TECHNICAL MANUAL

QuantiFluor® dsDNA System

Instructions for Use of Product
E2670

Revised 12/18
TM346

QuantiFluor® dsDNA System

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The QuantiFluor® dsDNA System[a] contains a fluorescent DNA-binding dye (504nm$_{Ex}$/531nm$_{Em}$) that enables sensitive quantitation of small amounts of double-stranded DNA (dsDNA) in a purified sample. The assay is highly selective for dsDNA over other nucleic acids and is linear over a range of 0.05–200ng of dsDNA input (0.05–200ng/µl from 1µl of original sample). **Note:** Lower dsDNA concentrations may be quantitated adjusting the standard curve accordingly.

The dye-based system provides concentrated QuantiFluor® dsDNA dye, dilution buffer and DNA standard. Simply dilute the dye 1:400, add standards and unknown samples, and read on a fluorometer. The QuantiFluor® dsDNA System can be used with any single-tube fluorometer (e.g., Quantus™ Fluorometer [see Section 3]) or may be scaled up for use in instruments that can read multiwell plates (e.g., GloMax® Discover System [see Section 4]) at the appropriate excitation and emission wavelengths.

2. Product Components and Storage Conditions

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

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

- 25ml 20X TE Buffer (pH 7.5)
- 1ml QuantiFluor® dsDNA Dye
- 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 vortexed well 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 7.C.

3. Protocol for Quantitating dsDNA in a Single Tube Using the Quantus™ Fluorometer

Materials to Be Supplied by the User

- nuclease-free water
- thin-walled 0.5ml PCR tubes (Cat.# E4941 or Axygen Cat.# PCR-05-C)
- Quantus™ Fluorometer (Cat.# E6150)

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.

Instructions for use of the Quantus™ Fluorometer can be found in the *Quantus™ Fluorometer Operating Manual* #TM396 available at: **www.promega.com/protocols**

Note: Other single-tube fluorometers can be used with the QuantiFluor® dsDNA System if capable of measuring the following wavelengths ($504nm_{Ex}/531nm_{Em}$) and calibrated using manufacturer's instructions.

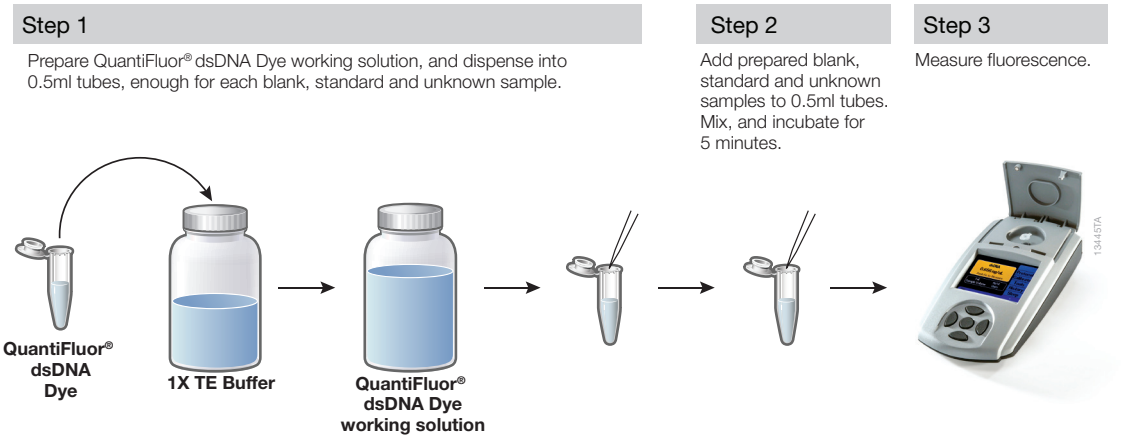

Figure 1. Overview of the single-tube format quantitation using the QuantiFluor® dsDNA System.

Note: If the Quantus™ Fluorometer was previously calibrated, you may not need to calibrate it again. Therefore, do not prepare blank and standard samples, and skip Steps 3, 4 and 8.

