

# 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  $\mu\text{L}$ . The Biomethod Mode of the UV-1900 is also introduced briefly.

K. Sobue

#### ■ 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  $\mu\text{L}$  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/ $\mu\text{L}$  when diluted 3 times and 1020 ng/ $\mu\text{L}$  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/ $\mu\text{L}$  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

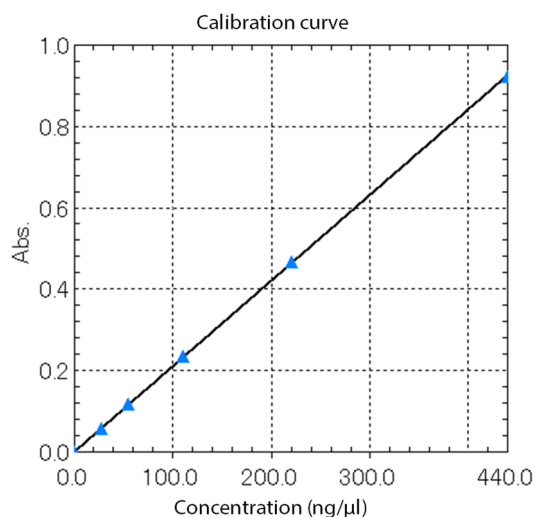


Fig. 2 Calibration Curve of Lambda-DNA Using TrayCell

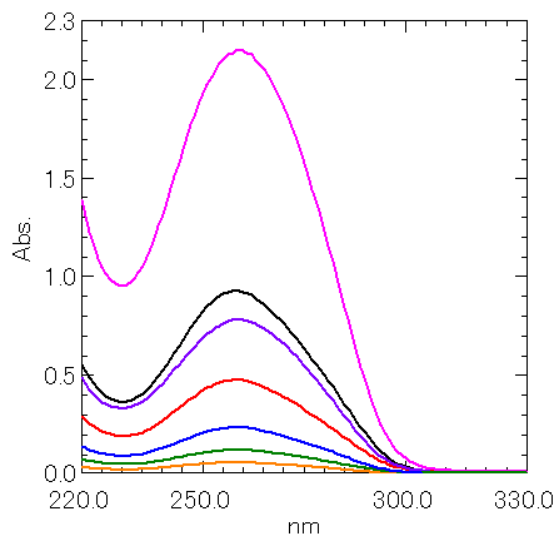


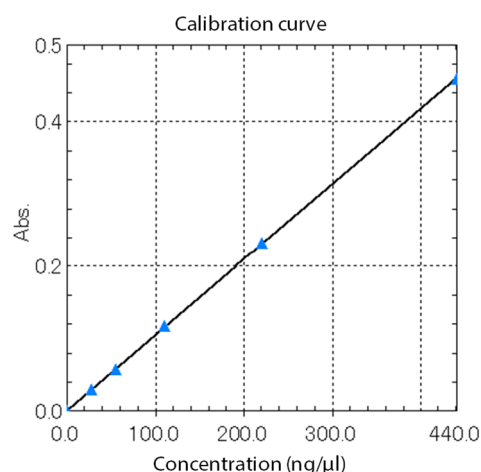
Fig. 3 Absorption Spectra of Lambda-DNA Using TrayCell  
Pink: Unknown Sample, Violet: Unknown Sample Diluted 3x,  
Black: 440 ng/ $\mu\text{L}$ , Red: 220 ng/ $\mu\text{L}$ , Blue: 110 ng/ $\mu\text{L}$ ,  
Green: 55 ng/ $\mu\text{L}$ , Orange: 27.5 ng/ $\mu\text{L}$

**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

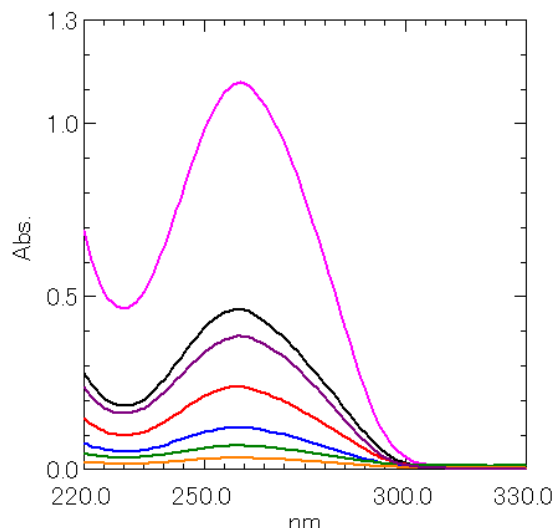
Next, the Lambda-DNA measured with TrayCell was measured using Nano Stick under the same conditions as in Table 1. The optical light path of Nano Stick is 0.5 mm, and a 3 µL sample was measured. The calibration curve in Fig. 4 had a slope of 0.0010 Conc. Abs, and the second power of the coefficient of 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**

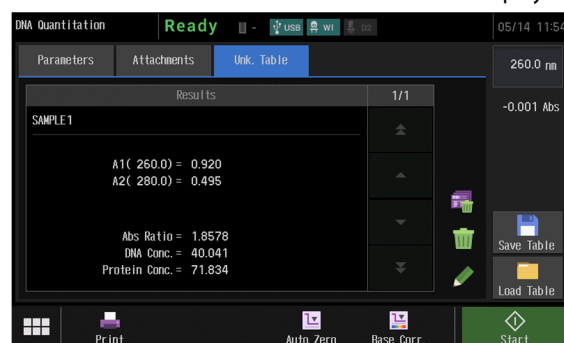
No.	Absorbance (260 nm)	Absorbance (260 to 320 nm)
1	0.467	0.461
2	0.472	0.457
3	0.471	0.464
4	0.465	0.458
5	0.468	0.458
6	0.471	0.459
7	0.471	0.459
8	0.469	0.459
9	0.470	0.459
10	0.468	0.460
Average	0.469	0.459
Standard deviation	0.0022	0.0020
CV value (%)	0.47	0.43



**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.

TrayCell is a trademark of Hellma GmbH & Co. KG. Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".

First Edition: May 2018



Shimadzu Corporation

www.shimadzu.com/an/

**For Research Use Only. Not for use in diagnostic procedures.**

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. Shimadzu disclaims any proprietary interest in trademarks and trade names used in this publication other than its own. See <http://www.shimadzu.com/about/trademarks/index.html> for details.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.