare the QuantiFluor® dsDNA Dye, standard and unknown samples.
  - If using the Quantus™ Fluorometer or QuantiFluor®-ST Fluorometer, only two points are needed (blank and highest dsDNA concentration). A true standard curve is not required when using the Quantus™ Fluorometer or QuantiFluor®-ST Fluorometer because these instruments generate a standard curve based on those two points and calculate the dsDNA concentrations of the unknown samples.
1. Mix each unknown or standard sample with an equal volume of QuantiFluor® dsDNA Dye working solution based on the preferred quantitation method.

### Single-Tube Formats:

- a. **0.5ml PCR Tube** (200µl assay volume): Dilute unknown samples to 100µl total volume with 1X TE buffer. Add 100µl of QuantiFluor® Dye working solution (prepared in Section 3) to each cuvette containing 100µl of unknown, blank or standard sample, and mix briefly by pipetting.
- b. **10 × 10 Cuvette** (2ml assay volume): Dilute unknown samples to 1ml total volume with 1X TE buffer. Add 1ml of QuantiFluor® Dye working solution (prepared in Section 3) to each cuvette containing 1ml of unknown, blank or standard sample, and mix briefly by pipetting.

### Multiwell Plate Format

- c. **96-Well Plate** (200µl assay volume): Dilute unknown samples to 100µl total volume with 1X TE buffer. Add 100µl of QuantiFluor® Dye working solution (prepared in Section 3) to each well containing 100µl of unknown, blank or standard sample, and mix briefly.



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 (see Section 5, Step 4).

2. Incubate assays for 5 minutes at room temperature, protected from light.

## 5. Protocol (continued)

### 3. Measure fluorescence (504nm<sub>Ex</sub>/531nm<sub>Em</sub>).

When using the Quantus™ Fluorometer, select the dsDNA protocol on the instrument.

When using the PCR tube- or cuvette-based format with the QuantiFluor®-ST Handheld Fluorometer, use the Blue optical channel.

For the multiwell plate format, use the Blue Fluorescence Optical Kit (490nm<sub>Ex</sub>/510–570nm<sub>Em</sub>) for the GloMax®-Multi+ Detection Systems.

#### Notes:

1. If using the QuantiFluor®-ST Handheld Fluorometer in the PCR tube or cuvette format, set the standard value of the instrument by pressing STD VAL and entering 200.
2. Instructions for proper calibration of the Quantus™ Fluorometer can be found in the *Quantus™ Fluorometer Operating Manual #TM396*. Calibration instructions for the QuantiFluor®-ST Handheld Fluorometer can be found in the *QuantiFluor® Handheld Fluorometers Operating Manual #TM338*, and instructions for use of the GloMax®-Multi+ Detection Systems can be found in the *GloMax® Multi+ Detection System with Instinct® Software Technical Manual #TM340*, available at:

**[www.promega.com/protocols](http://www.promega.com/protocols)**

### 4. Calculate the DNA concentration of your unknown samples using the fluorescence values measured in Step 3.

Some fluorometers, such as the Quantus™ Fluorometer or QuantiFluor®-ST Handheld Fluorometer, automatically calculate the concentrations of unknown samples using a two-point line (a blank and a standard). The Quantus™ Fluorometer will calculate the concentration automatically and report the value after each measurement. The QuantiFluor®-ST Handheld Fluorometer will display unitless values, and the user will have to correct for the dilution factor. For example, if the QuantiFluor®-ST Fluorometer was calibrated using a 200ng standard, 1µl of an unknown sample was prepared as instructed in Section 5, Step 1.a, and the resulting value displayed on the instrument is “100”, there is 100ng of dsDNA in the 1µl of unknown sample. Therefore, the original concentration of the unknown sample is 100ng/µl. If 2µl was quantitated, then the original unknown sample is 100ng ÷ 2µl = 50ng/µl. If 5µl was quantitated, then the original unknown sample is 100ng ÷ 5µl = 20ng/µl. To convert from ng/µl to ng/ml, multiply by 1,000.

For other instruments, calculate the dsDNA concentration as described in the following steps:

- a. Subtract the fluorescence of the blank sample (1X TE buffer) from the fluorescence measurements for all unknown and standard samples.
- b. Plot the standard curve values, with the final concentration of dsDNA after adding the QuantiFluor® dsDNA Dye in ng/ml on the X axis and fluorescence in relative fluorescence units (RFU) on the Y axis.
- c. Fit a line to the standard curve values, and display the linear regression calculation for that line. This will take the form of  $y = mx + b$ , or [fluorescence = (slope × dsDNA concentration) + Y intercept].
- d. Calculate the concentration for each blank-subtracted unknown sample by using fluorescence as y in this equation and determining the value of x.
- e. Multiply the resulting number by the dilution factor, if applicable.

