bioanalytics • microfluidics • membrane separations

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APPLICATION NOTE

Quantitation of dsDNA Using The Low Volume, Disposable FluoroVette® Flowcell

ALine's FluoroVette® (patent pending) is a low volume, disposable, UV transparent flowcell for use in applications involving fluorescence detection. It fits into all standard fluorometers using ALine's patent-pending Adapter Assembly. The design of the flowcell makes it suitable for a variety of applications, including quantitation of double-stranded DNA (dsDNA).

This application note details the use of the FluoroVette® flowcell in conjunction with the fluorescent nucleic acid stain PicoGreen® for the quantitation of dsDNA in the 100-20,000pg range.

Detecting and quantitating small amounts of DNA is one of the most common procedures in molecular biology. It is an essential part of evaluating the yields of DNA during purifications and amplification procedures such as PCR, assessing efficiency in the construction of cDNA libraries, and the generation of primer extension products, and detecting residual DNA in samples such as drug and protein preparations.

The most commonly used technique for measuring nucleic acid concentration is the determination of absorbance at 260 nm (A260). The major disadvantages of the absorbance method are its relative insensitivity (an A260 of 0.1 corresponds to a 5 ug/mL dsDNA solution), its inability to distinguish between signals from DNA and RNA, and its potential for interference from contaminants found in nucleic acid preparations.

PicoGreen® (Invitrogen, Carlsbad, CA) is a nucleic acid stain that fluoresces on binding to double-stranded DNA. The reagent has been developed as the basis of a highly sensitive assay kit. The assay has found wide acceptance within the research community for several reasons. First, and foremost, PicoGreen® dye–based fluorescence assays are up to 10,000-fold more sensitive than UV absorbance measurements. They are also less affected by the presence of common UV contaminants, such as proteins, free nucleotides or very short oligonucleotides, making quantitation of intact oligonucleotides and nucleic acids much more accurate in complex mixtures such as serum or whole blood. The assay utilizes a simple protocol that requires only that reagent be mixed with sample, making it ideal for automated, high-throughput measurements. It is also easily adaptable to apparatus utilizing spectrofluorometric detection. Overall, the performance characteristics of PicoGreen®-based dsDNA quantitation reagents make them very suitable for protocols involving small amounts of dsDNA such as in molecular biology applications.

Figure 1. **FluoroVette® flowcell shown inside the Adapter Assembly, fits any single sample Fluorometer.**

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Materials

FluoroVette® Flowcell from ALine, Inc.
FluoroVette® Adapter Assembly from ALine, Inc.
Quant-iT PicoGreen® dsDNA Assay Kit P11496 (Invitrogen Inc. Carlsbad, CA)
Calf Thymus DNA (Invitrogen Inc. Carlsbad, CA)
Turner BioSystems TBS-380 Mini-Fluorometer or the Hoefer DQ300 Fluorometer

Methods

On the day of the experiment, a 2X stock of the PicoGreen® dsDNA Quantitation Reagent was prepared by making a 1:200 dilution of the concentrated dye solution in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5 (1xTE). Calf Thymus DNA was diluted with 1xTE to create a dilution series of dsDNA for quantitation. The dsDNA samples were quantitated comparing two different methods-absorbance at 260 nm, and fluorescence utilizing the PicoGreen® dye reagent and detecting the fluorescent signal in the FluoroVette® flowcell loaded into a bench-top fluorometer. For fluorescent quantitation, the 2X PicoGreen® dye reagent was mixed in Eppendorf tubes with an equal volume of the dsDNA dilution sample and allowed to incubate at room temperature for 5 minutes before analysis. Fifty microliters of the PicoGreen®-dsDNA sample mix was loaded in the FluoroVette® flowcell, taking care that the sample in the chamber appeared homogenous and that air-bubbles were absent. As per the manufacturer's instructions, control samples without any dsDNA were used to blank the fluorometer and the most concentrated sample in the series was used to calibrate the fluorometer for maximum signal (1000 AUFS). Because of this calibration scheme, we decided to generate two standards curves, one for higher dsDNA concentrations covering the range from 40-400 ng/mL, and another separate series for lower concentrations ranging from 2.5-80 ng/mL. We did so in order to demonstrate how the fluorometer can be adjusted to obtain the optimal signal for the dsDNA being studied. Replicate introductions of the same sample into the FluoroVette® chamber were conducted in order to confirm the precision of the reading. The assay had good precision; the CV between readings on the same sample was less than 5%. A single set of fluorescent readings were used to generate standard curves.

Results

The standard absorbance at 260 nm method was used to generate a standard curve and to determine the concentration of the dsDNA in the calf thymus sample. The analysis indicated that the concentration of dsDNA in the calf thymus stock was 8 mg/mL. Figure 2 below shows the dose response curve obtained using this method of dsDNA quantitation. Using 0.02 as a reliable absorbance measurement, the sensitivity of this method was 1 ug/mL.

The same calf thymus dsDNA stock was used to construct samples for analysis using PicoGreen® and the FluoroVette® flowcell. Two different dilutions series were constructed, one containing a higher range of dsDNA concentrations and a second containing a lower range of dsDNA concentrations. We conducted a separate analysis for each dilution series. Fluorescent readouts using the Turner TBS-380 Mini-Fluorometer call for the user to blank the system, and then to calibrate it using a sample reflecting the highest concentration of analyte that might be encountered in the assay. The later step determines how much gain the instrument will apply in analyzing all test samples. Consequently, we used the 400 ng/mL dsDNA sample to calibrate the

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instrument for the higher concentration series, and the 80 ng/ml dsDNA sample to calibrate for the lower concentration series.

