TECHNICAL MANUAL

QuantiFluor® ssDNA System

Instructions for Use of Product
E3190



Revised 6/17
TM376

QuantiFluor® ssDNA 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

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1. Description

The QuantiFluor® ssDNA System[a] contains a fluorescent DNA-binding dye (492nm$_{Ex}$/528nm$_{Em}$) that enables sensitive quantitation of small amounts of single-stranded DNA (ssDNA) in purified samples. The assay is linear over a range of 0.2–400ng ssDNA input (0.2–400ng/µl from 1µl of original sample).

The dye-based system provides concentrated QuantiFluor® ssDNA dye, dilution buffer and DNA standard. Simply dilute the dye, add standards and unknown samples, and read on a fluorometer. The QuantiFluor® ssDNA 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® ssDNA System	**1ml**	**E3190**

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

- 25ml 20X TE Buffer (pH 7.5)
- 1ml QuantiFluor® ssDNA Dye
- 100µg ssDNA Standard, 100µg/ml (20mer oligonucleotide)

Storage Conditions: Store the QuantiFluor® ssDNA Dye at –30°C to –10°C, protected from light. Store the ssDNA Standard, 100µg/ml, at –30°C to –10°C. Store the 20X TE Buffer (pH 7.5) at –30°C to +30°C.

Instructions for handling and disposal of the QuantiFluor® ssDNA Dye are found in Section 8.E.

3. Protocol for Quantitating ssDNA 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:** shrimp DNase (USB Cat.# 78314) if samples contain a mixture of ssDNA and dsDNA; see Section 8.D

Warm all assay components to room temperature before use. The QuantiFluor® ssDNA Dye is dissolved in 100% DMSO and frozen at or below 4°C. Prior to dilution, thaw dye at room temperature, protected from light.

The QuantiFluor® ssDNA System enables accurate detection of ssDNA over a broad dynamic range. Quantitating unknown samples requires calibration of the instrument with a blank and a single standard sample. Use a ssDNA standard appropriate for the expected range of nucleic acid concentrations for your unknown samples. For samples in the range of 10–400ng/µl, prepare working solution and standard sample by following instructions for the "High Standard Calibration." For lower level samples in the range 0.2–10ng/µl, prepare working solution and standard sample according to the "Low Standard Calibration" instructions. Instructions for use of the Quantus™ Fluorometer can be found in the *Quantus™ Fluorometer Operating Manual* #TM396 available at:
www.promega.com/protocols

Notes:

1. When using the low-concentration calibration standard, the minimum amount of detectable ssDNA will depend on factors such as the plasticware and reader used to measure fluorescence.
2. Other single-tube fluorometers can be used with the QuantiFluor® ssDNA System if capable of measuring the following wavelengths ($492nm_{Ex}/528nm_{Em}$) and calibrated using manufacturer's instructions.

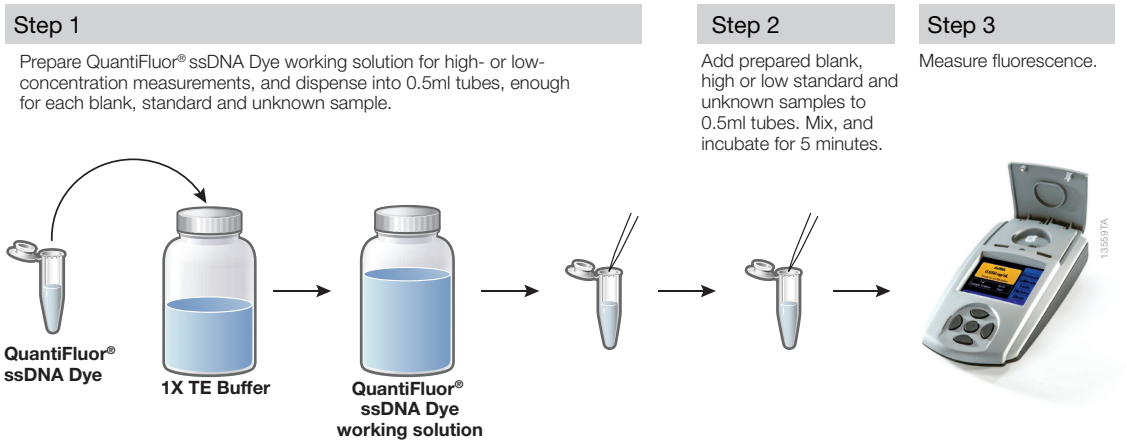


Figure 1. Overview of the single-tube format quantitation using the QuantiFluor® ssDNA System.