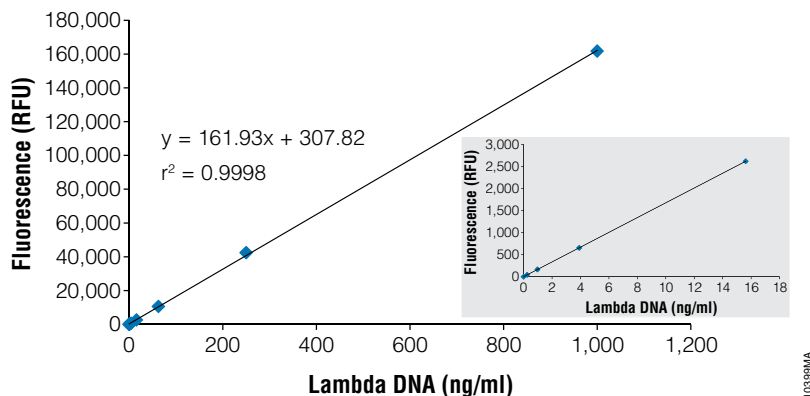
## 6. Representative Data

Representative data for the QuantiFluor® dsDNA Dye are shown in Table 2 and Figure 1.

**Table 2. Representative Data for the dsDNA Standard Curve and QuantiFluor® dsDNA Dye.**

| Lambda DNA Concentration After Adding QuantiFluor® dsDNA Dye (ng/ml) | Average Fluorescence (RFU) <sup>1</sup> |
|----------------------------------------------------------------------|-----------------------------------------|
| 0                                                                    | 0                                       |
| 0.2                                                                  | 42                                      |
| 1.0                                                                  | 163                                     |
| 3.9                                                                  | 652                                     |
| 16                                                                   | 2,620                                   |
| 62.5                                                                 | 10,590                                  |
| 250                                                                  | 42,479                                  |
| 1,000                                                                | 161,813                                 |

<sup>1</sup>Background fluorescence has been subtracted. n = 3



**Figure 1. Representative dsDNA standard curve in a 96-well-plate format.** The final amounts of the Lambda DNA Standard in the 96-well, 200 $\mu$ l assay format are listed in Table 2. **Inset.** Expanded view of the low end of the standard curve.

## 6. Representative Data (continued)

Example calculation using 1 µl of unknown sample in triplicate wells:

The standard and unknown samples have these average fluorescence values (in RFU):

|                         |        | Amount of dsDNA per Milliliter |       |       |       |       |        |        |         |
|-------------------------|--------|--------------------------------|-------|-------|-------|-------|--------|--------|---------|
|                         |        | 0ng                            | 0.2ng | 1.0ng | 3.9ng | 16ng  | 62.5ng | 250ng  | 1,000ng |
| <b>Standard Samples</b> |        | 418                            | 460   | 581   | 1,070 | 3,038 | 11,008 | 42,897 | 162,231 |
| <b>Unknown Sample</b>   | 15,418 |                                |       |       |       |       |        |        |         |

1. Subtract the 1X TE buffer blank (average of blank standards) from all samples:

|                         |        | Amount of dsDNA per Milliliter |       |       |       |       |        |        |         |
|-------------------------|--------|--------------------------------|-------|-------|-------|-------|--------|--------|---------|
|                         |        | 0ng                            | 0.2ng | 1.0ng | 3.9ng | 16ng  | 62.5ng | 250ng  | 1,000ng |
| <b>Standard Samples</b> |        | 0                              | 42    | 163   | 652   | 2,620 | 10,590 | 42,479 | 161,813 |
| <b>Unknown Sample</b>   | 15,000 |                                |       |       |       |       |        |        |         |

