

PicoGreen

Catalog No.: 2124-01K Size: 1000 ul

Storage

4°C, protect from light

Component

Component	Size
PicoGreen	1000 ul

Introduction

PicoGreen is a fluorescent dye that binds specifically to double-stranded DNA (dsDNA), with an excitation wavelength of 480 nm and an emission wavelength of 520 nm, compatible with most fluorometers and fluorescence microplate readers.

PicoGreen fluoresces only upon binding to dsDNA and is unaffected by nucleotides or single-stranded nucleic acids. The fluorescence intensity is directly proportional to dsDNA concentration with no sequence dependence, making it ideal for quantitative dsDNA detection within a range of 25 pg/mL to 1 µg/mL.

Usage Instructions

1. Preparation of Working Solution

The PicoGreen stock solution is supplied as a concentrate in anhydrous DMSO. Dilute the stock 1:100 in 1× TE buffer to prepare a 2× dye working solution. Mix the working solution 1:1 with the sample to achieve a final 1× PicoGreen concentration.

Note: PicoGreen readily adsorbs to glass surfaces. Prepare in plastic containers and protect from light. Use within a few hours of preparation for optimal results.

2. Protocol

① Prepare Standard:

Dissolve 1 mg of calf thymus DNA dry powder (Sigma-Aldrich; standardized in Tris/NaCl buffer system) in 1 mL of ddH₂O to prepare a 1 mg/mL DNA standard solution.

② Prepare working solution:

Dilute 10 ul of PicoGreen stock solution in 990 ul of 1x TE buffer as a 2x working solution.

③ Standard dilution:

- Stock Dilution:**
Dilute the 1 mg/mL DNA standard 1:100 in 1× TE buffer to obtain 10 µg/mL. Repeat the 1:100 dilution to obtain 100 ng/mL.
- Two-Fold Serial Dilution:**
Dilute the 100 ng/mL solution to 80 ng/mL (800 µL standard + 200 µL 1× TE). Then perform sequential two-fold dilutions in 1× TE buffer to generate standards at 40, 20, 10, 5.0, 2.5, and 1.25 ng/mL.

④ Standard Curve:

- Mix equal volumes of each standard (1.25–80 ng/mL) with 2× dye working solution. Incubate at room temperature for 5 min, protected from light.
- Measure fluorescence (Ex 480 nm / Em 520 nm) using a fluorometer with micro cuvette or a fluorescence microplate reader with 96-well plate. Use 1× TE buffer as blank. Maintain constant temperature throughout.
- Subtract blank readings from all values and perform linear regression of corrected fluorescence versus concentration (ng/mL) to generate the standard curve.

Note: Avoid introducing air bubbles when loading samples. Gently tap the cuvette to remove any bubbles before measurement.

⑤ Sample Measurement:

Mix sample with 2× dye working solution at 1:1 ratio and measure under the same conditions as step (4). Pre-dilute samples in 1× TE buffer if necessary to fall within the linear detection range. Determine dsDNA concentration by interpolation from the standard curve.

Precautions

- Protect from light and
- Prepare fresh before each use.
- Temperature variation may affect fluorescence intensity. Standards and samples must be measured at identical temperature (20–25°C).
- Safety:** Wear a lab coat and gloves when handling this reagent.