1. **Prepare 1X TE Buffer:** Dilute 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. **Prepare Working Solution:** Dilute the QuantiFluor® dsDNA Dye 1:400 in 1X TE buffer to make the QuantiFluor® dsDNA Dye working solution. For example, add 10µl of QuantiFluor® dsDNA Dye to 3,990µl of 1X TE buffer, and mix.

   Note: The QuantiFluor® Dye working solution is stable for at least 2 hours at 25°C.
3. **Prepare Blank Sample:** Add 200µl of QuantiFluor® dsDNA Dye working solution to an empty 0.5ml PCR tube. This will be the blank sample used in Step 8. Protect tube from light.
4. **Prepare 200ng Standard Sample:** Add 2µl of the provided DNA Standard (100ng/µl) to 200µl of QuantiFluor® dsDNA Dye working solution in an empty 0.5ml PCR tube. This will be the standard sample used in Step 8. Vortex well, and protect tube from light.
5. **Prepare Unknown Sample:** Add 1–20µl of unknown samples to 200µl of QuantiFluor® dsDNA Dye working solution in 0.5ml PCR tubes. For example, add 1µl of sample to 200µl of QuantiFluor® dsDNA Dye working solution. Protect tube from light.

   Note: See Section 7.D Tips for Pipetting Small Volumes if you are pipetting 1µl samples. Alternatively, minimize pipetting error by using >1µl of sample.
6. Incubate the prepared samples at room temperature for 5 minutes, protected from light.
7. Select the dsDNA protocol on the Quantus™ Fluorometer.
8. If needed, calibrate the Quantus™ Fluorometer by reading the blank (prepared in Step 3) and standard (prepared in Step 4) samples in the Calibration screen, then select “Save.”

3. Protocol for Quantitating dsDNA in a Single Tube Using the Quantus™ Fluorometer (continued)

9. Enter the volume of the unknown sample and desired concentration units.

   Note: This volume is the amount of unknown sample that is added for the quantitation. For example, if 2µl of sample was mixed with 200µl of QuantiFluor® dsDNA Dye working solution, then the volume entered on this screen should be 2µl.

10. Measure fluorescence of the unknown sample using the Quantus™ Fluorometer. The number displayed represents the concentration of the original sample.

4. Protocol for Quantitating dsDNA in Multiwell Plates

Materials to Be Supplied by the User

- multiwell detection instrument capable of measuring fluorescence (e.g., GloMax® Discover System [Cat.# GM3000])
- Nuclease-Free Water (Cat.# P1195)
- black, flat-bottom 96-well plates
- 1.5ml tubes

Instructions for use of the GloMax® Discover System can be found in the *GloMax® Discover System Operating Manual* #TM397, available at: **www.promega.com/protocols**

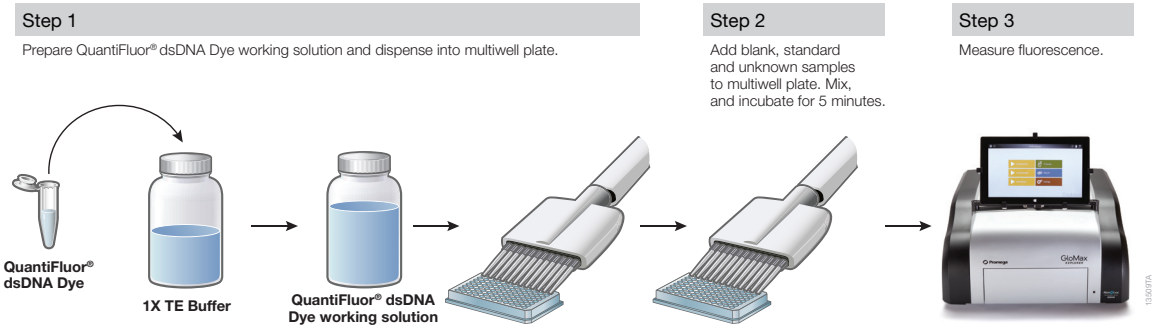

Figure 2. Overview of multiwell plate quantitation protocol using the QuantiFluor® dsDNA System.

