

Absorbance DNA Quantitation Using BMG LABTECH's POLARstar Omega Microplate Reader



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Application Note 168

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- Allows rapid DNA quantitation
- High degree of linearity from 0.1 to 100 µg/mL
- New data analysis software, MARS, with integrated extinction coefficients for dsDNA, ssDNA and RNA

Introduction

One of the most common methods for nucleic acid detection is the measurement of solution absorbance at 260 nm (A₂₆₀) due to the fact that nucleic acids have an absorption maximum at this UV wavelength. Although a relatively simple and time-honored method, A₂₆₀ suffers from low sensitivity and interference from nucleotides and single-stranded nucleic acids. Furthermore, compounds commonly used in the preparation of nucleic acids absorb at 260 nm leading to abnormally high quantitation levels¹. However, these interference and preparation compounds also absorb at 280 nm leading to the calculation of DNA purity by performing ratio absorbance measurements at A₂₆₀/A₂₈₀.

$$\frac{A_{260}}{A_{280}} = 1.7 \text{ to } 2.0 \text{ for "pure" DNA}$$

Therefore, constructing an absorbance ratio between these two absorbance wavelengths can provide an estimate of sample purity. As a general rule any preparations with an A₂₆₀/A₂₈₀ greater than approximately 1.7 is called "pure"^{1,2}.

The absorbance measurement is governed by Beer's Law.

$$A = \epsilon bc$$

Where A is absorbance, ϵ is the molar extinction coefficient, b is the path length, and c is the analyte concentration. With the spectrometer, the POLARstar Omega (Figure 1) offers easy handling for DNA measurements. The integrated path length correction and the new data analysis software, MARS, allow for fast determination of DNA concentration in samples.



Fig. 1: BMG LABTECH's POLARstar Omega multimode detection reader with fast absorbance spectrometer

Alternative methods to quantitate DNA than absorbance are new fluorescent techniques that are much more sensitive and specific for DNA. The PicoGreen® dsDNA Quantitation Reagent from Molecular Probes® for example is a highly sensitive fluorescent assay for double stranded DNA (dsDNA) detection that can routinely reach quantitation levels of 100 pg and even lower with optimization³.

Materials and Methods

All materials were obtained through normal distribution channels from the manufacturers stated.

- UV-Star plates, 96-well, Greiner Bio-One, Cat. No. 655801, Frickenhausen, Germany
- Deoxyribonucleic acid, Activated from calf thymus, lyophilized powder, Cat. No. D-4522, Sigma-Aldrich, Munich, Germany
- Distilled water
- POLARstar Omega, BMG LABTECH, Offenburg, Germany

In addition, consumables such as pipette tips and microcentrifuge tubes were used as needed from various manufacturers. The DNA from calf thymus was solved in distilled water to a final concentration of 1 mg/mL. From this stock solution further dilutions were performed yielding different DNA standards ranging from 0.1 to 100 µg/mL.

Four replicates of 350 µL aliquots of each standard were pipetted into the 96-well UV plate. Additionally, 16 replicates of 350 µL aliquots of distilled water were pipetted into the plate to serve as a blank. The prepared 96-well plate was inserted into the instrument and was read in the POLARstar Omega using the spectrometer.

There are two possibilities for the measurement. You can either choose to select up to eight specific wavelengths (in this case one wavelength at 260 nm) or you can measure a spectrum of the sample. An example for a DNA spectral range using different concentrations of DNA is given in Figure 2.

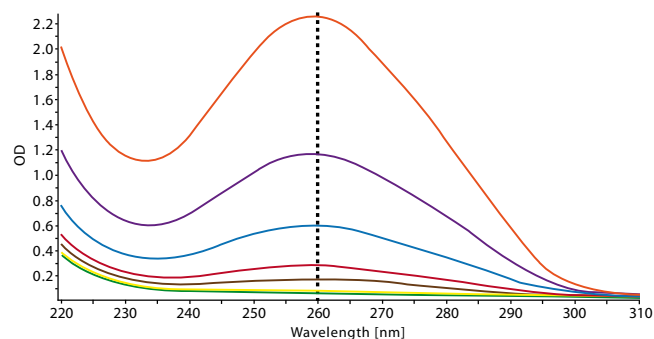


Fig. 2: Absorbance spectrum of different concentrations of calf thymus DNA recorded on the POLARstar Omega. Detection range is between 220 and 310 nm and resolution was set at 1 nm.

