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Application News

No.A484

Spectrophotometric Analysis

Micro-Volume DNA Quantitation Using TrayCell

Introduction

Purity confirmation and quantitation of nucleic acids and proteins are typically conducted using an ultraviolet-visible spectrophotometer. As the samples are often available only in small quantities, a cell that permits micro-volume measurement is required. Measurement of 25 µL samples is typically conducted using an ultra-micro black cell. Here we introduce an example of micro-volume analysis of nucleic acids in a 3 – 5 µL sample using the Hellma TrayCell.

Fig. 2 UV-1800

Hellma TrayCell

A 3 – 5 µL sample is necessary for analysis using the standard TrayCell having a 1.0 mm optical path length. As shown in Fig. 1, measurement is easily conducted by just pipetting the sample onto the top of the cell, then placing the lid on top, and setting the cell in the sample chamber of the spectrophotometer.

Fig. 1 Photograph of TrayCell and Sample Pipetting

DNA Analyses Using TrayCell

We conducted analysis of Lambda-DNA, a type of double-stranded DNA commonly used for nucleic acid analysis, using the TrayCell along with the analytical conditions shown in Table 1. The photometric values obtained in ten repeat measurements using 260 nm as the measurement wavelength, in addition to the standard deviation of those values, are shown in Table 2. Also, the overlaid spectra obtained from measurement of different concentrations of the nucleic acids are shown in Fig. 3, and the calibration curve generated from measurement of the nucleic acids is shown in Fig. 4.

A standard deviation of about 0.015 was obtained in 10 repeat measurements of a sample in which the concentration was adjusted to provide an absorbance of 1.0. Further, a CV value of 1.34 %, a calibration curve formula Y=0.00201x + 0.00827, and coefficient of determination value r^2=0.99989 confirm that measurement was conducted with excellent precision.

Table 1 Analytical Conditions

Instrument	: Shimadzu UV-1800 Ultraviolet-Visible Spectrophotometer
Wavelength Range	: 220 - 350 nm
Scan Speed	: Medium
Sampling Pitch	: 1.0 nm
Measurement Value	: Absorbance
Slit Width	: 1 nm (fixed)

Table 2 Absorbance Values and Standard Deviations of Nucleic Acids Measured Ten Times at 260 nm

Absorbance Values at 260 nm	
1	1.074
2	1.092
3	1.072
4	1.065
5	1.086
6	1.092
7	1.087
8	1.101
9	1.104
10	1.110
Average	1.088
Standard deviation	0.014629
CV value	1.34 %

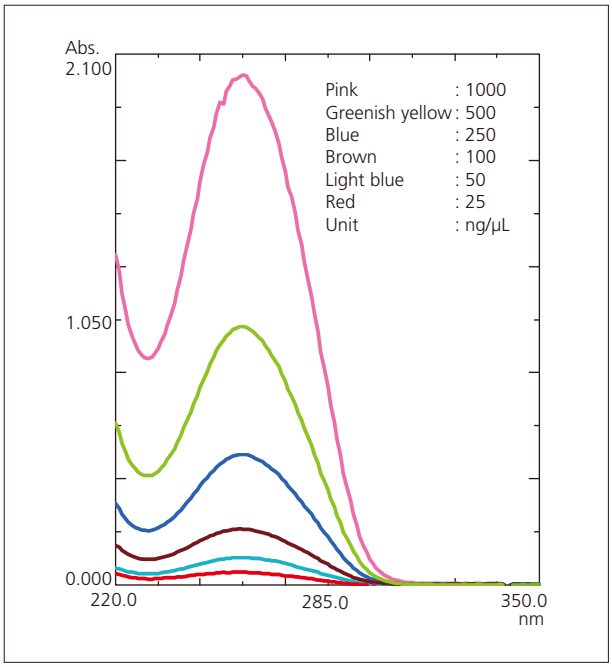

Fig. 3 Spectra of DNA at Different Concentrations

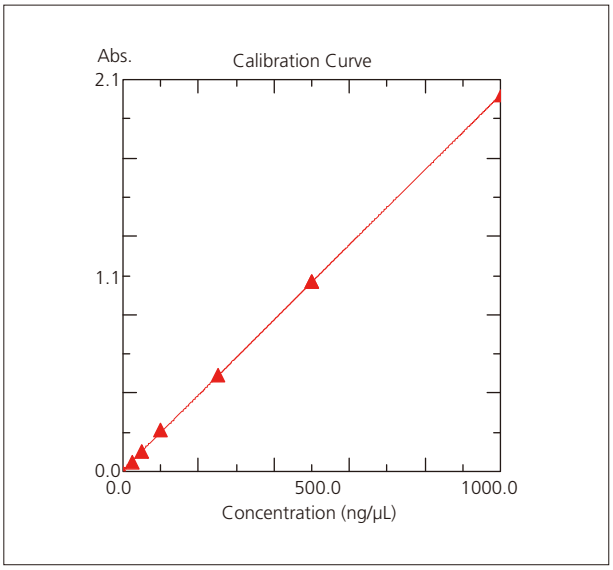

Fig. 4 Calibration Curve of Nucleic Acids

Conclusion

This study demonstrates that use of the Shimadzu UV-1800 ultraviolet-visible spectrophotometer with the TrayCell permits accurate and easy quantitation of a micro-volume sample.

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