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.

The following protocol shows an example 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.

1. **Prepare 1X TE Buffer:** Dilute 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. **Prepare Working Solution:** Dilute the QuantiFluor® dsDNA Dye 1:400 in 1X TE buffer to make the QuantiFluor® dsDNA Dye working solution. For example, add 10µl of QuantiFluor® dsDNA Dye to 3,990µl of 1X TE buffer, and mix.

   Note: The QuantiFluor® Dye working solution is stable for at least 2 hours at 25°C.
3. **Prepare a Standard Curve:** The following recommended standards result in 0.05–200ng/well and are designed for optimal pipetting accuracy, transferring 10µl of standard to each well.
   a. Label seven 1.5ml tubes for each standard as follows: 200, 50, 12.5, 3.1, 0.78, 0.2, 0.05.
   b. Prepare dsDNA standards by vortexing the Lambda DNA Standard, 100ng/µl, and serially diluting as shown in Table 1. Take care to not introduce air bubbles.

Table 1. Preparing Recommended dsDNA Standard Curve Samples.

Standard	Volume of dsDNA Standard	Volume of 1X TE Buffer (µl)	Final dsDNA Concentration (ng/µl)
A	20µl	80	20
B	25µl of Standard A	75	5.0
C	25µl of Standard B	75	1.25
D	25µl of Standard C	75	0.31
E	25µl of Standard D	75	0.078
F	25µl of Standard E	75	0.02
G	25µl of Standard F	75	0.005

4. Pipet 200µl of QuantiFluor® dsDNA Dye working solution into each well that is intended for an unknown, blank or standard sample.
5. Dispense 10µl of the dsDNA standards prepared in Table 1 above (labeled Standards A–G) to rows A–G of the multiwell plate (Figure 3). We recommend pipetting duplicates or triplicates of the standards.
6. For the blank, pipet 10µl of 1X TE Buffer into row H.

4. Protocol for Quantitating dsDNA in Multiwell Plates (continued)

7. Add 1–20µl of unknown sample to the remaining wells.

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.

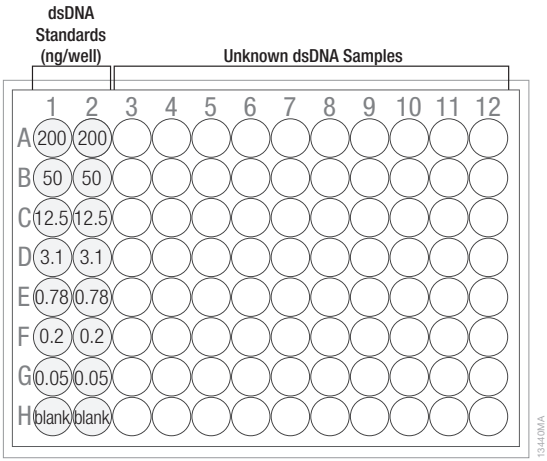

Figure 3. Dispense standard dilutions and blank samples in duplicate into Columns 1 and 2 of a multiwell plate.

8. Mix the plate thoroughly using a plate shaker or by pipetting the contents of each well.

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

10. Measure fluorescence ($504nm_{Ex}/531nm_{Em}$) using a plate reader. If using the GloMax® Discover System, select the preloaded protocol: "QuantiFluor dsDNA System."

11. Calculate the dsDNA concentration as follows: Subtract the fluorescence of the blank sample (1X TE Buffer) from all of the standard and unknown samples. 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 and multiply the resulting number by the dilution factor, if applicable (see Section 5). Alternatively, copy and paste your raw fluorescence data into our online tool: **www.promega.com/resources/tools/quantifluor-dye-systems-data-analysis-workbook/**

5. Representative Data

Representative data for the QuantiFluor® dsDNA Dye are shown in Table 2 and Figure 4.

