Product Information

PicoGreen

Catalog Number: P2023

Product Size: 1 mL

Parameters

Ex/Em: 480/520 nm (binding with dsDNA)

Storage

Store at 4°C and protect from light. Expiration date marked on the outer packing.

Description

PicoGreen is an ultra sensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in solution. Detecting and quantitating small amounts of DNA is extremely important in a wide variety of biological applications. These include standard molecular biology techniques, such as synthesizing cDNA for library production and purifying DNA fragments for subcloning, such as quantitating DNA amplification products and detecting primers. The most commonly used technique for measuring nucleic acid concentration is the determination of absorbance at 260 nm (A_{260}). The major disadvantages of the absorbance method are the large relative contribution of nucleotides and single-stranded nucleic acids to the signal, the 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 (an A_{260} of 0.1 corresponds to a 5 µg/mL dsDNA solution). The quantitative detection of PicoGreen is simple and convenient, becoming the standard for the detection of residual DNA in biological products.

PicoGreen only emits fluorescence when it binds to dsDNA, and the intensity of the fluorescence is proportional to the DNA concentration. PicoGreen can detect dsDNA in the range of 25 pg/mL-1000 ng/mL with a linear relationship (R^2> 0.99).

Protocol

Preparing the Reagent

The PicoGreen dsDNA Quantitation Reagent is stored as 1 mL stock solution in anhydrous DMSO (dimethyl sulfoxide). In the experiment, prepare an aqueous working solution of the PicoGreen reagent by making a 200-fold dilution of the concentrated DMSO solution in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). For example, to prepare enough working solution to assay 20 samples in a 2 mL final volume,add 100 µL PicoGreen reagent to 19.9 mL TE. We recommend preparing this solution in a plastic container rather than glass, as the reagent may adsorb to glass surfaces. Protect the working solution from light by covering it with foil or placing it in the dark, as the PicoGreen is susceptible to photodegradation. For best results, this solution should be used within a few hours of its preparation.

Method

1. Preparing the standard working solution:

1 mg calf thymine DNA dry powder from Sigma, add 1 mL double distilled water to prepare a 1 mg/mL standard solution.

2. Preparing the working solution 5 µL PicoGreen was added to

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1 mL TE buffer (Note: PicoGreen was diluted 200 times with 1 × TE buffer, use it now, avoid light.)

3. Standard solution dilution

(1) Dilution of stock solution: take 10 μL (1 mg/mL) standard solution and add to 990 μL TE buffer, diluted to 10 μg/mL, add 10 μL (10 μg/mL) standard solution to 990 μL TE buffer, diluted to 100 ng/mL.

(2) Multiple dilution: Add 800 μL (100 ng/mL) of the standard solution to 200 μL TE buffer at a concentration of 80 ng/mL, and add 500 μL (80 ng/mL) of the standard solution to 500 μL. TE buffer, diluted to 40 ng/mL; successively diluted to 20 ng/mL, 10 ng/mL, 5.0 ng/mL, 2.5 ng/mL.

4. DNA standard curve. After dilution, 100 μL of each gradient standard solution and dye working solution were mixed, and kept at room temperature for 5 min in the dark. Use the FB-15 portable fluorometer to measure the fluorescence value of the sample: Add the mixed solution to the micro cuvette and bubbles were not introduced into the sample. Flick the outside of the micro test plate to disperse the bubbles. Use 1 × TE buffer as a blank control to measure the fluorescence values of the sample and the blank control; or directly use a 96-well plate for fluorescence detection with an excitation wavelength of 480 nm and an emission wavelength of 520 nm and the fluorescence intensity corresponding to the concentration of the standard solution (ng/mL) is used for linear regression to prepare the standard curve.

5. Measure the fluorescence value of the sample. Calculate the concentration of the sample according to the prepared standard curve of DNA concentration.

Notes

1. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.

2. To ensure the best results, PicoGreen working fluid is best prepared when using .

3. For your safety and health, please wear lab coats and disposable gloves.

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