

PicoGreen Quantification

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1 General

The PicoGreen kit (dye, DNA standard and buffer catalogue number P7589¹ cost £320 on 26/01/2012).

It is sensitive enough to detect 50 pg of DNA regardless of assay volume (200 ul for plate or 2 ml for cuvette).

To make the standard, we can prepare the desired concentrations in separate eppendorfs and dilute them 100-fold for the final assay. We can input their original dilution in the software to calculate concentrations so that our samples that are diluted equally are quantified without conversion. For the plate, the minimum possible concentration that is quantified is $50 \text{ pg} / 200 \text{ ul} = 0.25 \text{ pg/ul}$ original concentration cannot be accurately quantified with a 100-fold dilution.

2 Procedure

Make a 400-fold dilution of the PicoGreen in TE that was used for the DNA.

- For one plate (100 samples x 200 ul per well) use 19.950 ml TE and 50 ul of PicoGreen.
- For two plates, 39.9 ml TE and 100 ul PicoGreen.

Pipette 2 ul of all DNA samples to be quantified on each well of the plate.

Pipette 2 ul of all ladder standards, except for the 100 standard (4 ul) and the 6.25 standard (1 ul). Leave one well empty as a 0 point for the ladder.

Add 198 ul recently made PicoGreen solution to each well.

Ready for the plate reader.

3 Preliminaries

3.1 Assay Buffer

Dilute the 20X TE buffer with DNase-free water. It is 25 ml so you can add 475 ml DNase-free water.

¹<http://products.invitrogen.com/ivgn/product/P7589>

3.2 Picogreen

The manual makes a 200-fold dilution of PicoGreen to prepare a working solution, and uses it in a 1:1 ratio with the samples to be quantified, so the final PicoGreen dilution is 400-fold.

3.3 DNA Standard

The lambda DNA standard is at 100 ug /ml

To make standards:

100 ug/ml: pipette 100 ul original standard into a new eppendorf

50 ug/ml: pipette 50 ul of above and 50 ul TE into new eppendorf

25 ug/ml: pipette 50 ul of above and 50 ul TE into new eppendorf

12.5 ug/ml: pipette 50 ul of above and 50 ul TE into new eppendorf

6.25 ug/ml: pipette 50 ul of above and 50 ul TE into new eppendorf