Optimizing the PicoGreen® Assay on BMG LABTECH's CLARIOstar® multimode microplate reader using LVF Monochromators™

Mark Gröne and Franka Maurer, BMG LABTECH, Ortenberg, Germany

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- Double stranded DNA quantification performed down to 2 µl using the LVis Plate
- High linearity and sensitivity over a broad DNA range
- CLARIOstar's LVF Monochromators™ show filter-like performance

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 CLARIOstar® using the low volume LVis Plate (Fig. 1), as well as 96- and 384-well microplates. For dsDNA Quanit-iT™ PicoGreen® measurements, the CLARIOstar® used either optimized filters or BMG LABTECH's new LVF Monochromators™.

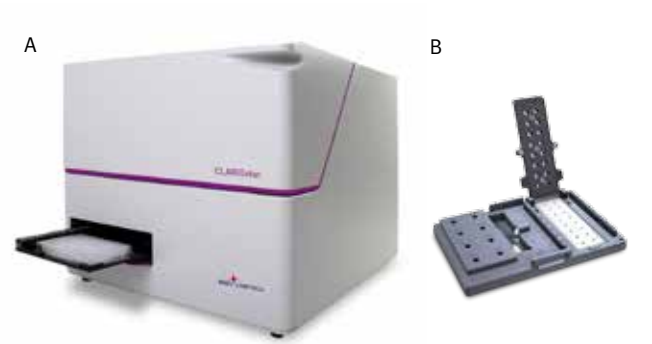

Fig. 1: (A) CLARIOstar® multimode microplate reader and (B) LVis Plate from BMG LABTECH.

Materials and Methods

- ☐ Quant-iT™ PicoGreen® dsDNA assay, LifeTechnologies (Cat. No. P11496)
- ☐ CLARIOstar® microplate reader, BMG LABTECH (Fig. 1)
- ☐ 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

Filter:	Ex: 485 nm & Em: 520 nm
Monochromator:	Optimized after spectral scanning of fluorophore (Ex: 483-14 nm & Em: 530-30 nm)
Number of flashes:	20-200
Gain and focus:	An automated gain and focus adjustment was performed

Results and Discussion

Fluorescence scan of PicoGreen® reagent bound to DNA

In order to determine useful monochromator settings, an excitation and emission scan was done for the DNA bound PicoGreen® reagent. The spectral scan result is shown in Fig. 2.

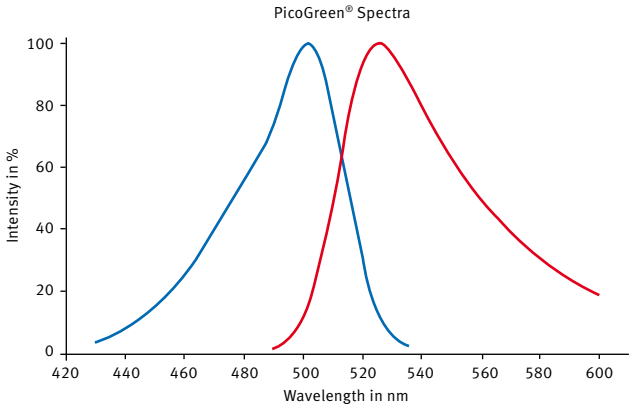

Fig. 2: Spectral scan of PicoGreen® reagent bound to DNA. The excitation scan (blue line) was recorded between 400 and 540 nm with a fixed emission wavelength at 550 nm. The emission was scanned between 490 and 600 nm (red line) while a fixed excitation wavelength of 460 nm was used.

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. The monochromator was finally set up with 483-14 for excitation and 530-30 for emission. As a comparison, the PicoGreen® assay was also measured using filters as described in the materials and methods section.

In 96-well plates the standard curves obtained using either filters or the monochromator are very similar (Fig. 3). With filters the slope of the standard curve is slightly higher.

Excellent linearity over the whole tested DNA concentration range was obtained for both filters (R^2 = 0.9999) or monochromator (R^2 = 0.9998) measurement.