2. Determine the linear regression from the scatter plot (Figure 1).  
 $y = 161.93x + 307.82$
3. Calculate the DNA concentration of the unknown sample in the 200 µl assay volume by solving for x in the linear regression equation, where:  
 $y = 15,000$   
 $x = (y - 307.82) / 161.93 = 90.7 \text{ ng/ml}$
4. Account for any dilution of the unknown sample (i.e., dilution factor = assay volume ÷ starting sample volume). For example, first, convert the concentration in the tube or well (90.7 ng/ml) into ng/µl (i.e., divide by 1,000); next, determine the total number of nanograms in the tube or well (multiplied by the assay volume of 200 µl), and finally, divide by the starting sample volume. If 1 µl of sample was added per well, the sample concentration is  $(90.7 \text{ ng/ml} \div 1,000) \times 200 \mu\text{l} \div 1 \mu\text{l} = 18.1 \text{ ng}/\mu\text{l}$ . If 5 µl of sample was added per well, the sample concentration is  $(90.7 \text{ ng/ml} \div 1,000) \times 200 \mu\text{l} \div 5 \mu\text{l} = 3.6 \text{ ng}/\mu\text{l}$ .

## 7. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: [www.promega.com](http://www.promega.com). E-mail: [techserv@promega.com](mailto:techserv@promega.com)

| <b>Symptoms</b>                 | <b>Causes and Comments</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|---------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Low or no fluorescence detected | Check that the correct filter set was used for the QuantiFluor® dsDNA Dye. For the QuantiFluor® dsDNA Dye, read the fluorescence at 504nm <sub>Ex</sub> /531nm <sub>Em</sub> (see Figure 3 for excitation and emission spectra).                                                                                                                                                                                                                                                                                        |
|                                 | The QuantiFluor® dsDNA Dye is light-sensitive. Exposure to light will reduce the sensitivity of the assay. Store QuantiFluor® dsDNA Dye and working solution protected from light.                                                                                                                                                                                                                                                                                                                                      |
|                                 | Confirm that dye was added. Add an equal volume of QuantiFluor® dsDNA Dye working solution to each sample.                                                                                                                                                                                                                                                                                                                                                                                                              |
|                                 | Check that unknown and standard samples were diluted appropriately. Increase the concentration of the unknown and standard samples, if necessary.                                                                                                                                                                                                                                                                                                                                                                       |
|                                 | Confirm that the unknown sample calculations were performed correctly and, if applicable, the concentrations calculated from the linear regression were multiplied by the dilution factor (Section 5, Step 4).                                                                                                                                                                                                                                                                                                          |
|                                 | Check that the unknown sample was within the sensitivity range of the assay and standard curve. Determine the average fluorescence and standard deviation of the blank standards. Subtract the average fluorescence of the blank standard from the average fluorescence of the unknown and standard samples. These blank-subtracted values should be more than three standard deviations (as determined for the blank standards) above the average fluorescence for the blank standards.                                |
|                                 | The high end of the standard curve was not within the dynamic range for the QuantiFluor® dsDNA Dye. Evaluate the blank-subtracted fluorescence of the standard curve. The values should be proportional to the dilution factors used to create the standard curve. If the increase in fluorescence is not proportional to the increase in dsDNA amount, the QuantiFluor® dsDNA Dye may be saturated (see Section 8.A). Recreate the standard curve, and decrease the concentration of the highest standard curve point. |

## 7. Troubleshooting (continued)

| <b>Symptoms</b>                                                                                                                                 | <b>Causes and Comments</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|-------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| No or low fluorescence detected in the standard samples                                                                                         | <p>Evaluate the performance of the fluorometer with a dsDNA sample of known concentration (e.g., Lambda DNA Standard) using the appropriate excitation and emission wavelengths for the QuantiFluor® dsDNA Dye.</p> <hr/> <p>The QuantiFluor® dsDNA Dye was exposed to light. Exposure to light will reduce the sensitivity of the assay. Store QuantiFluor® dsDNA Dye and working solution protected from light.</p> <hr/> <p>Check that the standard samples were diluted appropriately.</p> <hr/> <p>Mix the dsDNA standards with the QuantiFluor® dsDNA Dye working solution just prior to measurement. Extended exposure to light will decrease the amount of fluorescence detected.</p>                                                                                                                                                                                                                                                                                                                                                                                                  |
| Fluorescence too high                                                                                                                           | <p>Check that the unknown and standard samples were diluted appropriately. Decrease the concentration of the unknown and standard samples, if necessary.</p> <hr/> <p>Adjust the gain setting on your fluorometer so that the highest point on the standard curve is approximately 90% of maximum signal. This is not necessary for the GloMax®-Multi or GloMax®-Multi+ Detection Systems because these instruments will adjust automatically. The Quantus™ Fluorometer and QuantiFluor®-ST Handheld Fluorometer do not require gain adjustment.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| dsDNA concentration determined using the QuantiFluor® dsDNA Dye differed from concentration determined using an alternative quantitation method | <p>DNA concentrations determined using the QuantiFluor® dsDNA Dye and optical density readings at 260nm will be different due to inherent differences between methodologies. An optical density reading at <math>A_{260}</math> reflects the amount of light that is neither transmitted nor reflected and is proportional to the amount of all nucleic acid (dsDNA, ssDNA, RNA and nucleotides) in the sample. The QuantiFluor® dsDNA Dye intercalates into dsDNA and therefore, the amount of fluorescence is proportional to the amount of dsDNA.</p> <hr/> <p>If comparing concentrations determined using another dye-based quantitation method, carefully examine the blank-subtracted fluorescence of the two standard curves. The values should be proportional to the dilution factors used to create the standard curve. If the increase in fluorescence is not proportional to the increase in dsDNA amount, the fluorescent dye(s) may be saturated (see Section 8.A). Recreate the standard curve, and decrease the concentration of the highest point of the standard curve.</p> |

