Labrox® Application Note #012

Labrox® multimode plate reader:

Low volume DNA quantification by absorbance in a µDrop™ Plate using 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 very common nucleic acid detection method is spectrophotometric absorbance measurement at 260 nm, as DNA has an absorption maximum at this UV wavelength. DNA concentration is determined as the absorbance value at 260 nm (1). Often the available amount of DNA is very limited, requiring the possibility of its quantitation in 1-2 µl samples.

Volumes as small as 2 µl can be measured in µDrop™ Plate. Compared to a normal cuvette the pathlength of the µDrop Plate low-volume area is very short: 0.50 mm vs. 10 mm. By decreasing the pathlength the sample volume can also be decreased (2). In this application note, we show that the Labrox reader (Figure 1) is capable of determining DNA concentration in samples as small as 1 µl using a µDrop™ Plate.

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
- µDrop™ Plate (Thermo Scientific)
- Micropipettes and tips
- Labrox Multimode Plate Reader

Methods

DNA was diluted in water to make a 1 mg/ml stock solution. Samples of 1, 2, 3, 4 and 5 µl of this solution were added to the µDrop Plate wells (triplicates). Water samples (blanks), in the same volumes, were also added (triplicates).
The samples absorbance was measured at 260 nm and the results exported and analyzed in Excel.

Measurement Parameters:

PLATE: General 96

OPERATION: ABS Spectrum

Wavelength: 260 nm

No Mirror

EXC FILTER: Empty

EXC SPOT SIZE: 4 (Default)

Flashes: 25

Flash energy: 25 mJ

The µDrop Plate has a fixed nominal pathlength of 0,5 mm. To be able to follow the Lambert-Beers Law, a factor of 10 mm/ pathlength has to be added to compare the results to 10 mm cuvette measurements (2). DNA concentration of the different samples was determined using the following formula:
[DNA]= Abs 260 (S-B) x 50 µg/mlx(10/0,50)
(S-B is sample OD - blank OD).

Results

The obtained results are shown in Table 1.

Table 1. Results of the OD measurements of 1, 2, 3, 4 and 5 µl samples of a 1 mg/ml DNA solution in the Labrox reader using a µDrop Plate.

DNA Vol.	OD (S-B)	Aver OD (S-B)	SD	SD %	[DNA] µg/ml	[DNA] mg/ml
1 µl	1,02	1,00	0,016	1,6	998	**0,998**
	0,99					
	0,99					
2 µl	0,97	0,98	0,007	0,7	980	**0,98**
	0,98					
	0,98					
3 µl	1,00	1,01	0,014	1,4	1009	**1,009**
	1,02					
	1,03					
4 µl	1,05	1,03	0,021	2,0	1030	**1,03**
	1,03					
	1,01					
5 µl	0,99	0,99	0,009	0,9	990	**0,99**
	0,98					
	1,00					

Discussion

The results presented in Table 1 show that the calculated concentration of DNA, based on the measurements with the Labrox reader, is extremely close to the expected (the concentration of the DNA solution utilized, 1 mg/ml). This is true for all the measured volumes: 1, 2, 3, 4 and 5 µl samples provided approximately the same results, between 0,98 and 1,03 mg/ml.

Conclusion

Labrox multimode plate reader is ideal for determining DNA concentration in low volume samples using a µDrop Plate. Labrox reader is able to measure DNA samples as small as 1 µl with extreme accuracy.

References

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

2. Thermo Scientific µDrop Plate User Manual.

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