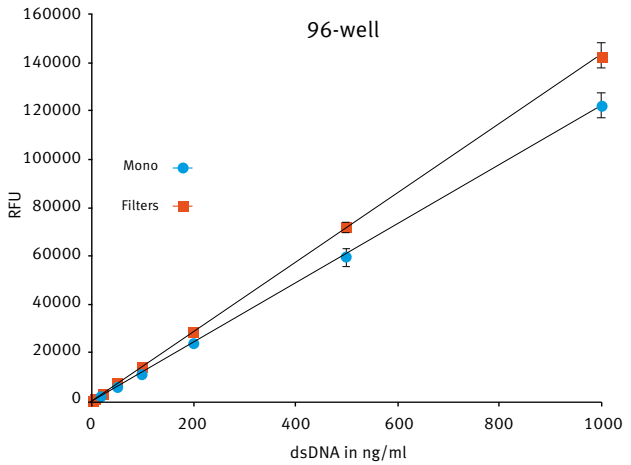

Fig. 3: PicoGreen® assay comparison using either filters (red) or LVF Monochromators™ (blue) in 96-well format. The final volume in the well was 200 µl.

To miniaturize the assay the well volume was decreased to 20 µl. The resulting standard curve in 384-well small volume plate (Fig. 4) was comparable to the curve obtained for 96-well plates.

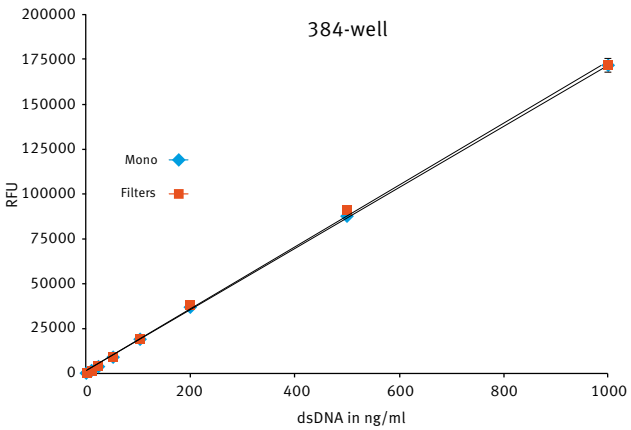

Fig. 4: PicoGreen® assay comparison using either filters (red) or LVF Monochromators™ (blue) in 384-well format. The final volume in the well was 20 µl.

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. Originally developed to do DNA and protein UV absorbance quantification the LVis Plate, used in the CLARIOstar®, 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.

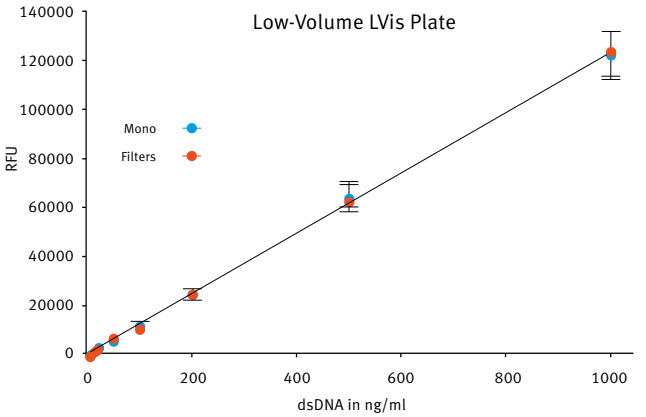

Fig. 5: PicoGreen® assay comparison using either filters or LVF Monochromators™ in the LVis Plate. The final volume in the well was 2 µl.

Conclusion

The standard curve data show that the PicoGreen® assay can be measured on the CLARIOstar® with high linearity and sensitivity over a broad DNA concentration range. The new LVF Monochromators™ show 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 in the CLARIOstar® to determine the DNA concentration of samples.

Germany:	**BMG LABTECH GmbH**	**Tel: +49 781 96968-0**
Australia:	BMG LABTECH Pty. Ltd.	Tel: +61 3 59734744
France:	BMG LABTECH SARL	Tel: +33 1 48 86 20 20
Japan:	BMG LABTECH JAPAN Ltd.	Tel: +81 48 647 7217
UK:	BMG LABTECH Ltd.	Tel: +44 1296 336650
USA:	BMG LABTECH Inc.	Tel: +1 877 264 5227
Internet:	**www.bmglabtech.com**	**applications@bmglabtech.com**