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Application News

No. A577

Spectrophotometric Analysis

Quantitation of Nucleic Acids
– Trace Measurement Using TrayCell and Nano Stick –

Ultraviolet-visible (UV-VIS) spectrophotometers are used in quantitative and qualitative analysis of substances in many fields. Purity confirmation and quantitation of nucleic acids, proteins, and other substances are also performed in the life sciences, but measurement at the trace level is demanded, as only small samples are available in many cases.

This article introduces an example of quantitation of nucleic acid with a Shimadzu UV-1900 UV-VIS spectrophotometer with two type of cells (TrayCell™, Hellma Analytics and Nano Stick, SINCO) which enable measurement of sample quantities of several µL. The Biomethod Mode of the UV-1900 is also introduced briefly.

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Quantitation of Nucleic Acid Using TrayCell

Fig. 1 shows the appearance of the UV-1900. Features include a space-saving design (W450 × D501 × H244 mm) and ergonomic hardware. Operation is performed by a color touch panel, and the user interface (UI) makes it possible to understand the system condition and use method at a glance.

Lambda-DNA, a type of double stranded DNA that is widely used in analyses of nucleic acids, and five standard samples with different concentrations were prepared. A sample of unknown concentration was also prepared by ethanol precipitation. TrayCell has two types of caps, enabling use with an optical light path of 1.0 mm or 0.2 mm. In this study, the cap for the 1.0 mm light path was used, and 4 µL of the sample was dripped and measured under the conditions in Table 1. The calibration curve in Fig. 2 showed a slope of 0.0021 Conc. Abs, and the second power of the coefficient of correlation was 0.9999. The concentration of the unknown sample was 373 ng/µl when diluted 3 times and 1020 ng/µl when measured in the undiluted state. Fig. 3 shows the samples used in measurement of the calibration curve and their spectra. Table 2 shows the results of 10 repeated measurements of the 440 ng/µl sample. The coefficient of correlation and CV value indicate that even micro samples can be measured accurately by using TrayCell.

Fig. 1 Appearance of UV-1900

Table 1 Measurement Conditions

Instrument	: UV-1900
Wavelength (Calibration curve)	: 260 nm, 320 nm
Wavelength range	: 220 to 330 nm
Scan speed	: Low
Sampling pitch	: 1.0 nm

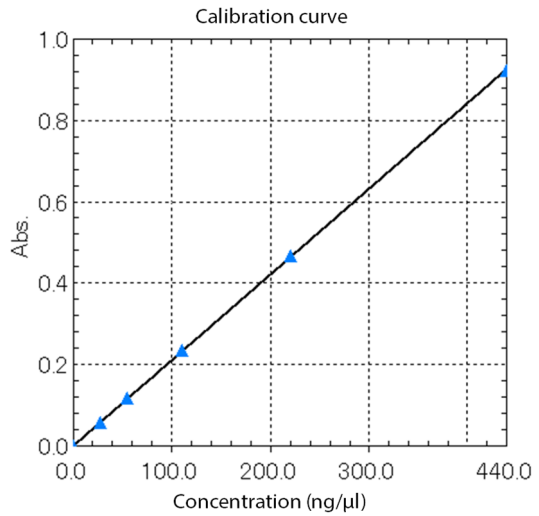

Fig. 2 Calibration Curve of Lambda-DNA Using TrayCell

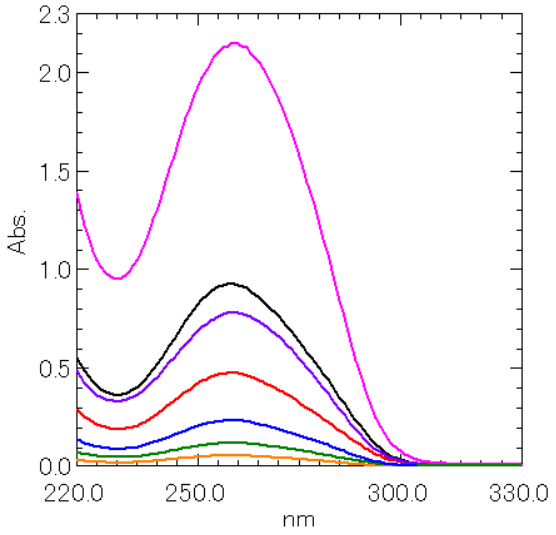

Fig. 3 Absorption Spectra of Lambda-DNA Using TrayCell
Pink: Unknown Sample, Violet: Unknown Sample Diluted 3x, Black: 440 ng/µl, Red: 220 ng/µl, Blue: 110 ng/µl, Green: 55 ng/µl, Orange: 27.5 ng/µl

Table 2 Results of Repeated Measurements Using TrayCell

No.	Absorbance (260 nm)	Absorbance (260 to 320 nm)
1	0.933	0.932
2	0.931	0.929
3	0.935	0.931
4	0.935	0.929
5	0.934	0.934
6	0.935	0.933
7	0.933	0.930
8	0.936	0.933
9	0.926	0.927
10	0.941	0.939
Average	0.934	0.932
Standard deviation	0.0038	0.0034
CV value (%)	0.41	0.36

