

# Application News

## No. A481

### Spectrophotometric Analysis

## Quantitation of DNA Using Nano Stick

### ■ Introduction

An ultraviolet-visible spectrophotometer can be used for quantitation and purity confirmation of nucleic acids and proteins. As such substances are typically available only in very small quantities, a cell that can accommodate trace level samples is required for measurement. Up to now, an ultra-micro black cell has been used for measurement of 25  $\mu$ L sample volumes. Here, we introduce an example of trace-level measurement of nucleic acids using a SCINCO Nano Stick microliter cuvette, which permits measurement of 2  $\mu$ L samples.

### ■ SCINCO Nano Stick

The Nano Stick pathlength is 0.5 mm according to the standard specifications, and requires a sample volume of at least 2  $\mu$ L. The reference is pipetted into the cell and measured first, and then the same operation is conducted for the sample. Measurement is easily conducted after placing the upper cover on top and setting the Nano Stick in the sample compartment of the spectrophotometer.

### ■ Biomethod Mode of UV-1800

The UV-1800 (see Fig. 2) supports the six biomethod modes shown in Fig. 3. The measurement method can be selected from among 1. DNA Quantitation, 2. Lowry Method, 3. BCA Method, 4. CBB Method, 5. Biuret Method, and 6. UV Method.

The [1. DNA Quantitation] measurement screen is shown in Fig. 4. After entering the necessary items shown in Fig. 4 and pressing the [START] button, the absorbance values are read for the wavelengths set for  $\lambda_1$  and  $\lambda_2$ , and the absorbance ratio, DNA concentration and protein concentration are instantly displayed.



Fig. 1 Photograph of Nano Sticks and Sample Pipetting



Fig. 2 UV-1800

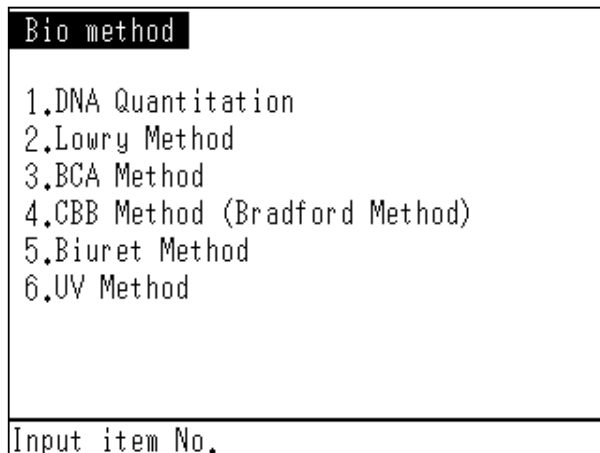


Fig. 3 Biomethod Selection Screen

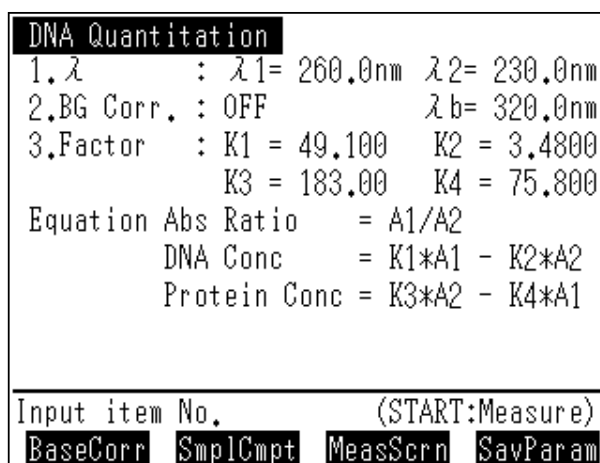


Fig. 4 DNA Quantitation Screen

■ Analyses of DNA Using Nano Stick

Using the Nano Stick and the analytical conditions shown in Table 1, we conducted analysis of Lambda-DNA, a kind of double-stranded DNA which is often used for nucleic acid analysis. Table 2 shows the photometric absorption values at 260 nm obtained for ten replicate measurements of a 0.500 absorbance standard. The table also shows the calculated standard deviation and % CV. The very low standard deviation of  $7.08 \times 10^{-3}$  along with the small CV (1.27 %) demonstrate the excellent accuracy and repeatability of this measurement system for DNA analysis.

In addition, Fig. 5 shows the overlaid spectra obtained for different concentrations of nucleic acid calibration standards ranging from 25 to 1000 ng/μL. Fig. 6 is the calibration curve constructed from these nucleic acid standards. The calibration curve exhibited a slope of 1.19 mAbs/(ng/μL) and coefficient of determination of 0.9997 demonstrating excellent resolution and linear response through the large 1000 ng/μL calibration range.