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

   High Standard Calibration: Dilute the QuantiFluor® ssDNA Dye 1:400 in 1X TE buffer to make the QuantiFluor® ssDNA Dye working solution. For example, add 10µl of QuantiFluor® ssDNA Dye to 3,990µl of 1X TE buffer, and mix thoroughly.

   Low Standard Calibration: Dilute the QuantiFluor® ssDNA Dye 1:2,000 in 1X TE buffer to make the QuantiFluor® ssDNA Dye working solution. For example, add 2µl of QuantiFluor® ssDNA Dye to 3,998µl of 1X TE buffer, and mix thoroughly.

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

3. **Prepare Blank Sample:** Add 200µl of QuantiFluor® ssDNA 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 ssDNA Standard Sample:**

   High Standard Calibration: Prepare a 400ng standard by adding 4µl of the provided ssDNA Standard (100ng/µl) to 200µl of QuantiFluor® ssDNA Dye working solution in an empty 0.5ml PCR tube. Mix, and protect tube from light.

   Low Standard Calibration: Prepare a 10ng standard by first diluting the ssDNA Standard (100ng/µl) 1:100 in 1X TE buffer (10µl of ssDNA Standard + 990µl of 1X TE buffer). Next, add 10µl of diluted standard to 200µl of QuantiFluor® ssDNA Dye working solution.

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

5. **Prepare Unknown Sample:** Add 1–20µl of unknown samples to 200µl of QuantiFluor® ssDNA Dye working solution in 0.5ml PCR tubes. For example, add 1µl sample to 200µl QuantiFluor® ssDNA Dye working solution. Vortex well, and 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 ssDNA protocol on the Quantus™ Fluorometer. Depending on which standard calibration you prepared, select "High" or "Low".

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".

9. 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® ssDNA 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 ssDNA in Multiwell Plates

Materials to Be Supplied by the User

- multiwell plate detection instrument capable of measuring fluorescence (e.g., GloMax® Discover System [Cat.# GM3000])
- Nuclease-Free Water (Cat.# P1195)
- black flat-bottom 96-well plates
- **optional:** shrimp DNase (USB Cat.# 78314) if samples contain a mixture of ssDNA and dsDNA; see Section 8.D.

Warm all assay components to room temperature before use. The QuantiFluor® ssDNA 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 GloMax® Discover System can be found in the *GloMax® Discover System Operating Manual* #TM397, available at: **www.promega.com/protocols**

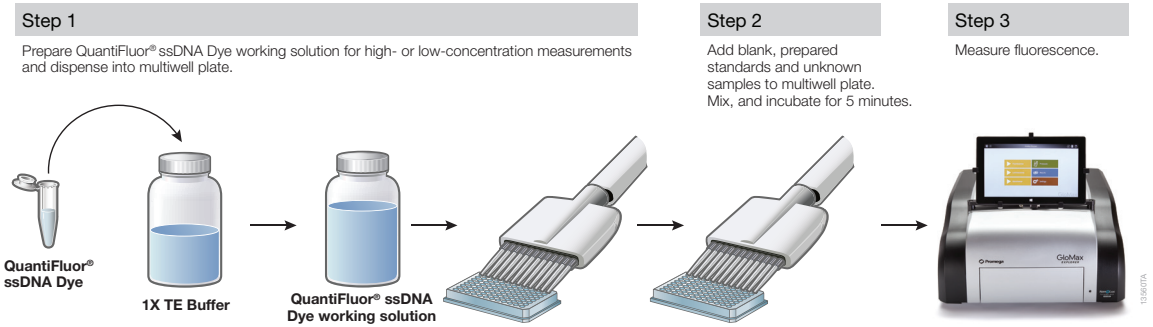


Figure 2. Overview of the multiwell plate quantitation protocol using the QuantiFluor® ssDNA System.