## Symptoms

dsDNA concentration determined using the QuantiFluor® dsDNA Dye differed from concentration determined using an alternative quantitation method (continued)

## Causes and Comments

Determine the average fluorescence and standard deviation of the blank standards. Subtract the average fluorescence of the blank standards from the average fluorescence of the unknown and standard samples. The blank-subtracted fluorescence should be more than three standard deviations (as determined for the blank standards) above the average fluorescence for the blank standards.

Nonlinear standard curve

Check that standard samples were diluted appropriately. If the high or low end of curve is nonlinear, then adjust the standard sample dilutions such that the standard curve is linear.

Adjust the gain setting on your fluorometer so that the highest point on the standard curve is approximately 90% of maximum signal. This is not necessary for the GloMax®-Multi or GloMax®-Multi+ Detection Systems because these instruments will adjust automatically. The Quantus™ Fluorometer and QuantiFluor®-ST Handheld Fluorometer do not require gain adjustment.

Check that the lower concentration standards are within the sensitivity range for the assay and assay format. Determine the average fluorescence and standard deviation of the blank standards. Subtract the average fluorescence of the blank standards from the average fluorescence of the unknown and standard samples. These blank-subtracted values should be greater than three standard deviations (as determined for the blank standards) above the average fluorescence for the blank standards.

Check that the unknown sample is within the sensitivity range of the assay and standard curve. Determine the average fluorescence and standard deviation of the blank standards. Subtract the average fluorescence of the blank standard from the average fluorescence of the unknown and standard samples. These blank-subtracted values should be more than three standard deviations (as determined for the blank standards) above the average fluorescence for the blank standards.

The QuantiFluor® dsDNA Dye is light-sensitive. Exposure to light will reduce the sensitivity of the assay. Store QuantiFluor® dsDNA Dye and working solution protected from light.

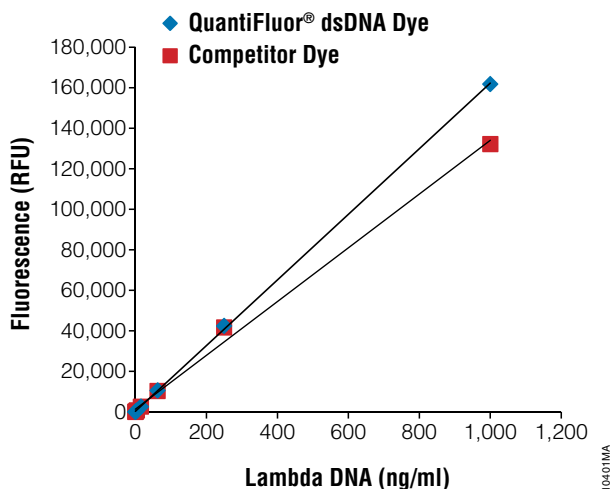
Check that the standard samples were diluted appropriately.

A concentration gradient may have formed if the Lambda DNA Standard, 100µg/ml, arrived frozen. Store Lambda DNA Standard, 100µg/ml, at 2–10°C overnight, then warm to room temperature and mix well before use. Do not refreeze the Lambda DNA Standard, 100µg/ml.

