UV absorbance DNA quantitation

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- **High degree of linearity from 0.1 to 100 µg/mL DNA**
- **MARS evaluation software offers integrated extinction coefficients for dsDNA, ssDNA and RNA**
- **Measure a full absorbance spectrum in less than 1 second per well**

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 levels. 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.

$$\frac{A260}{A280} = 1.7 \text{ to } 2.0 \text{ for "pure" DNA}$$

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".

Comparing Results of a *Spectrophotometer and a Microplate Reader*

The absorbance measurement is governed by Beer's Law.

$$A = \varepsilon bc$$

Where A is absorbance, ε is the molar extinction coefficient, b is the path length, and c is the analyte concentration. When the molar coefficient and path length are constant, absorbance is proportional to the concentration.

For a standard cuvette reader, the path length is usually defined as 1 centimeter. Therefore, with a conventional absorbance reading an A260 of 1.0 OD corresponds to 50 µg/ml dsDNA solution. In a microplate reader, the same DNA concentration measured will lead to a smaller OD value (about 0.7 OD) because of the smaller path length in a microplate well. The integrated spectrometer in the BMG instrumentation offers a path length correction feature that allows fast determination of DNA concentration in samples as well as results comparable to cuvette-based measurements.

Alternative methods to quantitate DNA than absorbance are fluorescent techniques that are much more sensitive and specific for DNA. The Quant-iT PicoGreen® dsDNA Quantitation Reagent from Life Technologies® for example is a highly sensitive fluorescent assay for double stranded DNA (dsDNA) detection.

Materials & Methods

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

- UV-Star plates, 96-well, Greiner Bio-One
- Deoxyribonucleic acid, Activated from calf thymus, lyophilized powder, Sigma-Aldrich
- Distilled water
- spectrometer-based BMG LABTECH microplate reader

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 UV absorbance was measured using the following settings.

Instrument settings

	SPECTROstar® Nano	FLUOstar®/ POLARstar® Omega	CLARIOstar®	PHERAstar® *FS*
Detection mode	Absorbance			
Spectro-meter settings	Select the wavelengths: 260 and 280 nm or Measure a spectrum between 220 and 400 nm			
Predefined Protocols available	√	√	√	√

There are two possibilities for the measurement. You can either choose to select up to eight specific wavelengths (in this case wavelengths at 260 nm and at 280 nm) or you can measure a spectrum of the sample. An example for a DNA spectral scan using different concentrations of DNA is given in Figure 1.

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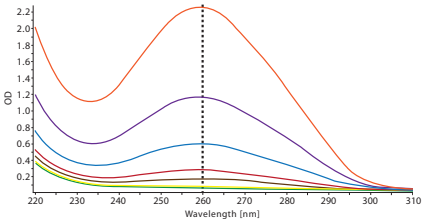


Fig. 1: Absorbance spectrum of different concentrations of calf thymus DNA recorded on the POLARstar Omega. Detection range is between 220 and 310 nm and resolution was set at 1 nm.

Results & Discussion

The data from the measurement was evaluated using the MARS data analysis software 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 2).

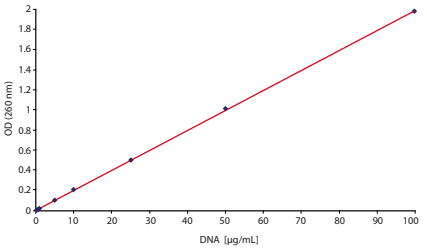


Fig. 2: Linear regression fit performed on the DNA standard curve in the concentration range from 0.1 to 100 µg/mL. An R^2-Value of 0.99988 was obtained indicating a high degree of linearity throughout the concentration range.

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).

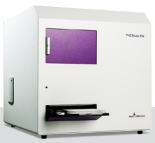
Table 1: Extinction coefficients of different nucleic acids.

Nucleic acids	Extinction coefficient [cm-1 · 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, BMG LABTECH instruments offer 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 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 220-1000 nm helps to identify impurities and it can be measured within one second per well.



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