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

Lambda DNA Mass (ng/well)	Average Fluorescence (RFU)[1]
0	0
0.05	42
0.2	163
0.78	652
3.1	2,620
12.5	10,590
50	42,479
200	161,813

[1]Background fluorescence has been subtracted. n = 3

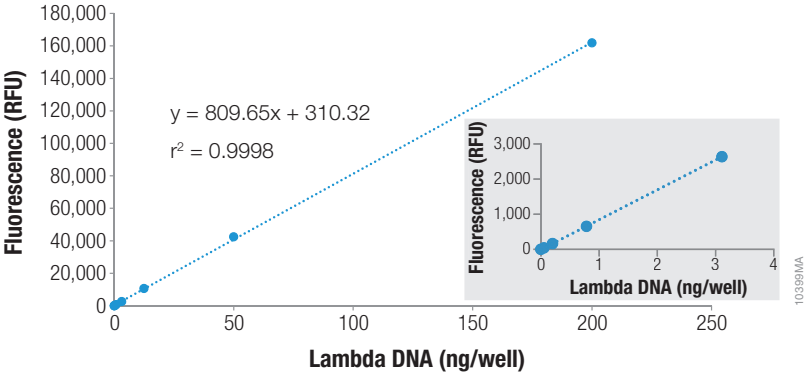

Figure 4. Representative dsDNA standard curve in a 96-well-plate format. The final amounts of the Lambda DNA Standard in the 96-well format are listed in Table 2. **Inset.** Expanded view of the low end of the standard curve.

5. Representative Data (continued)

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

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

	Unknown Sample	Standard Samples							
		0ng	0.05ng	0.2ng	0.78ng	3.1ng	12.5ng	50ng	200ng
Fluorescence	15,418	418	460	581	1,070	3,038	11,008	42,897	162,231

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

	Unknown Sample	Standard Samples							
		0ng	0.05ng	0.2ng	0.78ng	3.1ng	12.5ng	50ng	200ng
Fluorescence	15,000	0	42	163	652	2,620	10,590	42,479	161,813

2. Determine the linear regression from the scatter plot (Figure 4).
   $y = 809.65x + 310.32$
3. Calculate the DNA concentration of the unknown sample in the 200µl assay volume by solving for x in the linear regression equation, where:
   $y = 15{,}000$
   $x = (y - 310.32)/809.65 = 18.1\text{ng}$
4. Account for any dilution of the unknown sample. For example, if 1µl of sample was added per well, the sample concentration is 18.1ng/µl. If 5µl of sample was added per well, the sample concentration is 18.1ng ÷ 5µl = 3.6ng/µl.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
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$_{Ex}$/531nm$_{Em}$ (see Figure 6 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. Recreate the standard curve, and decrease the concentration of the highest standard curve point.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Low or no fluorescence detected in the standard samples	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.
	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.
	Check that the standard samples were diluted appropriately.
	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.
Fluorescence too high	Check that the unknown and standard samples were diluted appropriately. Decrease the concentration of the unknown and standard samples, if necessary.
	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® Detection Systems because these instruments will adjust automatically. The Quantus™ Fluorometer does not require gain adjustment.
dsDNA concentration determined using the QuantiFluor® dsDNA Dye differed from concentration determined using an alternative quantitation method	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 A_{260} 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.
	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. Recreate the standard curve, and decrease the concentration of the highest point of the standard curve.

Symptoms	Causes and Comments
dsDNA concentration determined using the QuantiFluor® dsDNA Dye differed from concentration determined using an alternative quantitation method (continued)	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® Detection Systems because these instruments will adjust automatically. The Quantus™ Fluorometer does not 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.
	Analyze the data using either a linear regression or a power regression for accurate concentration determinations within the 10–400ng/µl portion of the standard curve. We recommend the use of a power regression for unknowns that are expected to be <10ng/µl. Alternatively, copy and paste your raw fluorescence data into the Promega online tool, which uses a power regression: **www.promega.com/resources/tools/quantifluor-dye-systems-data-analysis-workbook** or contact Technical Services for additional assistance.
	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.

