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Application Note

Fluorometric Quantitation of dsDNA

An essential element of cellular and molecular biology is the ability to quantitate DNA in large numbers of samples at a sensitivity that enables determination of small amounts of sample. Here we describe a method to quantitate dsDNA using the BioTek FL600 fluorescence microplate reader.

Introduction

Many techniques of cellular and molecular biology require the ability to quantitate dsDNA in large numbers of samples at sensitivities that only require a small amount of the total sample. Isolation of plasmids from bacterial cultures, genomic DNA from mammalian cells, cDNA synthesis for library production, and quantitation of PCR products for diagnostic purposes all require the direct quantitation of dsDNA. Also, many biochemical studies that involve the growth kinetics of cell cultures or cell cycle studies require normalization by DNA content.

The most commonly used method for the determination of nucleic acid concentration is the determination of absorbance at 260 nm (A_{260}) as described by Matiatis et al.(1). This method, while quite adequate for many situations, can suffer from the interfering absorbance of contaminating molecules. Many of these contaminates which include nucleotides, RNA, EDTA and phenol are commonly found in nucleic acid preparations. As a result, several fluorescent staining techniques have been developed to alleviate many of the problems associated with absorbance at 260 nm (2-4). One such stain is PicoGreen®, developed by Molecular Probes (Eugene, OR), which in conjunction with the BioTek FL600 fluorescence microplate reader offers high specificity, as well as, high sensitivity for dsDNA quantitation.

Materials and Methods

A PicoGreen dsDNA Quantitation Kit, catalogue number P-7589, was purchased from Molecular Probes (Eugene, Oregon). The 96 well black microplates with clear bottom, catalogue number 3603 were purchased from Costar, (Cambridge, Massachusetts).

A series of dilutions ranging from 0.0 to 1000 ng/ml of purified Lambda DNA was made using TE (10 mM Tris, 1 mM EDTA, pH 7.5) as the diluent. Working PicoGreen reagent was prepared by diluting the concentrated DMSO-PicoGreen stock solution, provided in the PicoGreen kit, 1:200 with TE according to the kit instructions (2). Equal amounts of DNA sample and working PicoGreen dsDNA quantitation reagent were mixed and incubated for 5 minutes at room temperature. Following incubation, 200 µl aliquots were pipetted into microplate wells in replicates of eight. Fluorescence was determined using a BioTek Instruments FL600 fluorescent plate reader with a 485 nm, 20 nm bandwidth, excitation filter and a 530 nm, 25 nm bandwidth emission filter. The sensitivity setting was at 38 and the data collected from the bottom using static sampling with a 0.35 second delay, 100 reads per well. Although in these experiments the plates were read immediately, if they remained sealed and protected from light the reaction was found to be stable for several hours.

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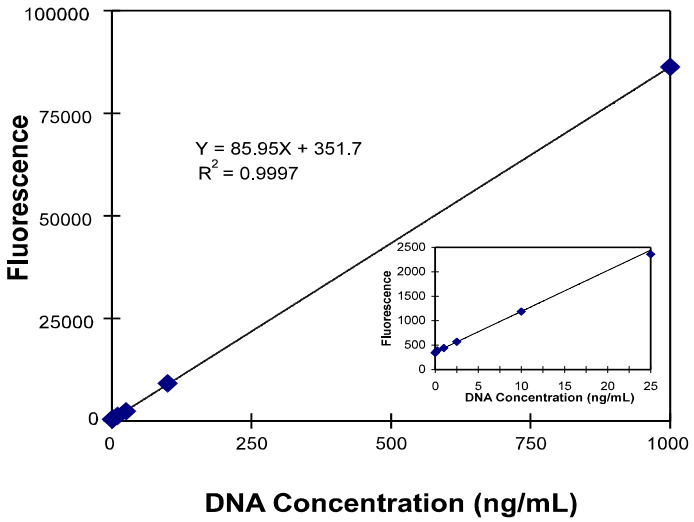
Results

The fluorescence intensity was determined for DNA concentrations ranging from 0 to 1000 ng/ml. Over this range the intensity increased in a linear fashion. Using Microsoft® Excel™, a least means squared linear regression analysis can be generated with a coefficient of determination (r^2) value of 0.999. The average coefficient of variance (%CV) of the standards was less than 5%, with the greatest percent variation taking place in the lower DNA concentrations tested (data not shown). In terms of sensitivity, the assay was found to be sensitive to the picogram level. Under appropriate sensitivity settings, DNA concentrations as low as 25 pg/ml were found to be statistically different ($P<0.05$) from the TE only, 0 ng/ml, control. Quantitation of dsDNA using the fluorescent properties of PicoGreen in conjunction with the BioTek Instruments FL600 allows researchers to quantitate as little as 5 pg/well (25 pg/ml in a 0.2 ml total volume). Thus providing reliable quantitation of dsDNA of concentrations ranging over four orders of magnitude.



Linearity of the Assay. Concentration curve from 0.025 to 1000 ng/ml with linear regression analysis. Insert figure depicts the data points for the lowest DNA concentrations (0.025 to 25 ng/ml). Filled diamonds (◆) represent the mean values of eight determinations at each concentration.

In order to achieve greater sensitivity measures can be undertaken to reduce background fluorescence. Removal of certain common contaminates can lead to an increase in sensitivity. Several compounds decrease the fluorescent signal when present in a PicoGreen-DNA assay. In particular, the presence of 200 nM sodium chloride or 50 mM magnesium chloride can decrease the signal by approximately 1/3. In cases where only low DNA concentrations are to be determined, a calibration curve utilizing lower concentrations can be used in conjunction with setting the fluorescence reader's gain such that the highest DNA concentration yields a fluorescence intensity near the instruments maximum. Alternatively, the use of different microplates that utilize lower fluorescent plastics would be expected to reduce background and therefore increase sensitivity.

The ability to perform this assay in microplates offers several advantages over the conventional tube-based fluorescence assays. Like most assays that are performed in microplates, the ability to use multi-channel pipettes greatly reduces the manual labor required to perform the assay. The microplate format also lends itself to "off the shelf" automation for laboratories with high volume requirements. The smaller reaction volumes in microplates will lead to lower per assay costs by reducing the amount of expensive reagents necessary to perform the assay.

References

(1) Maniatis T., E.F. Fritsch, and J. Sambrook (1982) Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Springs Harbor, NY.

(2) PicoGreen dsDNA Quantitation Reagent and Kit instruction, (1996) Molecular Probes, Eugene, OR.

(3) Labarca, C. and K. Paigen (1980) A Simple rapid, and sensitive DNA assay procedure. *Analytical Biochemistry* **102:**344-352.

(4) Ogura, M., C. Keller, K. Koo, M. Mitsuhashi (1994) Use of the Fluorescent Dye YOYO-1 to Quantify Oligonucleotides Immobilized on Plastic Plates. *BioTechniques* **16:**1032-1033

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