

## Fluorometric Application Note



## Detection of DNA from solution with PicoGreen<sup>®</sup> dsDNA Quantitation Reagent in the microplate format

## Summary

The Molecular Probes PicoGreen® dsDNA Quantitation Reagent is an ultrasensitive fluorescent stain used for the quantitation of double-stranded DNA in solution. In many molecular biology applications the detection and quantitation of small amounts of dsDNA is very important. In combination with Thermo Labsystems' Fluoroskan Ascent or Fluoroskan Ascent FL this reagent provides a rapid and easy determination of dsDNA, giving a wide dynamic range.

- **Thermo Labsystems products:**

- Fluoroskan Ascent, Cat. No. 5210480 (one dispenser included) or 5210470, Fluoroskan Ascent FL, Cat No. 5210460 (one dispenser included) or 5210450
- White 96-well strip plates, Cat. No. 95029510 or Black 96-well strip plates, Cat. No. 95029450. Alternatively White 384-well plates (Cat. No. 95040 010) can be used
- Finnpiettes® and tips

- **Other products:**

- PicoGreen® dsDNA Quantitation Kit, Molecular Probes, Inc. Cat. No. P-7589 or stand-alone reagent, Cat. No. P-7581
- Sterile distilled DNase-free water
- Plastic tubes

## Introduction

The Molecular Probes PicoGreen® reagent is an ultrasensitive fluorometric stain used for the quantitation of dsDNA in solution. In a wide variety of molecular biology applications the detection and quantitation of small amounts of dsDNA is very important. This reagent is much more sensitive than the most commonly used absorbance measurement at 260 nm ( $A_{260}$ ). When measuring the absorbance, single stranded nucleic acids and nucleotides interfere with the obtained signal. The commonly used bisbenzimidazole dyes of Hoechst, 33258 and 33342 are less affected by interfering substances than the absorbance measurement but the sensitivity is not as good as with PicoGreen®. Only one dilution of the reagent is needed for the whole dynamic range instead of two different fluorochrome dilutions which generally is needed with Hoechst 33258.

In combination with Thermo Labsystems' Fluoroskan Ascent or Fluoroskan Ascent FL the PicoGreen® reagent provides a rapid and easy determination of dsDNA, giving a wide dynamic range.

## Reagents and materials

PicoGreen® dsDNA Quantitation Kit, Molecular Probes Inc. (P-7589) or stand-alone 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)

## Instrumentation

Fluoroskan Ascent or Fluoroskan Ascent FL plate reader equipped with fluorescein

filters (Ex 485 nm Cat. No. 1424852 and Em 538 nm Cat. No. 1425382 or Em 518 nm Cat. No. 1425182).

## Others needed but not provided

- White or Black Strip plates (Thermo Labsystems Cat. No. 95029510 or Cat. No. 95029450) or White 384 Well plates (Cat. No. 95040 010).
- Finnpipettes and Finntips (Thermo Labsystems) for preparing and dispensing samples.
- Plastic tubes for making dilutions.
- Sterile, distilled DNase free water.

---

## Preparation of reagents

### 1 x TE buffer

Prepare 1 x TE working solution by diluting the concentrate 1:20 x in sterile, distilled DNase free water. Use this 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 and 25 µl/well for the 384 well plate.

### DNA Standard Curves

Dilute the provided Lambda DNA standard 1:50 in 1 x TE buffer. Alternatively prepare a 2 µg/ml DNA stock solution in TE buffer. An absorbance of 1 at 260 nm ( $A_{260}$  nm = 1.000) corresponds to 50 µg/ml of DNA.

For the high-range standard curve, prepare further dilutions of this 2 µg/ml DNA stock in 1 x TE buffer, which corresponds to 1 µg/ml in the final assay volume. Make further 1:10 dilutions (=200 ng/ml, 20

ng/ml and 2 ng/ml). Use TE buffer as a blank.

For the low-range standard curve, prepare a 50 ng/ml DNA stock in 1 x TE buffer and make further 1:10 dilutions of this stock (=5 ng/ml, 500 pg/ml and 50 pg/ml). Use TE buffer as a blank.

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

## Procedure

Pipette 100 µl blank, DNA standard dilutions or samples to the wells of a white or black 96-well strip plate. Add 100 µl of the diluted PicoGreen reagent. Mix well and allow to stand at room temperature for 2-5 min protected from light.

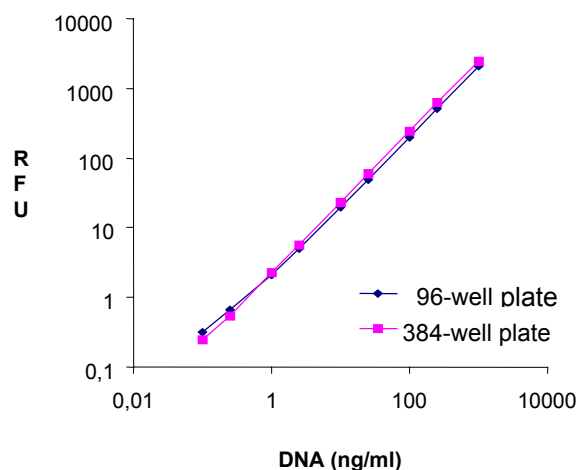
Measure with Fluoroskan Ascent or Fluoroskan Ascent FL. The wavelengths of the filters used are excitation 485 nm and emission 538 nm or 518 nm. Subtract the average blank value from standards and samples and generate the standard curve. Read the unknown samples from the curve.

### Fluoroskan Ascent and Fluoroskan Ascent FL procedure parameters

- In the Ascent software select the area to be measured under General parameters.
- Define the Layout (name the Blanks, Calibrators and Samples).
- Select a Shake step and set the corresponding parameters
- Select a Measure step, select filterpair 485/538 or 485/518.
- Select single measurement and use normal beam for the 96-well plate and small beam for the 384 well plate.
- Start the measurement procedure.

- In the Process menu select Curve Fit to generate the standard curve and to read the unknown samples.

## Results



*Figure 1. Detection of dsDNA with the PicoGreen® reagent and Fluoroskan Ascent using the filter combination Ex 485/Em 538. The assay was performed using either white 96-well strip plates (total volume 200 µl/well) or white 384-well plates (total volume 50 µl/well).*

## References

Molecular Probes, Product Information Sheet; PicoGreen® dsDNA Quantitation Reagent and Kit

(PicoGreen® is a registered trademark of Molecular Probes, Inc.)

Maje Welin  
Application Specialist  
Fluorometry

## Contact Information

Thermo Labsystems Oy  
Sorvaajankatu 15,  
P.O. Box 208  
FIN-00811 Helsinki, Finland

Tel. +358-9-329 100  
Fax. +358-9-3291 0415  
[www.labsystems.fi](http://www.labsystems.fi)