TECHNICAL MANUAL

QuantiFluor® ONE dsDNA System

Instructions for Use of Products
E4871, E4870 and E4891

Revised 10/22
TM405

Promega

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 DNA-binding dye (504nm$_{Ex}$/531nm$_{Em}$) that can sensitively quantitate 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 0.2–400ng of dsDNA input (0.2–400ng/µl from 1µl of original sample).

The dye-based system provides the QuantiFluor® ONE dsDNA Dye and DNA standard for quick quantitation without the need for preparing a working solution by diluting the dye. Simply add standards and unknown samples to the dye, and read on a fluorometer. The QuantiFluor® ONE 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® 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. 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)
- **optional**: K562 Genomic DNA (Cat.# E4931)

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; Cat.# E4931) is also available at a ready-to-use starting concentration for the QuantiFluor® ONE dsDNA add-and-read format.

Instructions for using the Quantus™ Fluorometer are found in the *Quantus™ Fluorometer Operating Manual* #TM396 available at: **www.promega.com/protocols**

Notes:

1. We recommend the use of a P2 pipettor for accurate pipetting of 1µl volumes in the following steps. See Section 9 Tips for Pipetting Small Volumes if you are pipetting 1µl samples. Alternatively, minimize pipetting error by using >1µl of sample.
2. Other single-tube fluorometers can be used with the QuantiFluor® ONE dsDNA System if capable of measuring the following wavelengths ($504nm_{Ex}/531nm_{Em}$) and calibrated using manufacturer's instructions.

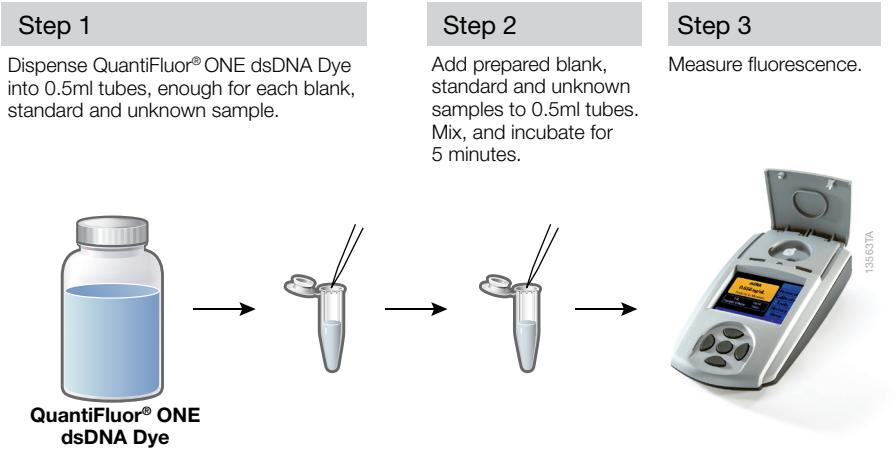

Figure 1. Overview of the single-tube format quantitation using the QuantiFluor® ONE dsDNA System.

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

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 2, 3 and 7.

1. Warm all assay components to room temperature before use.
2. **Prepare Blank Sample:** Add 200µl of QuantiFluor® ONE dsDNA Dye to an empty 0.5ml PCR tube. This will be the blank sample used in Step 8. Protect tube from light.
3. **Prepare 400ng Standard Sample:** Add 1µl of the provided QuantiFluor® ONE Lambda DNA standard (400µg/ml) to 200µl of QuantiFluor® ONE dsDNA Dye in an empty 0.5ml PCR tube. This will be the standard sample used in Step 8. Vortex well, and protect tube from light.

   Note: Alternatively, minimize pipetting error by adding 2µl of the 400ng/µl DNA standard to 400µl of QuantiFluor® ONE dsDNA Dye.
4. **Prepare Unknown Sample:** Add 1–20µl of unknown samples to 200µl of QuantiFluor® ONE dsDNA Dye in 0.5ml PCR tubes. For example, add 2µl of sample to 200µl of QuantiFluor® ONE dsDNA Dye. Vortex well, and protect tube from light.

Note: Mix all tubes thoroughly by vortexing. To thoroughly mix using a pipette, set the pipettor to 180µl and pipet completely three times. Take care to not introduce bubbles during mixing, as air bubbles will adversely affect fluorescence values.

5. Incubate reactions for 5 minutes at room temperature, protected from light.
6. Select the ONE DNA protocol on the Quantus™ Fluorometer.
7. If needed, calibrate the Quantus™ Fluorometer by reading the blank (prepared in Step 1) and standard (prepared in Step 2) samples in the Calibration screen, then select **Save**.
8. Enter the volume of the unknown sample and desired concentration units.