7. Appendix

7.A. 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 5.

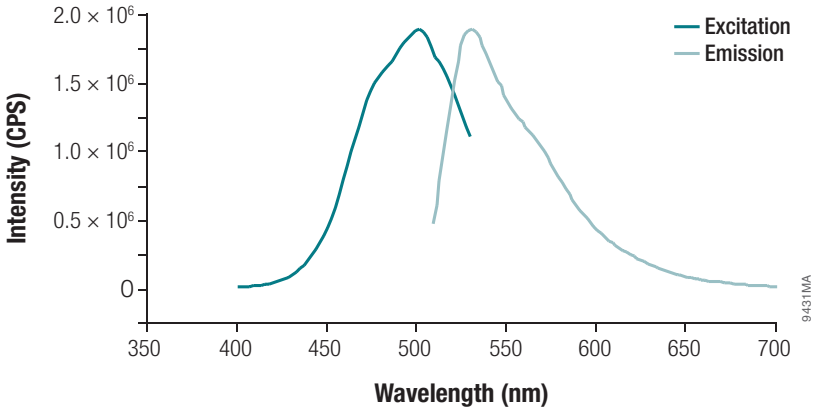

Figure 5. Excitation and emission spectra for the QuantiFluor® dsDNA Dye.

7.B. Interfering Compounds

Several compounds that are commonly used in nucleic acid preparation or 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.

Chemical	Concentration Shown to Affect the QuantiFluor® dsDNA Dye
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%

7.C. 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 a potential mutagen. Dispose of the QuantiFluor® dsDNA Dye according to local regulations.

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7.D. Tips for Pipetting Small Volumes

1. Hand-held pipettes are high precision instruments and should be handled carefully. Pipettes should also be routinely checked for accuracy every 6–12 months, and recalibrated or repaired by qualified professionals.
2. Use newer model pipettes with more modern "press fit" tips for the most accurate and reproducible results.
3. Basic pipetting guidelines:
   - When resetting a pipette to a new volume, turn the adjustment knob ½ turn higher than the target volume and then rotate back to the target volume.
   - Pipet using a slow, smooth action.
   - Hold the pipette vertically when drawing liquid in
   - Only immerse the pipette tip slightly when aspirating liquid.
   - When dispensing the liquid, hold the pipette vertically but keep the sidewall of the receiving vessel at a 45 degree angle. Pipette against the sidewall or into liquid that is already present.
4. Use the proper pipettor for the measured volume. When pipetting 1µl or 2µl, use a P2 pipettor for accuracy and reproducibility. Using aerosol or barrier tips with a P2 is not recommended.
5. All solutions, standards and unknowns should be at room temperature prior to pipetting. (See the following *Nature Methods Application Note* for more information: **www.nature.com/app_notes/nmeth/2007/071109/full/nmeth1086.html**)

For a comprehensive guide on pipetting, we recommend the following resource:
www.gilson.com/Resources/Gilson%20Guide%20To%20Pipetting%20Third%20Edition.pdf

7.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. Related Products

Product	Size	Cat.#
Quantus™ NGS Starter Package	1 each	E5150
QuantiFluor® ONE dsDNA System	100 reactions	E4871
	500 reactions	E4870
QuantiFluor® RNA System	1ml	E3310
QuantiFluor® ssDNA System	1ml	E3190

GloMax® Instruments

Product	Size	Cat.#
GloMax® Discover System	1 each	GM3000
GloMax® Explorer Fully Loaded Model	1 each	GM3500
GloMax® Explorer with Luminescence and Fluorescence	1 each	GM3510

Handheld Fluorometer

Product	Size	Cat.#
Quantus™ Fluorometer	1 each	E6150
0.5ml PCR Tubes	50 pack	E4941

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

9. Summary of Changes

The following changes were made to the 12/18 revision of this document:

1. Updated cross-references and section numbering.

[a]U.S. Pat. Nos. 8,598,198 and 9,206,474 and other patents and patents pending

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