QuantiFluor® dsDNA System

Instructions for Use of Product E2670.

Quick Protocol

Materials Required

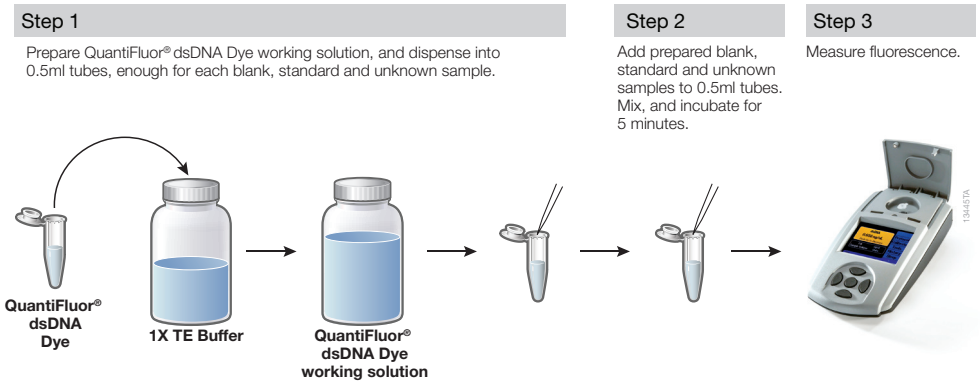
- QuantiFluor® dsDNA System (Cat.# E2670)
- Quantus™ Fluorometer (Cat.# E6150)
- thin-walled 0.5ml PCR tubes (Cat.# E4941 or Axygen Cat.# PCR-05-C)
- nuclease-free water

Warm all assay components to room temperature before use.

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

The *Quantus™ Fluorometer Operating Manual* #TM396 and *QuantiFluor® dsDNA System Technical Manual* #TM346 are available at: **www.promega.com/protocols**

Single-Tube Format Protocol



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. **Prepare 1X TE Buffer:** Dilute the 20X TE Buffer 20-fold with nuclease-free water.
2. **Prepare Working Solution:** Dilute the QuantiFluor® dsDNA Dye 1:400 in 1X TE buffer, and mix.
3. **Prepare Blank:** Mix 2µl of 1X TE buffer with 200µl of QuantiFluor® dsDNA Dye working solution in an empty 0.5ml PCR tube. Vortex well and protect tube from light.
4. **Prepare Standard:** Add 2µl of the provided DNA Standard (100ng/µl) to 200µl of QuantiFluor® dsDNA Dye working solution in an empty 0.5ml PCR tube. Vortex well and protect tube from light.
5. **Prepare Unknown(s):** Add 1–20µl of unknown samples to 200µl of QuantiFluor® dsDNA Dye working solution in 0.5ml PCR tubes. Vortex well, and protect tube from light.
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 (Step 3) and standard (Step 4) samples in the Calibration screen, then select “Save”.
9. Enter the volume of the unknown sample (1–20µl used in Step 5) and desired concentration units.
10. Measure fluorescence of the unknown sample and record the final sample concentration results.

QuantiFluor® dsDNA System

Instructions for Use of Product E2670.

Quick Protocol

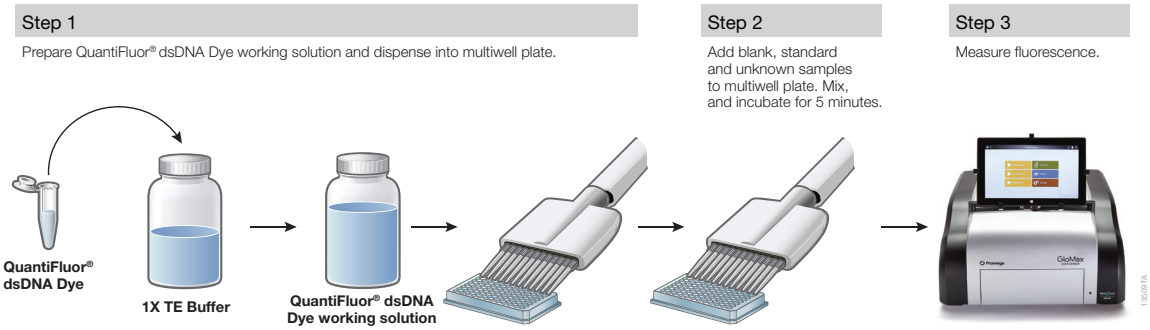
Materials Required

- 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

Warm all assay components to room temperature before use.

The *QuantiFluor® dsDNA System Technical Manual* #TM346 and *GloMax® Discover System Operating Manual* #TM397are available at: **www.promega.com/protocols**

Multiwell Plate Protocol



We recommend preparing a standard curve that extends above and below the likely concentration range for your unknown samples.

1. **Prepare 1X TE Buffer:** Dilute the 20X TE Buffer 20-fold with nuclease-free water.
2. **Prepare Working Solution:** Dilute the QuantiFluor® dsDNA Dye 1:400 in 1X TE buffer, and mix.
3. **Prepare a Standard Curve:** Using dsDNA standards, prepare samples that result in 0.05–200ng/well when dispensing 10µl of standard to each well.
4. Pipet 200µl of QuantiFluor® dsDNA Dye working solution into each well.
5. Dispense 10µl of the prepared dsDNA standards prepared as shown in Figure 1.
6. For the blank, pipet 10µl of 1X TE Buffer into row H.
7. Add 1–20µl of unknown sample to the remaining wells, recording the dilution factor.
8. Mix the plate thoroughly.
9. Incubate assays for 5 minutes at room temperature, protected from light.
10. Measure fluorescence (504nm$_{Ex}$/531nm$_{Em}$) using a plate reader. For the GloMax® Discover System, select "QuantiFluor dsDNA System."
11. Calculate the dsDNA concentration by copying and pasting your raw fluorescence data into our online tool: **www.promega.com/resources/tools/quantifluor-dye-systems-data-analysis-workbook/**

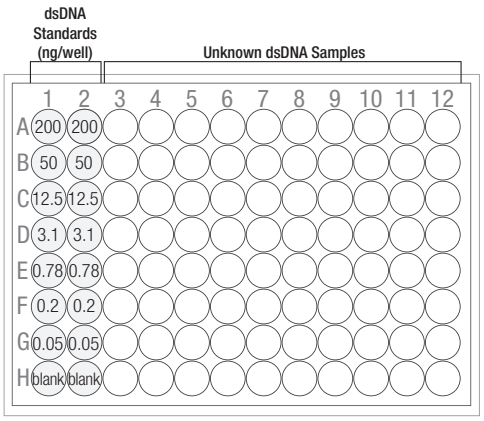


Figure 1. Dispense standard dilutions and blank samples in duplicate into Columns 1 and 2 of a multiwell plate.

Additional protocol information is found in Technical Manual #TM346, available online at: **www.promega.com**