QuantiFluor® ONE dsDNA System

Instructions for Use of Products E4870 and E4871.

Quick Protocol

Materials Required

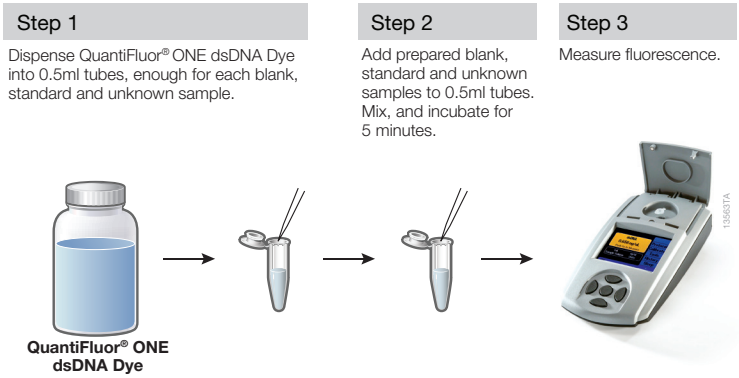
- QuantiFluor® ONE dsDNA System (Cat.# E4870, E4871)
- Quantus™ Fluorometer (Cat.# E6150)
- thin-walled 0.5ml PCR tubes (Cat.# E4941 or Axygen Cat.# PCR-05-C)
- nuclease-free water

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® ONE dsDNA System Technical Manual* #TM405 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 1, 2 and 6.

1. **Prepare Blank Sample:** Add 200µl of QuantiFluor® ONE dsDNA Dye to an empty 0.5ml PCR tube. Protect tube from light.
2. **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. Vortex well and protect tube from light.
3. **Prepare Unknown Sample(s):** Add 1–20µl of unknown samples to 200µl of QuantiFluor® ONE dsDNA Dye in 0.5ml PCR tubes. Vortex well, and protect tube from light.
4. Incubate the prepared samples at room temperature for 5 minutes, protected from light.
5. Select the ONE DNA protocol on the Quantus™ Fluorometer.
6. If needed, calibrate the Quantus™ Fluorometer by reading the blank (Step 1) and standard (Step 2) samples in the Calibration screen, then select “Save”.
7. Enter the volume of the unknown sample (1–20µl used in Step 3) and desired concentration units.
8. Measure fluorescence of the unknown sample and record the final sample concentration results.

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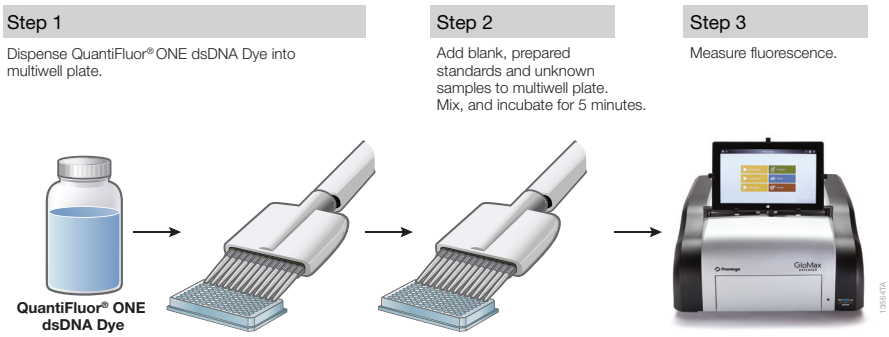
- multiwell detection instrument capable of measuring fluorescence (e.g., GloMax® Discover System [Cat.# GM3000])
- Nuclease-Free Water (Cat.# P1195)
- black, flat-bottom 96-well plates
- 1.5ml tubes

Warm all assay components to room temperature before use.

The *QuantiFluor® ONE dsDNA System Technical Manual* #TM405 and *GloMax® Discover System Operating Manual* #TM397 are 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 a Standard Curve:** Using dsDNA standards, prepare seven samples that result in 0.2–400ng/µl.
2. Pipet 200µl of QuantiFluor® ONE dsDNA Dye into each well.
3. Dispense 1µl of the prepared dsDNA standards prepared as shown in Figure 1.
4. For the blank, pipet 1µl of 1X TE Buffer into row H.
5. Add 1µl of unknown sample to the desired number of wells.
6. Mix the plate thoroughly.
7. Incubate assays for 5 minutes at room temperature, protected from light.
8. Measure fluorescence (504nm$_{Ex}$/531nm$_{Em}$) using a plate reader. For the GloMax® Discover System, select "QuantiFluor ONE dsDNA System."
9. 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/**

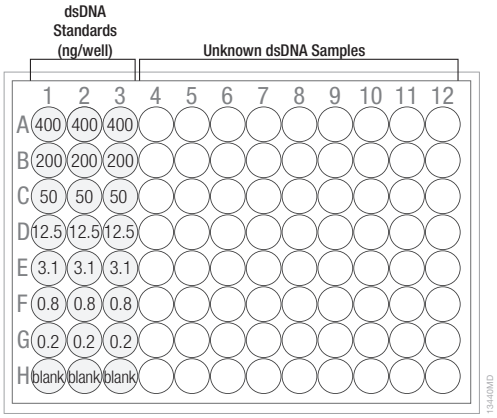


Figure 1. Dispense standard dilutions and blank samples in triplicate into Columns 1, 2 and 3 of a multiwell plate.

Additional protocol information is found in Technical Manual #TM405, available online at: **www.promega.com**