The QuantiFluor® ssDNA System enables accurate detection of ssDNA over a broad dynamic range. Quantitating unknown samples requires comparison to a ssDNA standard curve. Prepare a standard curve using the ssDNA Standard. Generate a standard curve appropriate for the expected range of nucleic acid concentrations for your unknown samples and your sample analysis setup. For samples that are 10–400ng/µl, prepare working solution and standard by following instructions for the "High-Concentration Standard Curve." For lower level samples in the range 0.2–10ng/µl, prepare working solution and standard according to the "Low-Concentration Standard Curve" instructions.

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: When using the low-concentration standard curve, the minimum amount of detectable ssDNA will depend on 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:** Prepare enough QuantiFluor® ssDNA Dye working solution to quantitate blank, standard and unknown samples. Protect the working solution from light by covering with foil or placing in the dark.

   High-Concentration Standard Curve (for samples 10–400ng/µl)**:** Dilute the QuantiFluor® ssDNA Dye 1:400 in 1X TE buffer to make the QuantiFluor® ssDNA Dye working solution. For example, add 10µl of QuantiFluor® ssDNA Dye to 3,990µl of 1X TE buffer, and mix thoroughly.

   Low-Concentration Standard Curve (for samples 0.2–10ng/µl)**:** Dilute the QuantiFluor® ssDNA Dye 1:2,000 in 1X TE buffer to make the QuantiFluor® ssDNA Dye working solution. For example, add 2µl of QuantiFluor® ssDNA Dye to 3,998µl of 1X TE buffer, and mix thoroughly.

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

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

3. **Prepare ssDNA Standard Curve:**

High-Concentration Standard Curve: The following recommended standards result in 6.25-400ng/well and are designed for optimal pipetting accuracy, transferring 10µl of standard to each well.

a. Prepare seven 1.5ml tubes labeled: 400, 200, 100, 50, 25, 12.5, 6.25.

b. Prepare ssDNA standards by serially diluting QuantiFluor® ssDNA Standard (100ng/µl) as shown in Table 1. Do not introduce air bubbles.

Table 1. Preparing Samples for a High-Concentration ssDNA Standard Curve.

Standard	Volume of ssDNA Standard	Volume of 1X TE Buffer	ssDNA Concentration (ng/µl)
A	40µl	60µl	40
B	50µl of Standard A	50µl	20
C	50µl of Standard B	50µl	10
D	50µl of Standard C	50µl	5.0
E	50µl of Standard D	50µl	2.5
F	50µl of Standard E	50µl	1.3
G	50µl of Standard F	50µl	0.63

Low-Concentration Standard Curve: The following recommended standards result in 0.16–10ng/well and are designed for optimal pipetting accuracy, transferring 10µl of standard to each well.

a. Prepare seven 1.5ml tubes labeled: 10, 5, 2.5, 1.3, 0.63, 0.31, 0.16.

b. Prepare ssDNA standards by serially diluting QuantiFluor® ssDNA Standard (100ng/µl) as shown in Table 2. Take care to not introduce air bubbles.

Table 2. Preparing Samples for a Low-Concentration ssDNA Standard Curve.

Standard	Volume of ssDNA Standard	Volume of 1X TE Buffer	ssDNA Concentration (ng/µl)
A	10µl	990µl	1
B	50µl of Standard A	50µl	0.5
C	50µl of Standard B	50µl	0.25
D	50µl of Standard C	50µl	0.13
E	50µl of Standard D	50µl	0.063
F	50µl of Standard E	50µl	0.031
G	50µl of Standard F	50µl	0.016

4. Pipet 200µl of QuantiFluor® ssDNA Dye working solution into each well that is intended for an unknown, blank or standard sample.

5. Dispense 10µl of the high-concentration ssDNA standards prepared in Table 1 (labeled Standards A–G) to rows A–G of the 96-well plate (Figure 3, Panel A). Alternatively, dispense 10µl of the low-concentration ssDNA standards prepared in Table 2 (labeled Standards A–G) to rows A–G of the 96-well plate (Figure 3, Panel B). We recommend pipetting duplicates or triplicates of the standards.

6. For the blank, pipet 10µl of 1X TE Buffer into row H.

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.