Table 1 Analytical Conditions

|                   |   |
|-------------------|---|
| Instrument        | : Shimadzu UV-1800 UV-VIS spectrophotometer |
| Wavelength Range  | : 220 nm - 350 nm                           |
| Scan Speed        | : Medium                                    |
| Sampling Pitch    | : 1.0 nm                                    |
| Photometric Value | : Absorbance                                |
| Slit Width        | : 1 nm (fixed)                              |

Table 2 Absorbance of DNA Measured Ten Times at 260 nm and the Standard Deviation

| Absorbance Values at 260 nm |          |
|-----------------------------|----------|
| 1                           | 0.545    |
| 2                           | 0.552    |
| 3                           | 0.558    |
| 4                           | 0.557    |
| 5                           | 0.556    |
| 6                           | 0.560    |
| 7                           | 0.564    |
| 8                           | 0.566    |
| 9                           | 0.565    |
| 10                          | 0.568    |
| Average                     | 0.559    |
| Standard Deviation          | 0.007078 |
| CV Value                    | 1.27 %   |

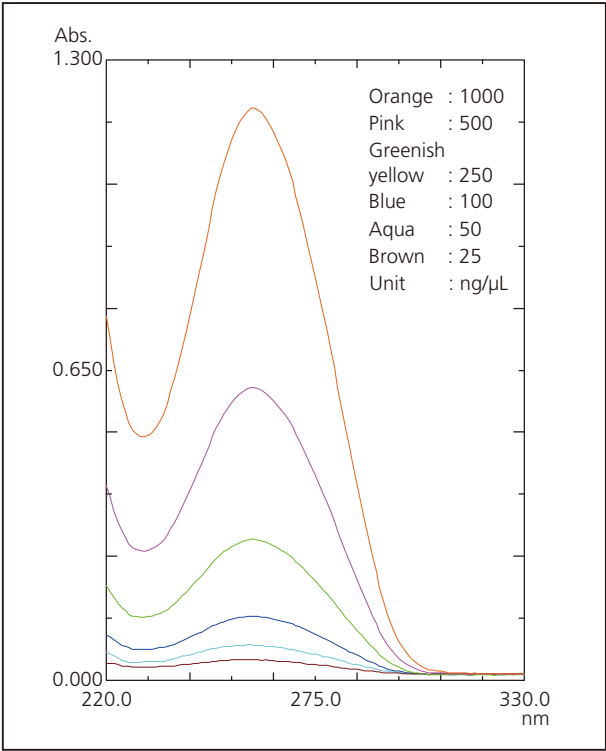


Fig. 5 Spectra of Different Concentrations of DNA  
Brown: 25 ng/μL, Aqua: 50 ng/μL, Blue: 100 ng/μL  
Greenish yellow: 250 ng/μL, Pink: 500 ng/μL Orange: 1000 ng/μL

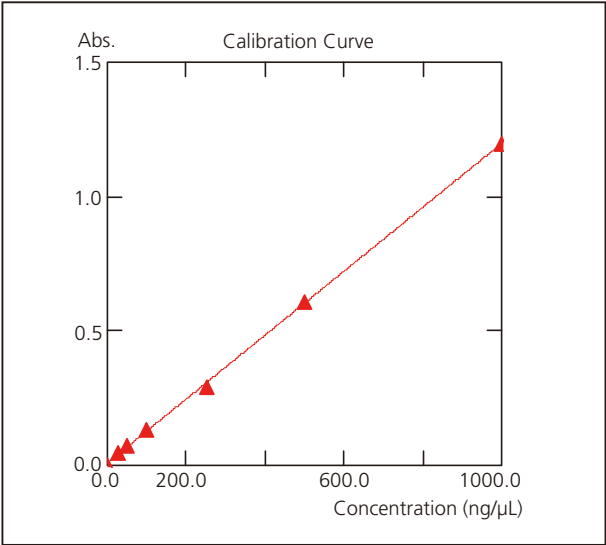


Fig. 6 Calibration Curve of DNA

■ Conclusions

It was found that the combination of a Shimadzu UV-1800 UV-VIS spectrophotometer and Nano Stick sampling accessory is a valuable and accurate method of DNA quantitation for any laboratory. The small micro-volume capacity of the Nano Stick combined with the sensitivity and stability of the UV-1800 provides for highly reliable and accurate repeat DNA measurements with solid quantitative results.