Results and Discussion

The data from the measurement was evaluated using the new data analysis software, MARS, from BMG LABTECH. The average value of the blank measurement was subtracted from the measurements made at each concentration and the results plotted. A linear regression fit was performed on the standard values (Figure 3).

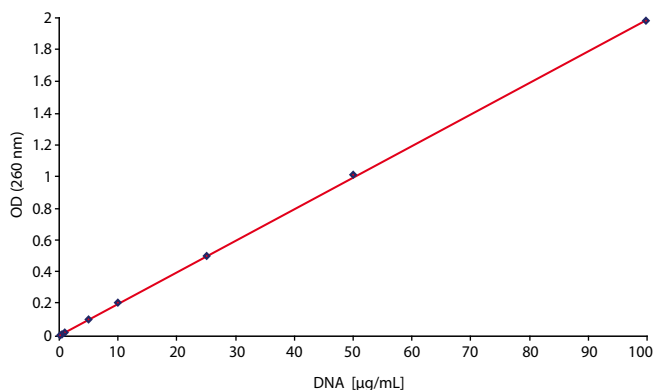


Fig. 3: Linear regression fit performed on the DNA standard curve in the concentration range from 0.1 to 100 µg/mL. An R^2 -Value of 0.99988 was obtained indicating a high degree of linearity throughout the concentration range.

The standard curve allows the back calculation of unknown samples. Sensitivity of < 0.3 µg/mL DNA (or about 0.1 µg DNA/well) was observed for measurements with selected 260 nm wavelength and spectrum measurements.

A further option in the new MARS data analysis software is the possibility to determine the DNA concentration of unknown samples without a standard curve. Based on the knowledge that 50 µg of double stranded DNA show an OD value of 1.0, the concentration is automatically calculated without the necessity of pipetting standards into the microplate. It should be taken into account that this method only works well when the path length correction feature is activated.

As double stranded and single stranded DNA or RNA have different extinction coefficients there are different MARS templates available for these different nucleic acids (Table 1).

Table 1: Extinction coefficients of different nucleic acids

Nucleic acids	Extinction coefficient [$\text{cm}^{-1} \cdot \text{M}^{-1}$]	MARS Data Analysis Software
double stranded DNA	50	dsDNA template
single stranded DNA	33	ssDNA template
RNA	40	RNA template

Conclusion

Because of its spectrometer, the POLARstar Omega offers easy handling for DNA absorbance measurements by simply selecting a wavelength of 260 nm or by measuring a spectrum which covers the absorbance maximum. Furthermore, with the help of the new data analysis software, MARS, it is possible to determine different nucleic acid concentrations depending on the extinction coefficient. The ratio of A260/A280 indicates how pure the DNA sample is and it can be measured just as easy and within the same measurement time as A260 alone. A full absorbance spectrum in the range of 220-850 nm helps to identify impurities and it can be measured within one second per well.

References

- [1] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning. A Laboratory Manual*. Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbour
- [2] Steffan, R.J., Goksoyr, J., Bej, A.K. and Atlas, R.M. (1988) Recovery of DNA from Soils and Sediments. *Appl. Environ. Microbiol.* **54** (12) 2908-2915.
- [3] Angersbach, S. and Earp, R. (2004) Molecular Probes PicoGreen® Assay Performed on BMG LABTECH POLARstar OPTIMA Microplate Reader, BMG LABTECH, www.bmg-labtech.com/application-notes/fluorescence-intensity/PicoGreen-fluorescence-DNA-103.cfm

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