Quantitation of Nucleic Acid Using Nano Stick

Next, the Lambda-DNA measured with TrayCell was measured using Nano Stick under the same conditions as in Table 1. The optical light path of Nano Stick is 0.5 mm, and a 3 µL sample was measured. The calibration curve in Fig. 4 had a slope of 0.0010 Conc. Abs, and the second power of the coefficient of correlation was 0.9999. The concentration of the unknown sample was 365 ng/µl when diluted 3 times and 1066 ng/µl when measured without dilution. Fig. 5 shows the samples used in measurement of the calibration curve and their spectra. Table 3 shows the results of 10 repeated measurements of the 440 ng/µl sample. The coefficient of correlation and CV value indicate that even micro samples can be measured accurately by using Nano Stick.

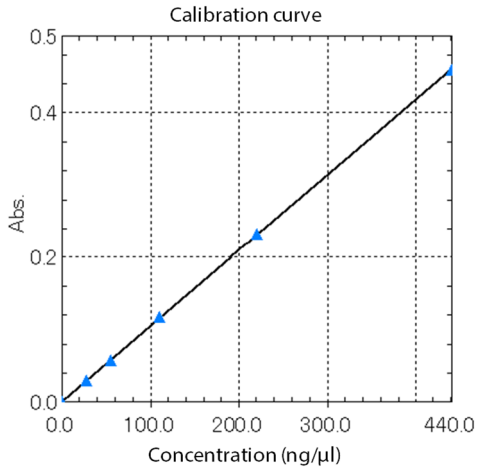

Fig. 4 Calibration Curve of Lambda-DNA Using Nano Stick

Table 3 Results of Repeated Measurements Using Nano Stick

No.	Absorbance (260 nm)	Absorbance (260 to 320 nm)
1	0.467	0.461
2	0.472	0.457
3	0.471	0.464
4	0.465	0.458
5	0.468	0.458
6	0.471	0.459
7	0.471	0.459
8	0.469	0.459
9	0.470	0.459
10	0.468	0.460
Average	0.469	0.459
Standard deviation	0.0022	0.0020
CV value (%)	0.47	0.43

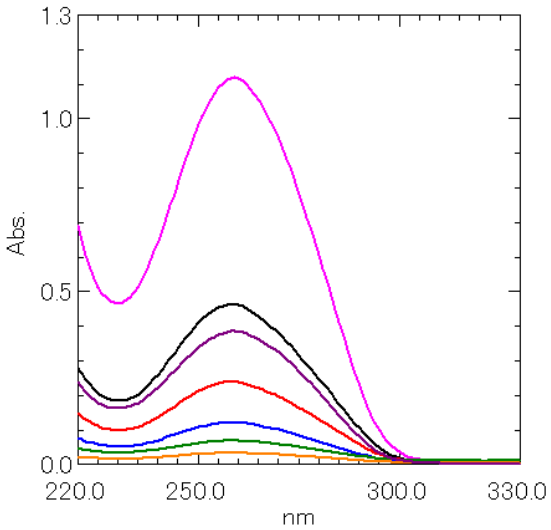

Fig. 5 Absorption Spectra of Lambda-DNA Using Nano Stick Pink: Unknown Sample, Violet: Unknown Sample Diluted 3x, Black: 440 ng/µl, Red: 220 ng/µl, Blue: 110 ng/µl, Green: 55 ng/µl, Orange: 27.5 ng/µl

Biomethod Mode of UV-1900

The Biomethod Mode of UV-1900 includes six measurement methods, 1. Nucleic Acid Quantitation, 2. Lowry Method, 3. BCA Method, 4. CBB Method (Bradford Method), 5. Biuret Method, and 6. UV Method, and enables simple quantitation corresponding to the purpose. The UV-1900 also has an operation panel screenshot function. Fig. 6 shows a quantitation results screen when using the Nucleic Acid Quantitation method. The absorbance ratio used in purity confirmations, the concentrations of DNA and proteins, and other measurement items can be calculated and displayed.

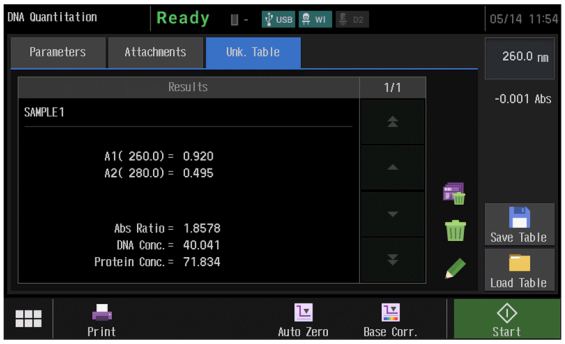

Fig. 6 Screen Showing Results of Nucleic Acid Measurement

Conclusion

The UV-1900 UV-VIS spectrophotometer and TrayCell or Nano Stick enable simple, accurate quantitation of trace level samples of several µL. Simple confirmation of the absorbance ratio, concentrations of DNA and protein concentration, and other measurement items is also possible by using the Biomethod Mode of the UV-1900.

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