   Note: This volume is the amount of sample that is added for the quantitation. For example, if 2µl of sample was mixed with 200µl of QuantiFluor® ONE dsDNA Dye, then the volume entered on this screen should be 2µl.
9. Measure fluorescence of the unknown sample using the Quantus™ Fluorometer. The number displayed represents 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
- **optional**: K562 Genomic DNA (Cat.# E4931)

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**

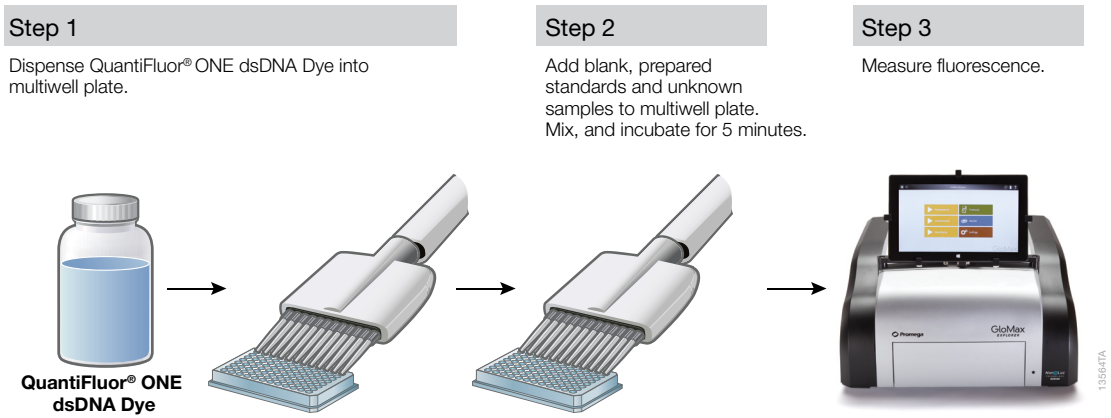

Figure 2. Overview of multiwell plate quantitation protocol using the QuantiFluor® dsDNA System.

This multiwell plate protocol is designed to use 1–20µl of sample + 200µl of QuantiFluor® ONE dsDNA Dye.

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

1. **Prepare a Standard Curve:**

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

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

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.

2. Pipet 200µl of the QuantiFluor® ONE dsDNA Dye to each well containing standard, blank and any wells of the plate that will be used for unknowns.
3. Pipet 1µl 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.
4. For the blank, pipet 1µl of 1X TE Buffer into row H in duplicate or triplicate.
5. Add 1µl of unknown sample to the desired number of wells.

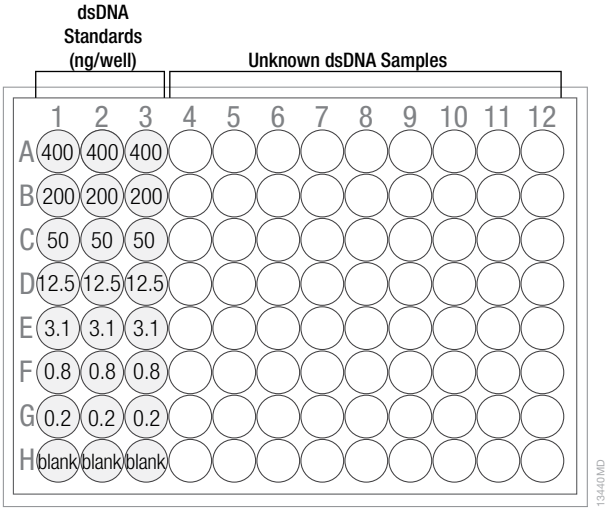

Figure 3. Dispense standard dilutions and blank samples in triplicate into Columns 1, 2 and 3 of a multiwell plate.

6. Mix the plate thoroughly using a plate shaker or by pipetting the contents of each well. For example, set a 200µl multichannel pipettor to 180µl, and mix each column on the plate three times by pipetting and then ejecting the volume **slowly**. Use caution to avoid introducing air bubbles, which will interfere with fluorescence.
7. Incubate assays for 5 minutes at room temperature, protected from light.
8. Measure fluorescence ($504nm_{Ex}/531nm_{Em}$). If using the GloMax® Discover System, select the preloaded protocol: **QuantiFluor ONE dsDNA System**.
9. 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/quantifluor-dye-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 2–20µl of sample with 200µl of QuantiFluor® ONE dsDNA Dye.
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® Detection Systems because these instruments will adjust automatically. The Quantus™ Fluorometer does not require gain adjustment.

Symptoms	Causes and Comments
dsDNA concentration determined using the QuantiFluor® ONE dsDNA Dye differed from concentration determined using an alternative method	DNA concentrations determined using the QuantiFluor® ONE dsDNA Dye and optical density readings at 260nm will be different due to inherent quantitative 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® Detection Systems because these instruments will adjust automatically. The Quantus™ Fluorometer does not 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.
	Pipetting was not accurate. If your standard curve was prepared using 1µl of each standard, refer to Section 9 Tips for Pipetting Small Volumes. Alternatively, prepare standard samples such that >1µl can be dispensed.
	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 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.

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. 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

10. 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
K562 Genomic DNA	80µg	E4931

11. Summary of Changes

The following changes were made to the 10/22 revision of this document:

1. Added Cat.# E4891 to cover page.
2. Made minor text edits.
3. Updated cover page and document font.

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

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