## 8. Appendix

### 8.A. QuantiFluor® dsDNA Dye and Competitor Dye Dynamic Range

Both the QuantiFluor® dsDNA Dye and competitor dye have similar dynamic ranges and become saturated near 1,000ng/ml in the 96-well, 200µl assay format (Figure 3). For the QuantiFluor® dsDNA Dye, fluorescence for the 1,000ng/ml standard (161,813RFU) nearly equals four times the fluorescence of the 250ng/ml standard ( $4 \times 42,479 = 169,916\text{RFU}$ ). For the competitor dye, fluorescence of the 1,000ng/ml standard (132,174RFU) is less than four times the fluorescence of the 250ng/ml standard ( $4 \times 41,685 = 166,740\text{RFU}$ ). Carefully evaluate the standard curve fluorescence values prior to performing linear regression and calculating the blank-subtracted fluorescence for the standard samples. These values should be proportional to the dilution factors used when creating the standard curve. For example, if the standard curve was created using serial twofold dilutions, the fluorescence should change in twofold increments and be proportional to the DNA concentration. If the increase in fluorescence is not proportional to the increase in dsDNA amount, the dye may be saturated. If this occurs, recreate the standard curve and decrease the concentration of the highest standard curve point.

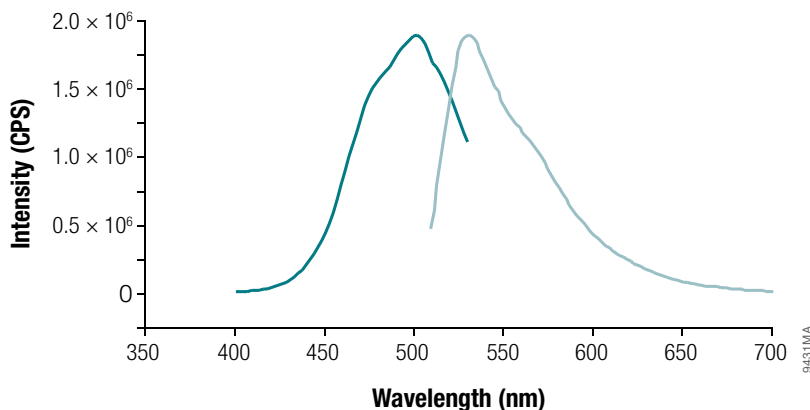


**Figure 2. Standard curves using the QuantiFluor® dsDNA Dye and a competitor dye.** Representative standard curves were generated using Lambda DNA Standard in a 96-well, 200µl assay format as described in Section 5. The standard curve DNA concentrations are 0.2, 1.0, 3.9, 16, 62.5, 250 and 1,000ng/ml. Fluorescence was measured using the GloMax®-Multi+ Detection System. The average fluorescence for the blank standards was subtracted from each sample. Under these conditions, the dynamic range for both dyes is approximately 100pg–200ng per well (in 200µl total volume), and the QuantiFluor® dsDNA Dye limit of detection is approximately 10pg per well as defined by greater than three standard deviations above background fluorescence.

## 8.B. Instrument Compatibility

Fluorescence measurements can be performed using any fluorescence reader capable of measuring excitation and emission at the appropriate wavelengths. For the QuantiFluor® dsDNA Dye, measure excitation and emission at 504nm and 531nm, respectively; excitation and emission spectra are shown in Figure 3.

The QuantiFluor® dsDNA Dye was developed using the fluorescence module of GloMax® Multi+ Detection System and the QuantiFluor®-ST Handheld Fluorometer. The Quantus™ Fluorometer was optimized for use with the QuantiFluor® Dyes, including the QuantiFluor® dsDNA Dye.



**Figure 3. Excitation and emission spectra for the QuantiFluor® dsDNA Dye.**

### 8.C. Interfering Compounds

Several compounds that are commonly used in nucleic acid preparation or can be found in eluates from nucleic acid purification may affect the QuantiFluor® dsDNA Dye. Table 3 lists compounds that have known effects on DNA quantitation using the QuantiFluor® dsDNA Dye and the concentrations at which they affect quantitation results.

**Table 3. Compounds that Interfere with the QuantiFluor® dsDNA Dye.**

| <b>Chemical</b>                  | <b>Concentration Shown to Affect the QuantiFluor® dsDNA Dye</b> |
|----------------------------------|-----------------------------------------------------------------|
| agarose                          | >0.01%                                                          |
| ammonium acetate                 | >50mM                                                           |
| bovine serum albumin (BSA)       | >1.3%                                                           |
| chloroform                       | >2.5%                                                           |
| DMSO                             | >20%                                                            |
| dNTP mix                         | >1.3%                                                           |
| ethanol                          | >20%                                                            |
| IgG                              | >2µg                                                            |
| small dsDNA fragments            | 300pg                                                           |
| Colorless GoTaq® Reaction Buffer | >20%                                                            |
| phenol                           | >2.5%                                                           |
| polyethylene glycol (PEG)        | >20%                                                            |
| RNA                              | 300pg                                                           |
| sodium acetate                   | >1mM                                                            |
| sodium dodecyl sulfate (SDS)     | >0.01%                                                          |
| ssDNA                            | 800pg                                                           |
| Triton® X-100                    | >0.01%                                                          |