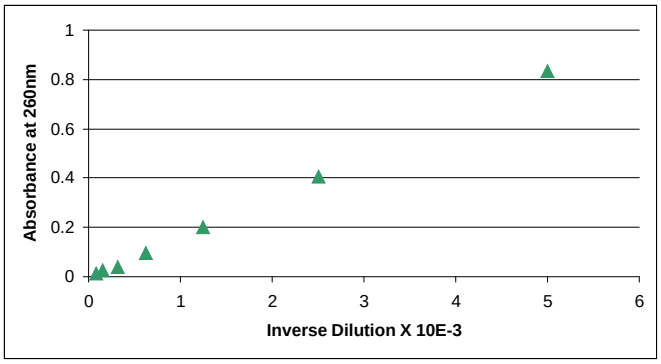

Figure 2: **Analysis of Calf Thymus DNA Using Absorbance at 260nm**.

The results of analyzing samples across these two dilution series are shown below in Figures 3 and 4. Both series yielded a linear dose response curve intercepting at zero. However, the two curves had different slopes. The line describing the high concentration dilution series had a correlation coefficient r=.9998, while the line describing the lower concentration dilution series had a correlation coefficient r=.9956. Closer examination of each curve demonstrates that the linear detection range of the PicoGreen® assay using the FluoroVette® flowcell in the TBS-380 Mini-Fluorometer extends over three orders of magnitude.

Using a signal cutoff of 2-fold over background we determined that the assay sensitivity for the higher concentration series was 4 ng/mL, while the sensitivity in the lower concentration series was 2.5 ng/mL This represents a 250-fold, and 400-fold improvement, respectively, in the sensitivity of this method over the standard A260 method shown above. The absorbance 260nm measurements made using standard cuvettes (1 cm pathway) require between 500-1000 microliters. This is contrasted with the measurements using the FluoroVette® flowcell which require sample volumes of between 25-50 microliters for manual loadings. We routinely used 50 microliters loading volume, and we used that value in making the following calculations. We calculate that standard absorbance 260nm across a 1 cm light path requires approximately 1000 ng dsDNA, whereas the FluoroVette® flowcell requires approximately 125 pg, a difference of 8000-fold. Overall, the FluoroVette® flowcell yields a several hundred-fold improvement in sensitivity over the standard A260 method while requiring a lower sample volume and substantially less dsDNA for detection.

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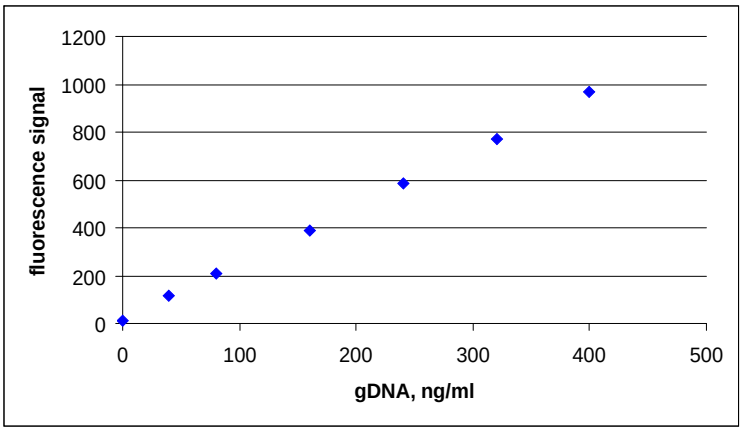

Figure 3. **Dose-Response Curve Using FluoroVette® Flowcell With A Higher Concentration Series**

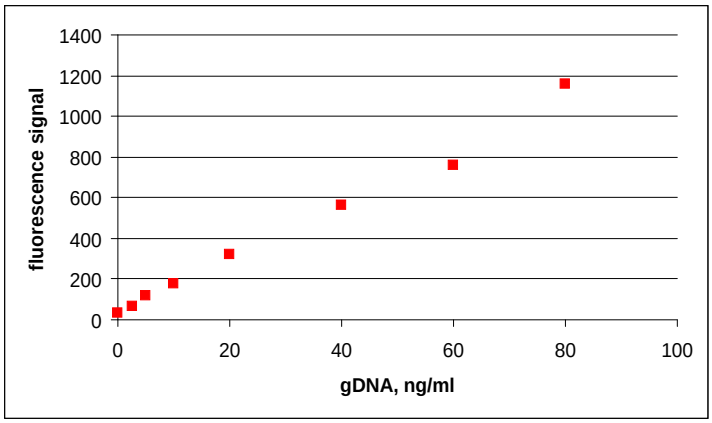

Figure 4. **Dose-Response Curve Using FluoroVette® Flowcell With A Lower Concentration Series**

Conclusion

The FluoroVette® flowcell is a convenient, sensitive, precise, and disposable companion to the PicoGreen® assay reagent in the high sensitivity quantitation of dsDNA. Its universal compatibility with all types of fluorometers, including small footprint mobile units such as the TBS-380 Mini-Fluorometer or the Hoefer DQ300 Fluorometer, highlights its adaptability to all potential applications involving fluorescent detection.