Measuring Double-Stranded DNA from DNA IQ™ System using the Quantus™ Fluorometer

Materials Required

- QuantiFluor® dsDNA System (Cat.# E2670)
- Quantus™ Fluorometer (Cat.# E6150)
- 0.5ml PCR tubes (Axygen Cat.# PCR-05-C, available through Fisher or VWR)

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 is available at: **www.promega.com/protocols/**

The DNA IQ™ System is a DNA isolation system designed specifically for forensic laboratories to extract DNA from a variety of sample types, both liquid and solid support. Often these forensic samples contain very low or limiting amounts of DNA. For forensic laboratories, it is important to accurately quantitate the amount of DNA present in these samples as downstream applications such as STR amplification require specific amounts of template for proper analysis and identification of the sample source. DNA IQ™ chemistries are available to purify forensic samples manually, on the Maxwell® 16 Instrument and on larger robotic systems.

The most commonly used technique for measuring nucleic acid concentration is the determination of absorbance at 260nm (A_{260}); however, there are major disadvantages of the absorbance method including: the large relative contribution of nucleotides and single-stranded nucleic acids to the signal, interference caused by contaminants commonly found in nucleic acid preparations, the inability to distinguish between DNA and RNA, and the relative insensitivity of the assay (traditional spectrophotometric assays cannot determine nucleic acid concentrations below 2 µg/ml). The use of sensitive, fluorescent nucleic acid stains alleviates many of these problems. Thus, for forensic laboratories these systems represent an accurate, sensitive, fast and inexpensive means of DNA quantitation.

This Application Note describes the protocol for quantitating DNA samples purified using the DNA IQ™ chemistries on the Quantus™ Fluorometer.

Protocol

1. 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. Add 100µl of the QuantiFluor™ dsDNA Dye Working Solution to a 0.5ml PCR tube containing 100µl of 1X TE. This will be the Blank. Protect from light.
3. Prepare the DNA Standard by adding 2µl of the provided DNA Standard to 98µl of 1X TE buffer, and mix. Add 100µl of the QuantiFluor™ dsDNA Dye Working Solution to the tube and mix. This will be the Standard. Protect from light.

4. Add up to 100µl of DNA IQ™ System-purified sample and 100µl of QuantiFluor® dsDNA Dye Working Solution to a 0.5ml PCR tube, and mix.

   Note: If the volume of the sample is less than 100µl, add 1X TE buffer to a final volume of 100µl. For example, dilute a 1µl sample with 99µl of 1X TE buffer, then add to 100µl of dye working solution for a total volume of 200µl.

5. Incubate the Blank, Standard and Samples for 5 minutes, protected from light.

6. Select the dsDNA protocol on the Quantus™ Fluorometer. Go to the Calibration screen, and read the Blank and Standard prepared in Steps 2 and 3. Save the calibration.

7. Enter the volume of unknown samples and desired concentration units.

   Note: This volume is the amount of sample that is added for the quantitation. For example, if 1µl of sample was added with 99µl of 1X TE, and then added to 100µl of dye working solution for a total volume of 200µl in the tube, then the volume entered on this screen should be 1µl.

8. Place the unknown sample into the tube holder and close the lid. The instrument will automatically measure fluorescence when the lid is closed and the calculated nucleic acid concentration will be displayed.

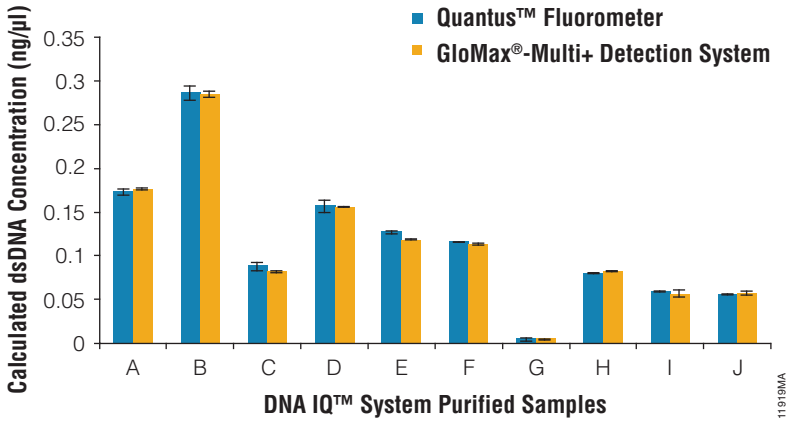

Figure 1. Comparing calculated dsDNA concentrations from DNA IQ™ System-chemistry samples on the Quantus™ Fluorometer and the GloMax®-Multi+ Detection System. Samples were run in duplicate on both instruments. Quantitation of samples run on the GloMax®-Multi+ Detection System can be performed using the QuantiFluor® Dye Systems Data Analysis Workbook.