TECHNICAL MANUAL

QuantiFluor® ONE dsDNA System

Instructions for Use of Products
E4871 and E4870

Revised 12/13
TM405

QuantiFluor® ONE 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® ONE dsDNA System[a] contains a fluorescent double-stranded DNA-binding dye ($504nm_{Ex}/531nm_{Em}$), prepared in an "add-and-read" format for dye and standard use, making sample quantitation simple. This system enables sensitive quantitation of small amounts of double-stranded DNA (dsDNA).

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

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
QuantiFluor® ONE dsDNA System	**100 reactions**	**E4871**

This system contains sufficient reagents for 100 assays at a reaction volume of 200µl. Includes:

- 1 × 20ml QuantiFluor® ONE dsDNA Dye
- 80µg QuantiFluor® ONE Lambda DNA (400µg/ml)
- 10ml 1X TE Buffer (pH 7.5)

PRODUCT	SIZE	CAT.#
QuantiFluor® ONE dsDNA System	**500 reactions**	**E4870**

This system contains sufficient reagents for 500 assays at a reaction volume of 200µl. Includes:

- 5 × 20ml QuantiFluor® ONE dsDNA Dye
- 400µg QuantiFluor® ONE Lambda DNA (400µg/ml)
- 25ml 1X TE Buffer (pH 7.5)

Storage Conditions: Store QuantiFluor® ONE dsDNA Dye and QuantiFluor® ONE Lambda DNA at –30°C to +10°C. Store the 1X TE Buffer (pH 7.5) at –30°C to +30°C.

See Section 7, Handling and Disposal, for instructions on the handling and disposal of QuantiFluor® ONE dsDNA Dye.

3. Materials to Be Supplied by the User

- black flat-bottom plates (for multiwell plate formats) or thin-walled tubes (e.g., Promega Cat.# E4942 or Axygen Cat.# PCR-05-C)
- fluorometer
- optional: K562 Genomic DNA (Promega Cat.# E4931)

4. Preparing a Standard Curve

Quantitation of unknown samples requires comparison to a dsDNA standard. Prepare a standard using the QuantiFluor® ONE Lambda DNA, or a DNA of similar molecular weight to your sample of interest. The QuantiFluor® ONE Lambda DNA provided with the QuantiFluor® ONE dsDNA System can be used as a DNA standard; however, 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; Promega Cat.# E4931) is also available at the "ready-to-use" starting concentration for the QuantiFluor® ONE dsDNA add-and-read format.

The following protocols are examples of how to prepare a standard curve for the Quantus™ Fluorometer and microplate fluorometer. We recommend preparing a standard curve that extends above and below the likely concentration range for your unknown samples and is within the linear range of the assay. In addition, a blank sample containing 1X TE Buffer and QuantiFluor® ONE dsDNA Dye should be used to assess the background level of the assay.

Notes:

- If the Quantus™ Fluorometer (Cat.# E6150) is used for detection, follow the instructions in the *Quantus™ Fluorometer Operating Manual* #TM396 to operate the instrument and prepare the QuantiFluor® ONE standard and unknown samples.
- If using the Quantus™ Fluorometer, only two points are needed (blank and highest dsDNA concentration). A true standard curve is not required when using the Quantus™ Fluorometer because this instrument generates a standard curve based on those two points and calculates the dsDNA concentration of the unknown samples.
- The minimum amount of dsDNA detected using the QuantiFluor® ONE dsDNA Dye reagent will depend on factors such as plasticware and the instrument used to measure fluorescence.

Instructions for proper calibration of the Quantus™ Fluorometer can be found in the *Quantus™ Fluorometer Operating Manual* #TM396. Instructions for use of the GloMax®-Multi+ Detection System can be found in the *GloMax®-Multi+ Detection System with Instinct® Software Technical Manual* #TM340. Instructions for use of the GloMax® Discover System are in the *GloMax® Discover System Technical Manual* #TM397. All manuals are available at: **www.promega.com/protocols/**

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4.A. Protocol for use with the Quantus™ Fluorometer

The Quantus™ Fluorometer protocol is designed to use 1µl of sample + 199µl of QuantiFluor® ONE dsDNA Dye for a total assay volume of 200µl. If more sample is desired (i.e., 2–10µl), adjust the volume of dye (e.g., to 198µl for a 2µl sample) for a final volume of 200µl. If using a sample volume >1µl, you also must update the QuantiFluor® ONE protocol ("ONE DNA") on the Quantus™ Instrument. Failure to update the QuantiFluor® ONE protocol will result in an incorrect concentration value. See the *Quantus™ Fluorometer Operating Manual* #TM396 for instructions.

We recommend the use of a P2 pipettor for accurate pipetting of 1µl volumes in the following steps.

