



## DNA-quantification using Varioskan and PicoGreen® dsDNA Quantitation Kit

This application note describes the use of Thermo's Varioskan spectro-photo- and spectrofluorometer as a dispensing and measurement tool for determining the genomic DNA concentration together with the PicoGreen® dsDNA quantitation kit.

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### Abstract

PicoGreen dsDNA Quantitation Reagent (Molecular Probes) is a sensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in molecular biological procedures such as cDNA synthesis for library production and DNA fragment purification for subcloning, as well as diagnostic applications, such as quantitating DNA amplification products and primer extension assays<sup>1</sup>.

The most commonly used technique for measuring nucleic acid concentration is the determination of absorbance at 260 nm (A<sub>260</sub>). The major disadvantages of the absorbance method are the large relative contribution of nucleotides, single-stranded nucleic acids and

proteins 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<sub>260</sub> of 0.1 corresponds to a 5 µg/mL dsDNA solution).

The combination of the Varioskan and PicoGreen kit is ideal for quantification of dsDNA. In a 96 well plate, a maximum of 88 preps can be analyzed, 8 wells are necessary for the standard curve. To simplify the assay procedure, the diluted PicoGreen reagent is dispensed with Varioskan in-built dispenser.

### Experimental

#### Reagents and materials

PicoGreen® dsDNA Quantitation Kit, Molecular Probes Inc. (P-7589) or standalone reagent (P-7581).

The kit contains:

- 1 ml PicoGreen dsDNA quantitation reagent, stock solution in DMSO (Component A)
- 25 ml 20 x TE buffer (Component B) 200 mM Tris-HCl, 20 mM EDTA, pH 7.5
- 1 ml Lambda DNA standard (100 µg/ml in TE buffer)

#### Preparation of reagents

1 x TE buffer

- Prepare 1 x TE working solution by diluting the concentrate 1:20 in sterile, distilled DNase free water. Use 1 x TE buffer as blank and for preparing all the DNA standard dilutions.

PicoGreen working solution

- Dilute the PicoGreen Reagent 1:200 in 1 x TE buffer in a plastic tube protected from light, and use it within a few hours after preparation. For the 96-well plate 100 µl of diluted reagent is needed/well. Make a little more for the prime option in the Varioskan.

DNA standard curves

- Prepare a 2 µg/ml DNA stock solu-

tion in TE buffer. Use this to make a standard curve as shown in the table:

| Final DNA concentration (ng/µl) | Vol.(µl) 1xTE | Vol.(µl) 2 µg/ml DNA Standard |
|---------------------------------|---------------|-------------------------------|
| 0                               | 100           | 0                             |
| 2.5                             | 97.5          | 2.5                           |
| 5                               | 95            | 5                             |
| 10                              | 90            | 10                            |
| 20                              | 80            | 20                            |
| 30                              | 70            | 30                            |
| 40                              | 60            | 40                            |
| 50                              | 50            | 50                            |

NOTE! Please see the Molecular Probes Product Information Manual received with the kit or stand alone reagent for complete, important information about the procedure, preparation and storage of the reagents.

#### Assay procedure

Pipette DNA standard dilutions or samples to the wells of a white or black 96-well plate. For more reliable results, the standard curve can be replicated. For the unknowns, 2 µl of sample must be pipetted in 98 µl of 1xTE. Before starting the protocol, the

dispenser of the Varioskan must be primed with 150 µl of the PicoGreen working solution.

#### SkantIt protocol

Make plate layout with 96 well plate containing DNA standards (Calibrators) and samples (Unknown).

The SkanIt protocol has 4 steps:

1. Dispense 100 µl PicoGreen working solution.
2. Shake 20s, 600 rpm, Diameter 1 mm.
3. Pause 5 minutes (Continuation: Time expired)
4. Fluorescence reading: Excitation at 485 nm. Emission at 535 nm.

After measurement see Results and choose "Curve Fit" from "Calculations" menu to generate standard curve for reading the concentrations of unknown samples.

#### Results and Discussion

In Figure 1 DNA standard curve generated in SkanIt software using "Curve Fit" option is shown.

In conclusion, Thermo Varioskan together with PicoGreen Quantitation Kit is a quick tool for determination of DNA concentration. The in-built dispenser of the instrument is recommended to be used for dispensing the diluted PicoGreen Reagent to achieve the most reliable and reproducible assay results.

#### References

<sup>1</sup>Molecular Probes, Product Information Manual; PicoGreen<sup>®</sup> dsDNA Quantitation Reagent and Kits (PicoGreen<sup>®</sup> is a registered trademark of Molecular Probes, Inc.)

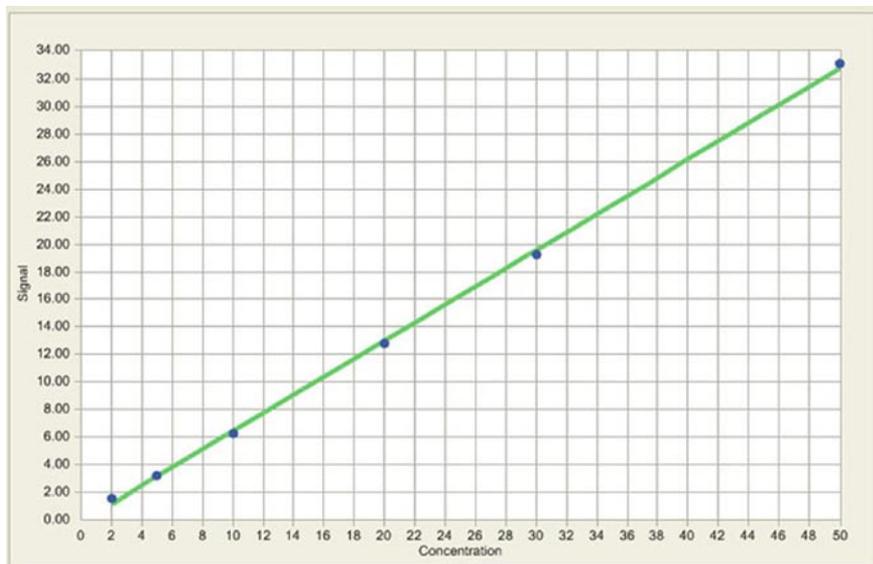


Figure 1. Standard curve measured in the Varioskan. Correlation coefficient R2 = 0,9996.

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