

PicoGreen dsDNA Quantitation

(Modified from Molecular Probes protocol: 15-January-2001)

This protocol describes the PicoGreen method for quantitating LM-PCR products using the SpectroMAX GeminiXS Spectrofluorometric Microplate reader (Molecular Devices). The PicoGreen reagent provides a sensitive, fluorescence-based assay for double-stranded (ds) DNA in solution. The PicoGreen dye is essentially non-fluorescent and exhibits >1000-fold fluorescent enhancement upon binding to dsDNA resulting in an assay that displays a linear correlation between dsDNA concentration and fluorescence. The assay is selective for dsDNA over RNA, oligonucleotides and single-stranded (ss) DNA and is ideal for measuring PCR product concentration. The assay can be assembled in 96 well plates and quickly read on any spectrofluorometric microplate reader.

Materials

PicoGreen dsDNA Quantitation Reagent and Kit (P-7589)

Component A: PicoGreen dsDNA quantitation reagent

Component B: 20X TE

Component C: Lambda DNA standard (100ug/ml)

LM-PCR products (resuspended in 20ul 20%DMSO)

Corning (3915) or Falcon (353241) Black 96-well flat-bottom plates

Reagent preparation (for 1 x 96-well plate)

Assay Buffer: 10mM Tris-HCl, 1mM EDTA, pH 7.5

Dilute the 20X TE (Component B) with nuclease free water to a 1X working solution. 11ml required.

550ul 20X TE

10.45ml nuclease free water

PicoGreen: Dilute the 200X PicoGreen Reagent (Component A) with 1X TE assay buffer to a 1X working solution. 5 ml is required. PicoGreen is made fresh and protected from light.

25ul 200X PicoGreen

4.975ml 1X TE assay buffer

DNA Standard Curve: lambda DNA (31.3ng/ml – 1000ng/ml). Prepare a 2ug/ml stock solution of lambda DNA by diluting 50-fold the lambda DNA stock (Component C, 100ug/ml) in 1X TE. 200ul is required.

4ul Lambda DNA stock

196ul 1X TE

Protocol for preparing high-range standard curve.

Volume (μL) of 2 $\mu\text{g}/\text{mL}$ DNA Stock	Volume (μL) of 1X TE	Volume (μL) of Diluted PicoGreen Reagent	Final DNA Concentration in PicoGreen Assay
50	0	50	1000 ng/ml
25	25	50	500 ng/ml
12.5	37.5	50	250 ng/ml
6.25	43.75	50	125 ng/ml
3.13	46.87	50	62.5 ng/ml
1.56	48.43	50	31.3 ng/ml

Sample analysis

- 1 For the high-range standard curve, dilute the 2 $\mu\text{g}/\text{mL}$ lambda DNA stock solution as described above, into wells A1-A6 of a black 96-well plate.
- 2 Prepare reagent blanks by adding 1ul of 20% DMSO to wells A7-A10, followed by 49 μl of 1X TE.
- 3 Prepare control DNA samples by adding 25 μl of the 2 $\mu\text{g}/\text{mL}$ lambda DNA stock solution to A11 & A12, followed by 25 μl of 1X TE.
- 4 Prepare unknown LM-PCR products by first adding 49ul 1X TE to wells B1-H12. Add 1ul of LM-PCR product from corresponding well location to each well containing the 1X TE using a 12-channel pipettor.
- 5 Add 50 μl of diluted PicoGreen working solution to each well (A1-H12). Mix well and incubate for 2 to 5 minutes at room temperature, protected from light.

Measure fluorescence

- 6 After incubation, measure the fluorescence using a SpectroMAX GeminiXS platereader (excitation 485 nm, emission 538 nm).
- 7 Insert the 96-well plate into the platereader and run PicoGreen dsDNA Quantitation protocol.

The protocol will automatically subtract the reagent blank value from each of the samples, and create a standard curve of fluorescence vs. DNA concentration from the corrected data. The protocol will also determine the DNA concentration of the LM-PCR products and generate a report. All DNA values are ng/ml, and will have been adjusted for the 1:100 dilution factor.