1. Prepare a 400ng standard used to calibrate the instrument by adding 1µl of QuantiFluor® ONE Lambda DNA or 1µl of a 400µg/ml DNA standard of similar molecular weight as the samples, into 199µl of QuantiFluor® ONE dsDNA Dye in an 0.5ml PCR tube. Mix thoroughly by vortexing or pipetting.

   Note: Alternatively, add 2µl of a 400µg/ml DNA standard to 398µl of QuantiFluor® ONE Dye Solution and mix thoroughly. Then transfer 200µl of the mixture into a new 0.5ml PCR tube.

2. Prepare a blank by adding 1µl of 1X TE Buffer to 199µl of QuantiFluor® ONE Dye solution in an 0.5ml PCR tube. Mix tube thoroughly by vortexing or pipetting.

Note: It is important to mix all tubes thoroughly by vortexing. Thorough mixing by pipet can be accomplished by setting the pipettor to 180µl and mixing completely three times. Take care to not introduce bubbles during mixing, as air bubbles will adversely affect fluorescence values.

3. Add 1µl of unknown sample to 199µl of QuantiFluor® ONE dsDNA Dye solution in an 0.5ml PCR tube. Mix thoroughly by vortexing or pipetting.
4. Incubate reactions for 5 minutes at room temperature, protected from light.
5. Select the QuantiFluor® ONE dsDNA protocol on the Quantus™ Fluorometer ("ONE DNA").
   Note: If "ONE DNA" is not listed in the instrument protocol menu you will need to update the Quantus™ Firmware. Please follow the instructions located at:
   www.promega.com/quantusfluorometerfirmware/
6. Calibrate the Quantus™ Fluorometer using blank and standard tubes.
7. Read unknowns on the Quantus™ Fluorometer and record the final sample concentration results.

4.B. Protocol for Multiwell Plates (200µl Assay Volume)

This multiwell plate protocol is designed to use 1µl of sample + 199µl of QuantiFluor® ONE dsDNA Dye for a total assay volume of 200µl. If more sample is desired (up to 10µl), adjust the volume of dye for a final volume of 200µl. Adjust standard and blank volumes accordingly so that the final concentration of QuantiFluor ® ONE dsDNA Dye is the same in all reactions.

1. Prepare seven 1.5ml tubes labeled: 400, 200, 50, 12.5, 3.1, 0.8 and 0.2.

4.B. Protocol for Multiwell Plates (200µl Assay Volume; continued)

2. Prepare standards taking care to not introduce air bubbles by preparing 1:1 and 1:4 serial dilutions of QuantiFluor® ONE Lambda DNA (400ng/µl) according to Table 1.

Table 1. Preparing a dsDNA Dilution Series for a Standard Curve from the 400ng/µl dsDNA Standard.

Standard	Volume of dsDNA Standard	Volume of 1X TE Buffer	Final dsDNA Concentration (ng/µl)
A	15µl of Undiluted Standard	0µl	400
B	10µl of Standard A	10µl	200
C	5µl of Standard B	15µl	50
D	5µl of Standard C	15µl	12.5
E	5µl of Standard D	15µl	3.1
F	5µl of Standard E	15µl	0.8
G	5µl of Standard F	15µl	0.2

Note: Use a P2 pipettor to more accurately pipet 1µl volumes in the following steps.

3. Pipet 199µl of the QuantiFluor® ONE dsDNA Dye to each well containing standard, blank and any wells of the plate that will be used for unknowns.
4. Pipet 1µl of the dsDNA standards prepared in Table 1 above (A–G) to rows A–G of the microplate in triplicate.
5. For the blank, pipet 1µl of 1X TE Buffer into row H in triplicate.
6. Add 1µl of unknown sample to the desired number of wells.
7. Set a 200µl multichannel pipettor to 180µl, and mix each column on the plate three times by pipetting and then ejecting the volume **slowly**. **Do not** allow air to enter the pipet tips, as this can cause wetting of the pipet tip filters. Use caution to avoid introducing air bubbles, which will interfere with fluorescence.
8. Incubate assays for 5 minutes at room temperature, protected from light.
9. Measure fluorescence ($504nm_{Ex}/531nm_{Em}$). For the multiwell plate format, use the Blue Fluorescence Optical Kit ($490nm_{Ex}/510–570nm_{Em}$) with the GloMax®-Multi+ System. For the GloMax® Discover System use wavelength settings of $475nm_{Ex}/500–550nm_{Em}$.
10. 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.