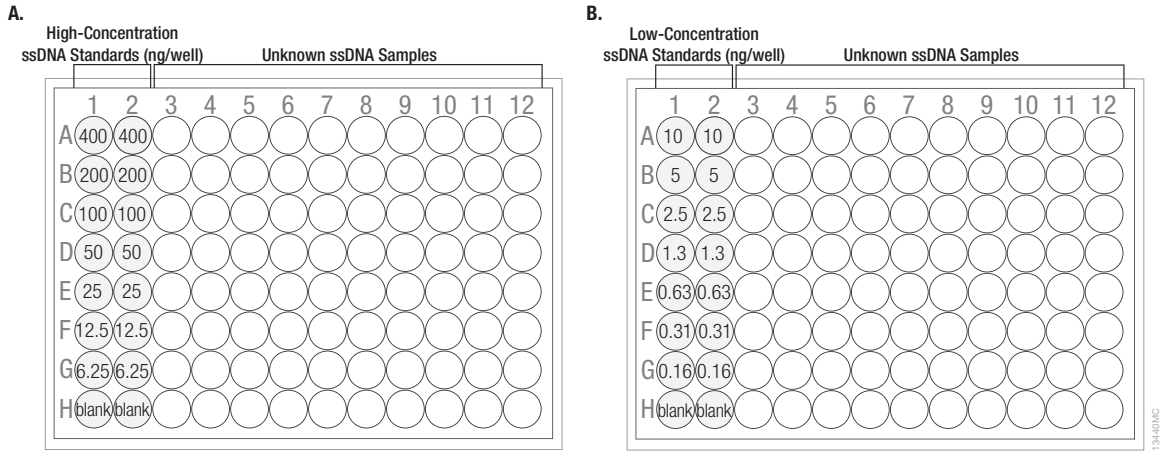


Figure 3. Dispense standard dilutions and blank samples in duplicate into Columns 1 and 2 of a multiwell plate. Panel A. High-concentration ssDNA standard and blank samples. **Panel B.** Low-concentration ssDNA standard and blank samples.

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

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

10. Measure fluorescence ($492nm_{Ex}/528nm_{Em}$) using your plate reader. If using the GloMax® Discover System, select the preloaded protocol: "QuantiFluor ssDNA System.".

11. Calculate the ssDNA 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. 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® ssDNA System are shown in Table 3 and Figure 4.

Table 3. Representative Data for the High-Concentration ssDNA Standard Curve and QuantiFluor® ssDNA Dye in the 96-Well, 200µl Assay Format

ssDNA DNA Standard Mass (ng/well)	Average Fluorescence (RFU)[1]
0	0
6.25	654
12.5	2,407
25	5,858
50	13,507
100	32,922
200	78,704
400	142,855

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

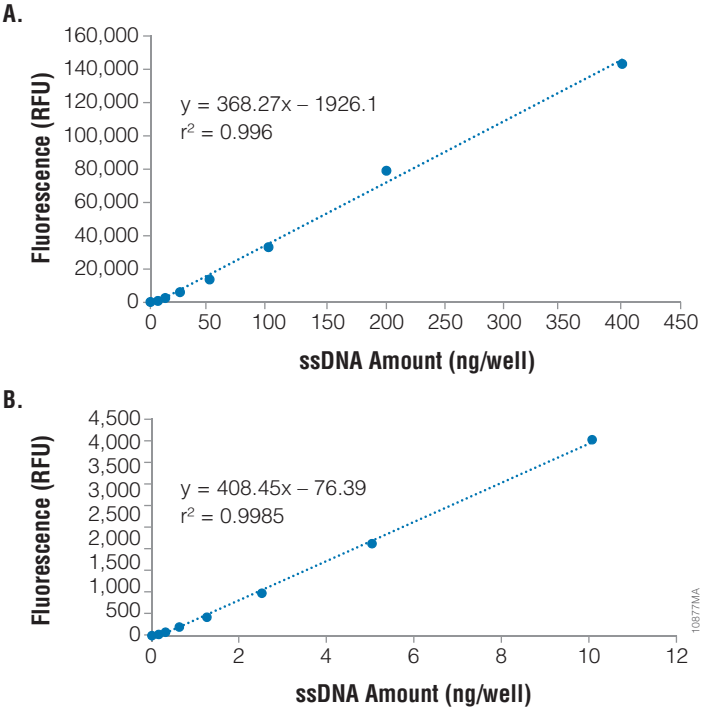


Figure 4. Representative ssDNA standard curves in a 96-well-plate format. Panel A. The high-concentration ssDNA standard curve. The final concentrations of ssDNA standard in the 96-well, 200µl assay format are listed in Table 3. **Panel B.** The low-concentration ssDNA standard curve.