### 8.D. Handling and Disposal

QuantiFluor® dsDNA Dye contains DMSO, which is an irritant and facilitates the entry of organic compounds into tissues. Wear gloves, safety glasses and a lab coat, and handle dyes with care. Because the QuantiFluor® dsDNA Dye binds to nucleic acid, it should be treated as potential mutagens. Dispose of the QuantiFluor® dsDNA Dye according to local regulations.

## 8.E. Composition of Buffers and Solutions

### 20X TE Buffer (pH 7.5)

0.2M Tris buffer (pH 7.5)

20mM EDTA (pH 8.0)

Prepare this solution in nuclease-free water. Adjust pH to 7.5.

## 8.F. Related Products

| Product                   | Size | Cat.# |
|---------------------------|------|-------|
| QuantiFluor® ssDNA System | 1ml  | E3190 |
| QuantiFluor® RNA System   | 1ml  | E3310 |

### GloMax® Instruments

| Product                                                                                               | Size   | Cat.# |
|-------------------------------------------------------------------------------------------------------|--------|-------|
| GloMax®-Multi+ Detection System with Instinct® Software:<br>Base Instrument with Shaking*             | 1 each | E8032 |
| GloMax®-Multi+ Detection System with Instinct® Software:<br>Base Instrument with Heating and Shaking* | 1 each | E9032 |
| GloMax®-Multi+ Fluorescence Module                                                                    | 1 each | E8051 |

\*Cat.# E8032 and E9032 cannot be sold separately and must be purchased with the detection module (Cat.# E8051).

| Product                           | Size   | Cat.# |
|-----------------------------------|--------|-------|
| GloMax®-Multi Fluorescence Module | 1 each | E7051 |

| Product                                                 | Size   | Cat.# |
|---------------------------------------------------------|--------|-------|
| GloMax®-Multi Jr Base Instrument*                       | 1 each | E6070 |
| GloMax®-Multi Jr with Luminescence Module               | 1 each | E6080 |
| Fluorescence Optical Kit, Blue (Ex 460nm, Em 515–570nm) | 1 each | E6071 |

\*Cat.# E6070 cannot be sold separately and must be purchased with a Fluorescence Optical Kit (Cat.# E6071).

| Product                                             | Size   | Cat.# |
|-----------------------------------------------------|--------|-------|
| GloMax® 20/20 Luminometer                           | 1 each | E5311 |
| GloMax® 20/20 Luminometer with Single Auto-Injector | 1 each | E5321 |
| GloMax® 20/20 Luminometer with Dual Auto-Injector   | 1 each | E5331 |
| GloMax® 20/20 Fluorescent Module, Blue              | 1 each | E5361 |



## 8.F. Related Products (continued)

### Handheld Fluorometers

| Product                                                                  | Size     | Cat.# |
|--------------------------------------------------------------------------|----------|-------|
| Quantus™ Fluorometer                                                     | 1 each   | E6150 |
| QuantiFluor®-ST Minicell Adapter Kit* (for measuring 50–250µl of sample) | 400 each | E6112 |
| Minicell Borosilicate Glass Cuvettes                                     | 400 each | E6091 |
| 10 × 10mm Square Polystyrene Cuvette (3.5ml capacity)                    | 100 each | E6092 |
| 10 × 10mm Square Methacrylate Cuvette (3.5ml capacity)                   | 100 each | E6093 |

\*Cat.# E6112 includes 400 borosilicate glass cuvettes.

| Product                                | Size  | Cat.# |
|----------------------------------------|-------|-------|
| Nuclease-Free Water                    | 50ml  | P1193 |
|                                        | 150ml | P1195 |
| TE Buffer, 1X, Molecular Biology Grade | 100ml | V6231 |
|                                        | 500ml | V6232 |

## 8.G. Summary of Changes

The following changes were made to the 5/15 revision of this document:

1. Corrected errors in Section 6, Representative Data, and Figure 3 (Section 8).

<sup>(a)</sup>U.S. Pat. No. 8,598,198 and other patents pending.

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