Quant-iT™ PicoGreen® dsDNA assay for nucleic acid quantification

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BMG LABTECH

- **Double stranded DNA quantification performed in down to 2 µl using the LVis Plate**
- **High linearity and sensitivity over a broad DNA range**
- **Easy to use UV absorbance alternative assay that can be miniaturized and adapted to high-throughput systems**

Introduction

The quantification of nucleic acids samples is a major need in any genetic and molecular biology laboratory. Next to well-known UV absorbance assays (e.g. 260/280 nm), a lot of different fluorescence intensity based DNA quantification assays are offered. These assays are not only more sensitive than absorbance assays, they are also able to differentiate between double-stranded and single-stranded DNA, as well as RNA.
Aside from sensitivity and specificity, the demand for very low volume measurements is emerging. This is challenging for assays as well as for the detection instrumentation. In this application note we present DNA quantification data obtained on the low volume LVis Plate, as well as 96- and 384-well microplates.

Materials & Methods

- Quant-iT™ PicoGreen® dsDNA assay, LifeTechnologies
- Low volume LVis Plate, BMG LABTECH
- Black 96-well and 384-well microplates, Greiner

Sample preparation

All necessary reagents such as detection reagent, TE buffer, DNA standard (Lambda DNA standard) are provided with the kit. From a 20x TE buffer stock solution a 1x TE buffer was prepared using HPLC grade water. The PicoGreen® reagent was diluted 1:200 by using 1x TE buffer. dsDNA standards were diluted with 1x TE buffer to obtain a concentration range of 2 to 1000 ng/ml. DNA standards were pipetted in 6 replicates, adding the same amount of PicoGreen® reagent into each well leading to the final volumes: 200 µl in 96-well plates, 20 µl in 384-well plates and 2 µl in the LVis Plate. The blank consisted of TE buffer.

Instrument settings

	FLUOstar®/ POLARstar® Omega	CLARIOstar®	PHERAstar® FS
Filter settings	Ex485 Em520	Ex485 Em520	Optic Module: FI 485 520
Monochromator settings		Ex: 483-14 Em: 530-30	

All other settings are default settings for a fluorescence endpoint test protocol.

Results & Discussion

Fluorescence scan of PicoGreen® reagent bound to DNA

In order to determine useful monochromator settings for the CLARIOstar, an excitation and emission scan was done for the DNA bound PicoGreen® reagent. The spectra scan result is shown in Fig. 1.

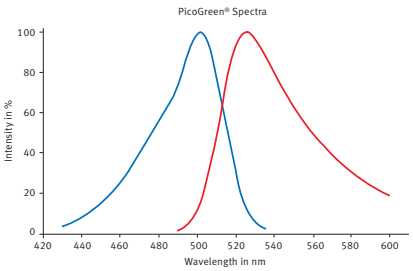

Fig. 1: CLARIOstar spectral scan of PicoGreen® reagent bound to DNA. The excitation scan (blue line) was recorded between 400 and 540 nm with a fixed emission wavelength at 550 nm. The emission was scanned between 490 and 600 nm (red line) while a fixed excitation wavelength of 460 nm was used.

The scan showed an excitation maximum at 502 nm and an emission maximum at 524 nm. For further measurements, the highest signal/blank ratio was determined by varying the band widths for excitation and emission.

96-well

In 96-well plates the standard curves obtained using either filters or the monochromator are very similar (Fig. 2). With filters the slope of the standard curve is slightly higher.

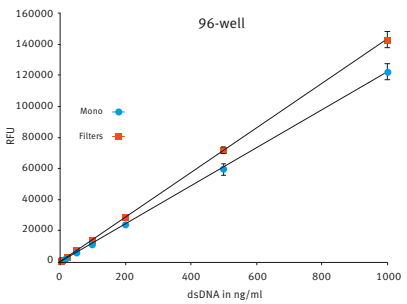

Fig. 2: PicoGreen® assay comparison using either filters (red) or the LVF Monochromator™ (blue) in 96-well format in the CLARIOstar. The final volume in the well was 200 µl.

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384-well

To miniaturize the assay, the well volume was decreased to 20 µl. The resulting standard curve in 384-well small volume plate (Fig. 3) was comparable to the data obtained for 96-well plates.

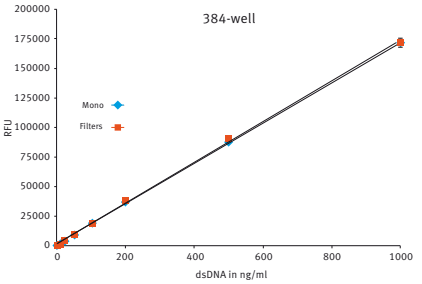

Fig. 3: PicoGreen® assay comparison using either filters (red) or the LVF Monochromator™ (blue) in 384-well format in the CLARIOstar. The final volume in the well was 20 µl.

Very good linearity was achieved for the CLARIOstar® using either filters (R^2 = 0.9999) or LVF Monochromators™ (R^2 = 0.9998).

LVis Plate results

With the LVis Plate it is possible to miniaturize the assay further. This special plate was developed to use a volume of as little as 2 µl (Fig. 4).

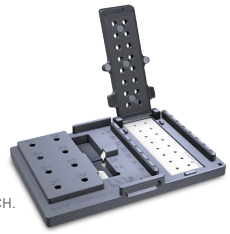

Fig. 4: LVis Plate from BMG LABTECH.

Originally developed to do DNA and protein UV absorbance quantification the LVis Plate, used in the CLARIOstar or PHERAstar® *FS*, is an excellent tool to determine the DNA content of samples in fluorescence intensity mode. Results for the PicoGreen® assay in the LVis Plate are shown in Fig. 5.

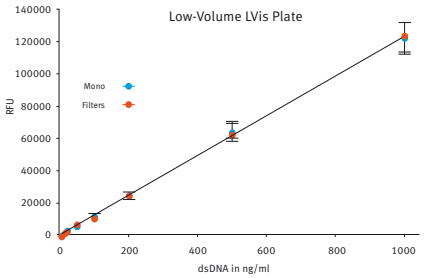

Fig. 5: PicoGreen® assay comparison using either filters or the LVF Monochromator™ in the LVis Plate. The final volume in the well was 2 µl.

Conclusion

The standard curve data show that the PicoGreen® assay can be measured with high linearity and sensitivity over a broad DNA concentration range. For the CLARIOstar, the LVF Monochromator™ shows filter-like performance. For example, the sensitivity in 384- well plates was calculated to be about 3 pg/well for both filters and LVF Monochromators™.

With the help of the PicoGreen® assay, any preferred volume, between 2 µl in the LVis Plate or standard volumes in 96- and 384-well plates, can be used to determine the DNA concentration of samples.