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	6.25ng	12.5ng	25ng	50ng	100ng	200ng	400ng
Fluorescence	12,050	690	1,644	3,097	6,548	14,197	33,612	79,394	143,545

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

	Unknown Sample	Standard Samples							
		0ng	6.25ng	12.5ng	25ng	50ng	100ng	200ng	400ng
Fluorescence	11,360	0	954	2,407	5,858	13,507	32,922	78,704	142,855

2. Determine the linear regression from the scatter plot (Figure 5).
   $y = 368.27x - 1926.1$
3. Calculate the DNA concentration of the unknown sample in the 200µl assay volume by solving for x in the linear regression equation, where
   $y = 11,360$
   $x = (y + 1926.1)/368.27 = 36.1ng$
4. Account for any dilution of the unknown sample. For example, if 1µl of sample was added per well, the sample concentration is 36.1ng/µl. If 5µl of sample was added per well, the sample concentration is 36.1ng ÷ 5µl = 7.2ng/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	Possible Causes and Comments
Low or no fluorescence detected	Check that the correct filter set was used for the QuantiFluor® ssDNA Dye. For the QuantiFluor® ssDNA Dye, read the fluorescence using $492nm_{Ex}/528nm_{Em}$ (see Figure 6 for excitation and emission spectra).
	The QuantiFluor® ssDNA Dye is light-sensitive. Exposure to light will reduce the sensitivity of the assay. Store the QuantiFluor® ssDNA Dye and working solution protected from light.
	Confirm that dye was added. Add an equal volume of QuantiFluor® ssDNA Dye working solution to each sample.
Fluorescence too high	Check that the unknown and standard samples were diluted appropriately. Increase the dilution factor 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.
ssDNA concentration too low	Check that unknown and standard samples were diluted appropriately. Decrease the dilution factor of the unknown and standard samples, if necessary.
	Confirm that the unknown sample calculations were performed correctly and, if applicable, any dilutions were recorded appropriately.

6. Troubleshooting (continued)

Symptoms	Possible Causes and Comments
ssDNA concentration too low (continued)	Check that the unknown sample was within the linear 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. 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.
	The high end of the standard curve is not within the dynamic range for the QuantiFluor® ssDNA 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 ssDNA amount, the QuantiFluor® ssDNA Dye may be saturated (see Section 8.A). Recreate the standard curve, and decrease the concentration of the highest standard curve point.
ssDNA concentration determined using the QuantiFluor® ssDNA Dye differed from concentration determined using an alternative quantitation method	Nucleic acid concentrations determined using the QuantiFluor® ssDNA 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.
	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 ssDNA amount, the QuantiFluor® ssDNA Dye may be saturated. Recreate the standard curve, and decrease the concentration of the highest point of the standard curve.

6. Troubleshooting (continued)

Symptoms	Possible Causes and Comments
ssDNA concentration determined using the QuantiFluor® ssDNA 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.
	Contaminating dsDNA may be present. Treat sample with shrimp DNase (see Section 7.C).
Nonlinear standard curve	Check that standard samples were diluted appropriately. If the high or low end of the curve was nonlinear, 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 linear 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. The blank-subtracted fluorescence 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® ssDNA Dye is light-sensitive. Exposure to light will reduce the sensitivity of the assay. Store the QuantiFluor® ssDNA Dye and working solution protected from light.
	Confirm the concentrations of the standard samples used to prepare the standard curve.

Symptoms	Possible Causes and Comments
No or low fluorescence detected in the standard sample	Evaluate the performance of the fluorometer with a ssDNA sample of known concentration (e.g., ssDNA Standard) using the appropriate excitation and emission wavelengths for the QuantiFluor® ssDNA Dye.
	The QuantiFluor® ssDNA Dye working solution was exposed to light. Exposure to light will reduce the sensitivity of the assay. Store the QuantiFluor® ssDNA Dye and working solution protected from light.
	Check that the standard samples were diluted appropriately.

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® ssDNA Dye, measure excitation and emission at 492nm and 528nm, respectively; emission and excitation spectra are shown in Figure 5.

