PicoGreen Assay
Invitrogen-Molecular Probes
Catalog number: P7589

(Document prepared by Ms Alex Aitken)

The PicoGreen reagent is a proprietary, asymmetrical cyanine dye. Free dye does not fluoresce, but upon binding to dsDNA it exhibits a >1000-fold fluorescence enhancement. PicoGreen is 10,000-fold more sensitive than UV absorbance methods, and highly selective for dsDNA over ssDNA and RNA.

1. Turn on the fluorescence plate reader at least 10 minutes before reading results. Use the following settings to read the PicoGreen results:

	Wavelength/bandwidth
Excitation:	~485nm/20nm
Emission:	~530nm/25nm

2. Prepare 1X TE buffer (10mM Tris-HCl, 1mM EDTA, pH 7.5) from the 20X TE stock, which is supplied in the PicoGreen kit (to make 50mL, add 2.5mL of 20X TE to 47.5mL sterile distilled DNase-free water). 50mL is sufficient for 250 assays.
3. Dilute DNA standards form 100µg/mL to 2µg/mL with 1X TE. For two standard curves prepare 600µL of a 2µg/mL stock by adding 12µL of the 100µg/mL stock to 588µL 1X TE.
4. Prepare the two standard curves in the microtiter plate as shown in the table:

Plate Well	Final [DNA] (ng/mL)	Vol. (µL) 2µg/mL DNA standard	Vol.(µL) 1X TE buffer	Final Total DNA (ng)/Well
Any	0	0	100	0
A1 & A2	25	2.5	97.5	5
B1 & B2	50	5	95	10
C1 & C2	100	10	90	20
D1 & D2	200	20	80	40
E1 & E2	300	30	70	60
F1 & F2	400	40	60	80
G1 & G2	500	50	50	100
H1 & H2	1000	100	0	200

5. For each unknown, add 1µL of sample to 99µL of 1X TE in the microplate well. Mix by pipetting up and down.
6. Prepare a 1:200 dilution of the PicoGreen reagent in 1X TE. For each standard and each unknown sample, a volume of 100µL will be needed. For examples, 2 standard curves with 8 points each will require 1.6mL. To calculate the total volume of diluted PicoGreen reagent needed, determine the total number of samples and unknowns you will be testing and multiply this number by 100µL (if using a multichannel pipet, make extra reagent). The PicoGreen reagent is light sensitive and should be kept wrapped in foil while thawing and in the diluted state. Vortex well.
7. Add 100µL of diluted PicoGreen to every standard and sample. Mix by pipetting up and down.
8. Cover the microplate with foil and allow to incubate at room temperature for 2-5 minutes.
9. Read the plate.
10. Generate a standard curve using the average values of the standards and determine the concentrations of DNA in the unknown samples.