Labrox
Application
Note #001

Labrox® multimode plate reader:

DNA quantification by absorbance with the Labrox plate reader

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Introduction

DNA concentration determination is needed for innumerous scientific applications such as PCR, electrophoresis and many others. A common nucleic acid detection method is spectrophotometric absorbance measurement. As DNA has an absorption maximum at this UV wavelength, its concentration is determined as the absorbance value at 260 nm (Green and Sambrook, 2012).

Labrox® multimode plate readers are developed for several applications. Versatile and easy to use they include various detection modes, among them (Abs). In this application note, we show that the Labrox reader (Figure 1) Absorbance methodology is efficient in determining DNA concentration.

Figure 1. Labrox multimode plate reader.

Materials

- Lyophilized DNA (Deoxyribonucleic Acid, Sodium Salt, Calf Thymus - CAS 73049-39-5 – Calbiochem, Merck Millipore 2618)
- Lonza AccuGENE™ Molecular Biology Water cat. BE51200
- 96 well UV transparent plates (Greiner bio-one, 96 well UV-Star®, F-bottom, chimney well, µClear®)
- Micropipettes and tips
- Labrox Multimode Plate Reader

Methods

DNA was diluted in water to make a 1 mg/ml stock solution. This DNA stock solution was further diluted to make a dilution series from 100 µg/ml to 0,5 µg/ml (see Table 1).
The DNA samples were added into the plates (200 µl/well,3 replicates of each sample). 200 µl water samples were included as blank samples (controls). The samples absorbance was measured at 260 nm and the absorbance spectrum scanning from 220 nm to 320 nm was performed.

Results

Data were analyzed using Microsoft Excel. The averages and standard deviations were calculated and absorbance values were normalized to correspond to measurements with a 10mm light path. The light path in the microplates using 200 microliters is 5,7 mm (value calculated from the total volume of the well and its depth). The final absorbance values were calculated by multiplying by the factor 1,75 (10/5,7) The water absorbance value was subtracted from all the samples (S/B) (Table 1)

Table 1. DNA dilution series absorbance at 260 nm.

DNA conc	Abs. 260	Abs. 260 norm.	S/B	SD (%)
100	1,30	2,28	2,19	0,4
50	0,78	1,37	1,27	0,4
25	0,44	0,77	0,67	0,3
10	0,22	0,38	0,28	0,3
5	0,14	0,24	0,15	2,3
2,5	0,10	0,17	0,08	1,6
1	0,08	0,14	0,04	0,8
0,5	0,07	0,12	0,02	9,3
0	0,05	0,10	0,00	2,2

The S/B results were plotted and a linear regression fit was performed on the DNA standard curve (Figure 2, page 2).

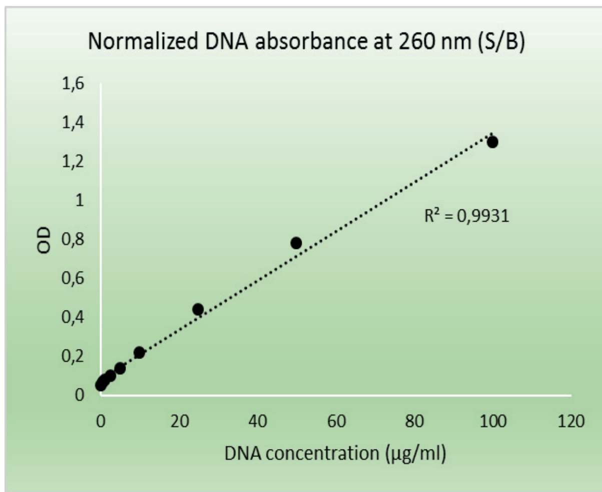

Figure 2. DNA absorbance linearity

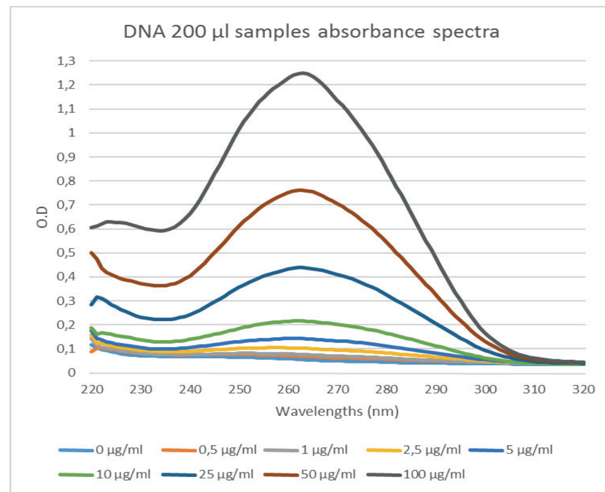

Figure 3. Absorbance spectra of different DNA concentrations recorded on the Labrox multireader. Detection range is between 220 and 320 nm and resolution was set at 1 nm.

The absorbance spectra (220-320 nm) obtained with the different DNA concentrations are shown in Figure 3.

Discussion

The results show that DNA regression line is linear between 0,5 – 100 µg/ml DNA and can be used for calculating DNA concentrations of unknown samples.
The obtained spectra are typical pure DNA spectra, showing the purity of the samples.

Conclusion

Labrox multimode plate reader is suitable for determining DNA concentration in 96 wells microplates using 200 µl samples. The instrument offers reliable and reproducible results. The absorbance spectrum can be read in less than one second, allowing a fast and easy assessment of the samples purity.

References

Michael R. Green, Joseph Sambrook. (2012) Molecular cloning: a laboratory manual, Fourth edition. NY, Cold Spring Harbor, Cold Spring Harbor Laboratory Press.

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