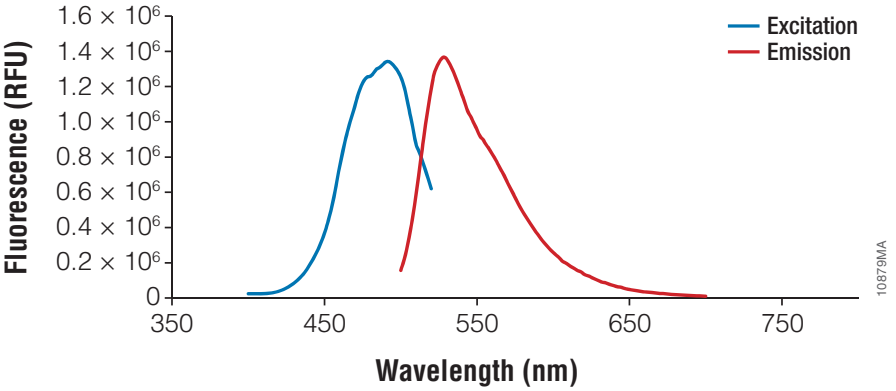


Figure 5. Excitation and emission spectra for the QuantiFluor® ssDNA Dye.

7.B. 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® ssDNA Dye. Table 4 lists compounds that have known effects on DNA quantitation using the QuantiFluor® ssDNA Dye and the concentrations at which they affect quantitation results.

Table 4. Compounds that Interfere with the QuantiFluor® ssDNA Dye.

Chemical	Concentration Shown to Affect the QuantiFluor® ssDNA Dye[1]	Change in QuantiFluor® ssDNA Dye Signal
agarose	0.08%	44% decrease
ammonium acetate	20mM	8% decrease
bovine serum albumin (BSA)	1%	17% decrease
chloroform	0.4%	22% increase
ethanol	10%	24% decrease
IgG	0.5%	15% decrease
sodium chloride	20mM	14% decrease
magnesium chloride	2mM	25% decrease
zinc chloride	1mM	1% increase
dsDNA	10ng	9% increase
RNA	60ng	11% increase
dNTPs	0.5mM	8% decrease
rNTPs	0.5mM	7% decrease
urea	3M	13% decrease
phenol	0.2%	9% decrease
polyethylene glycol	10%	3% increase
sodium acetate	15mM	6% decrease
sodium dodecyl sulfate (SDS	0.02%	17% decrease
Triton® X-100	0.2%	17% decrease

[1]Compounds were tested at the indicated concentration with an initial QuantiFluor® ssDNA Dye dilution of 1:200 and a final concentration of 990ng/ml ssDNA Standard in a 200µl assay.

7.C. Sample Considerations

For samples containing a mixture of ssDNA and dsDNA, we recommend a shrimp DNase treatment (USB Cat.# 78314) to degrade contaminating dsDNA and quantitate ssDNA more specifically.

Prior to quantitation:

1. Add $MgCl_2$ to a final concentration of 10mM to the unknown sample. For example, add 1µl of 500mM MgC_{12} solution to 50µl of unknown sample.
2. Add 1µl (2U) of shrimp DNase to each unknown sample. Mix gently. Do not vortex.
3. Incubate at room temperature (approximately 25°C) for 1 hour.
4. Add 1µl of 500mM EDTA to each tube to inactivate the enzyme.
5. Proceed to ssDNA quantitation as described in Section 5.

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. Handling and Disposal

QuantiFluor® ssDNA 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 dye with care. Because the QuantiFluor® ssDNA Dye binds to nucleic acid, the dye should be treated as a potential mutagen. Dispose of the QuantiFluor® ssDNA Dye according to local regulations.

7.F. Composition of Buffers and Solutions

20X TE Buffer (pH 7.5)

0.2M Tris buffer (pH 7.5)
20mM EDTA ($Na_2EDTA \cdot 2H_2O$) (pH 8.0)

8. Related Products

Product	Size	Cat.#
Quantus™ NGS Starter Package	1 each	E5150
QuantiFluor® ONE dsDNA System	100 reactions	E4871
	500 reactions	E4870
QuantiFluor® dsDNA System	1ml	E2670
QuantiFluor® RNA System	1ml	E3310

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 change was made to the 6/17 revision of this document:

1. Revised the protocol and associated representative data.
2. Removed discontinued product listings.

[a]Patent Pending.

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