Quantitating Maxwell® FFPE DNA Samples Using the QuantiFluor® dsDNA System and the Quantus™ Fluorometer

Promega Corporation

Materials Required

- QuantiFluor® dsDNA System (Cat.# E2670)
- Quantus™ Fluorometer (Cat.# E6150)
- 0.5ml PCR Tubes (Cat.# E4941)

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

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

Formalin-fixed, paraffin-embedded (FFPE) tissues are valuable samples that are typically prepared from biopsies for histological examination. Isolating DNA from FFPE tissues is made simple with the Maxwell® Instrument and associated FFPE tissue DNA purification kits, which are optimized to provide the best combination of speed, purity and yield for nucleic acid purification. Accurate quantitation of the extracted DNA is critical for many downstream applications; however, because many FFPE tissue sections are small, isolated DNA samples have concentrations well below the 2µg/ml detection limit of traditional spectrophotometric assays.

The Quantus™ Fluorometer and the QuantiFluor® dsDNA System provide a fast, easy and sensitive method for determining DNA concentration. The QuantiFluor® dsDNA System provides a fluorescent DNA-binding dye that enables sensitive and specific quantitation of small amounts of double-stranded DNA (dsDNA) in solution. The dye shows minimal binding to single-stranded DNA (ssDNA) and RNA, allowing specific quantitation of dsDNA.

Using the QuantiFluor® dsDNA System, we have detected sample dsDNA concentrations as low as 10pg/µl using 1µl of sample input per assay. It is possible to quantitate more dilute samples by adding more sample per assay. Up to 100µl of sample may be measured per 200µl assay.

This Application Note describes the protocol for using the QuantiFluor® dsDNA System with the Quantus™ Fluorometer to measure Maxwell® 16-extracted FFPE DNA samples. The Quantus™ Fluorometer measures sample volumes as little as 1µl in a 200µl assay volume without sacrificing instrument sensitivity.

Protocol

Note: Please refer to the *Quantus™ Fluorometer Operating Manual* #TM396 for information on instrument calibration. Once you have calibrated the instrument for the QuantiFluor™ dsDNA Dye System, you do not need to repeat the calibration, will not need to prepare blank and standard samples, and can omit Steps 2, 3 and 7 in this protocol.

1. **Prepare Working Solution:** Dilute the QuantiFluor® dsDNA Dye 1:200 in 1X TE buffer to make a working solution. For example, add 10µl of QuantiFluor™ dsDNA Dye to 1,990µl of 1X TE buffer and mix.

2. **Prepare Blank:** Add 100µl of the QuantiFluor® dsDNA Dye working solution and 100µl of 1X TE buffer to an empty 0.5ml PCR tube, and mix. This will be the blank used in Step 7. Protect from light.
3. **Prepare Standard:** Prepare a 2ng/µl DNA Standard solution by adding 2µl of the provided DNA Standard to 98µl of 1X TE buffer, and mix. Add 100µl of QuantiFluor® dsDNA Dye working solution, and mix. This will be the standard used in Step 7. Protect from light.
4. **Prepare Unkown(s):** Add 100µl of unknown sample and 100µl of QuantiFluor® dsDNA Dye working solution to a 0.5ml PCR tube, and mix.

   Note: If the volume of the unknown DNA sample is less than 100µl, add 1X TE buffer to a final volume of 100µl. For example, mix 1µl of sample with 99µl of 1X TE buffer, and then add 100µl of QuantiFluor® dsDNA Dye Working Solution for a total volume of 200µl.
5. Incubate the reactions at room temperature for 5 minutes, protected from light.
6. Select the dsDNA protocol on the Quantus™ Fluorometer.
7. If you need to calibrate the Quantus™ Fluorometer, read the blank and standard samples using the Calibration screen, then select “Save”.
8. Enter the volume of the unknown samples and desired concentration units.

   Note: This volume is the amount of sample that is added for quantitation. For example, if 1µl of sample was mixed with 99µl of 1X TE buffer and then added to 100µl of QuantiFluor® dsDNA Dye working solution for a total volume of 200µl in the quantitation tube, then the volume entered should be 1µl.
9. Measure fluorescence of the unknown samples.

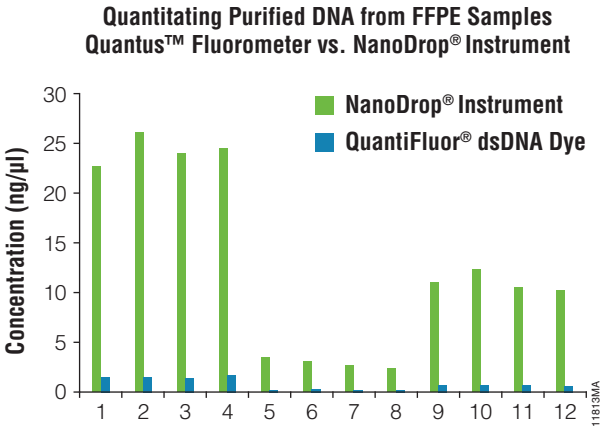

Figure 1. Measuring dsDNA concentration of Maxwell® 16-extracted FFPE samples using the QuantiFluor® dsDNA System and the Quantus™ Fluorometer. Twelve FFPE breast tissue samples were purified using the Maxwell® 16 FFPE DNA Kit and the DNA quantitated using a NanoDrop® spectrophotometer (A_{260}/A_{280}; green bars), and the Quantus™ Fluorometer with QuantiFluor® dsDNA dye (blue bars). Even with highly purified DNA the NanoDrop® consistently overestimates amount of DNA in solution.

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