    Alternatively, copy and paste your raw fluorescence data into the Promega online tool: **www.promega.com/resources/tools/quantifluordye-systems-data-analysis-workbook**

5. 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® ONE dsDNA Dye. Read fluorescence at 504nm(Ex)/531nm(Em).
	The QuantiFluor® ONE dsDNA Dye is light-sensitive. Exposure to light will reduce the sensitivity of the assay. Store the QuantiFluor® ONE dsDNA Dye in the provided amber bottle.
	Confirm that the unknown sample calculations were performed correctly.
	Check that the unknown sample was within the sensitivity range of the assay and standard curve.
No or low fluorescence detected in the unknown sample	Increase the volume of unknown sample. For example, try 2µl of sample with 198µl of QuantiFluor® ONE dsDNA Dye. If more than 10µl of sample is needed, it may be necessary to concentrate the sample. Less than 190µl of QuantiFluor® ONE dsDNA Dye in the 200µl assay volume may lead to insufficient fluorescence detected by your fluorometer. Divide the resulting concentration by the volume of unknown sample added to the assay to obtain the concentration in the original unknown sample.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
No or low fluorescence detected in the standard sample	Evaluate the performance of the fluorometer with a dsDNA sample of known concentration (e.g., QuantiFluor® ONE Lambda DNA) using the appropriate excitation and emission wavelengths for the QuantiFluor® ONE dsDNA Dye.
	Check that the standard samples were diluted appropriately, if preparing a standard curve.
	Mix the dsDNA standards with the QuantiFluor® ONE dsDNA Dye just prior to use. Extended exposure to light will decrease the amount of fluorescence detected.
Fluorescence is too high	Check that the standard samples were diluted appropriately, if preparing a standard curve.
	If necessary, dilute the unknown sample prior to quantitation. Multiply the calculated result by the dilution factor to determine the concentration of the undiluted unknown sample.
	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+ Detection System or GloMax® Discover System because these instruments will adjust automatically. The Quantus™ Fluorometer does not require gain adjustment.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
dsDNA concentration determined using the QuantiFluor® ONE dsDNA Dye differed from concentration determined using an alternative quantitative method	DNA concentrations determined using the using the QuantiFluor® ONE 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 all nucleic acid (dsDNA, ssDNA, RNA and nucleotides) in the sample. The QuantiFluor® ONE 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.
	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	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® Discover System because these instruments will adjust automatically. The Quantus™ Fluorometer does not require gain adjustment.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
Nonlinear standard curve (continued)	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.
	The QuantiFluor® ONE dsDNA Dye was exposed to light. Exposure to light will reduce the sensitivity of the assay. Store the QuantiFluor® ONE dsDNA Dye and working solution protected from light.
	Analyze the data using either a linear regression or a power regression for accurate concentration determinations within the 10ng/ml to 400ng/ml portion of the standard curve. We recommend the use of a power regression for unknowns that are expected to be < 10ng/ml. 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.

6. 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® ONE dsDNA Dye. Table 2 lists compounds that have known effects on DNA quantitation using the QuantiFluor® ONE dsDNA Dye and the concentrations at which they affect quantitation results.

Table 2. Compounds that Interfere with the QuantiFluor® ONE dsDNA Dye.

Chemical	Concentration of Compound Shown to Affect QuantiFluor® ONE dsDNA Dye*	Lambda DNA (ng/well)	Change in QuantiFluor® ONE dsDNA Dye Signal
Guanidine Thiocyanate	4M	200	20% decrease
Bovine Serum Albumin (BSA)	0.08%	0.2	39% increase
Sodium Chloride	2.5M	0.2	3% increase
Polyethylene Glycol (PEG 8000)	10%	0.0	4% increase
Ethanol	0.6%	200	4% decrease
ssDNA	6.3ng	0.2	60% increase
RNA	6.3ng	0.2	7% increase

*Compounds were tested by adding 1µl at indicated concentrations in a 200µl final assay volume.

7. Handling and Disposal

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

8. Composition of Buffers and Solutions

1X TE Buffer (pH 7.5)

10mM Tris buffer (pH 7.5)
1mM EDTA

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

9. Related Products

Product	Size	Cat.#
QuantiFluor® dsDNA System	1ml	E2670
QuantiFluor® dsDNA Sample Kit	1 each	E2671
QuantiFluor® RNA System	1ml	E3310
QuantiFluor® ssDNA System	1ml	E3190

Handheld Fluorometers

Product	Size	Cat.#
Quantus™ Fluorometer	1 each	E6150

GloMax® Instruments

Product	Size	Cat.#
GloMax® Discover System	1 each	GM3000
GloMax®-Multi+ Detection System with Instinct® Software	1 each	E8032 / E9032
GloMax®-Multi Jr Base Instrument	1 each	E6070

Miscellaneous

Product	Size	Cat.#
Nuclease-Free Water	50ml	P1193
	150ml	P1195
K562 Genomic DNA	80µg	E4931
0.5ml PCR Tubes	Pack of 50 tubes	E4941
	4 × Pack of 50 tubes	E4942

[a]Patent Pending.