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Application News

No. AD-0097

UV-1280, Nano Stick, Nucleic Acid

Micro Volume Quantitation Measurement of Nucleic Acid using Nano Stick with UV-1280

❑ Introduction

An ultraviolet-visible (UV-VIS) spectrophotometer can be used for quantitation of nucleic acids and proteins and such samples are typically available in small micro volume (less than 5 µl). A conventional 10 mm path length super micro cuvette requires at least 50 µl of sample and needs extra care in cleaning to prevent carryover. Hence a system that is able to measure micro volume with high reproducibility will be preferred. SINCO Nano Stick microliter cuvette is designed for micro volume measurements of nucleic acids and protein with a UV-VIS Spectrophotometer.

Figure 2: UV-1280

❑ SINCO Nano Stick

The dimensions of the SINCO Nano Stick is equivalent to a standard 10 mm path length cuvette which allows it to fit into the cuvette holder of the spectrophotometer. The sample volume required is at least 2 µl. It has a pathlength of 0.5 mm which generates a virtual dilution factor of 1:20 in comparison to a measurement with a standard 10 mm cuvette. A bubble checker is included to ensure that there is no dust or bubbles in the sample port, thus minimizing time for re-measurement.

Figure 1: SINCO Nano Stick

❑ Material and Methods

- Shimadzu UV-1280
- 1 mg/ml calf thymus DNA standard from SIGMA ALDRICH
- SINCO Nano Stick
- TE buffer solution (pH 8.0) from Nacalai Tesque

The DNA stock solution was serially diluted with TE buffer solution to provide samples with DNA concentration ranging from 25 ng/µl to 1000 ng/µl.

[Spectrum] mode of UV-1280 was used to measure the DNA samples and the analytical conditions is shown in Table 1. A concentration of 500 ng/µl sample was measured ten times at 260 nm using [DNA Quantitation] mode of UV-1280.

Table 1 Analytical Conditions

Instrument	: UV-1280 UV-VIS Spectrophotometer
Wavelength Range	: 220 nm – 350 nm
Scan Speed	: Medium
Sampling Pitch	: 1.0 nm
Photometric Value	: Absorbance
Slit Width	: 5 nm (fixed)

Here, an amount of 3 µl of sample was used for measurement. The TE buffer solution was pipetted onto the cell window. The upper cover was placed on top, and the Nano Stick was set in the sample compartment of the spectrophotometer as shown in Figure 3. Baseline correction was performed to zero the absorbance reading from the reference blank and the cell. The same procedure was conducted for the sample. The sample was measured by pressing the [START] button on UV-1280.

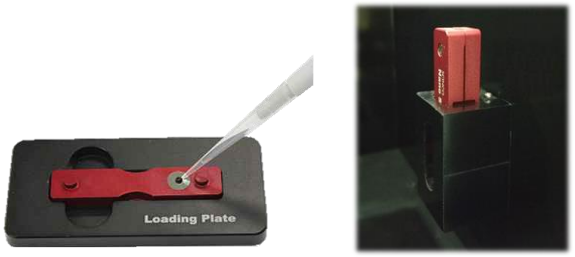

Figure 3: Sample preparation

❑ Results and Discussion

DNA has an absorbance peak at 260 nm and a valley at 230 nm. An absorbance value close to baseline was observed at 320 nm. Figure 4 showed the overlapped absorbance spectrum of the DNA standards ranging from 25 to 1000 ng/µl. Figure 5 is the calibration curve generated from these nucleic acid standards. A correlation coefficient r^2 of 0.9995 demonstrate the wide linearity range through 1000 ng/µl.

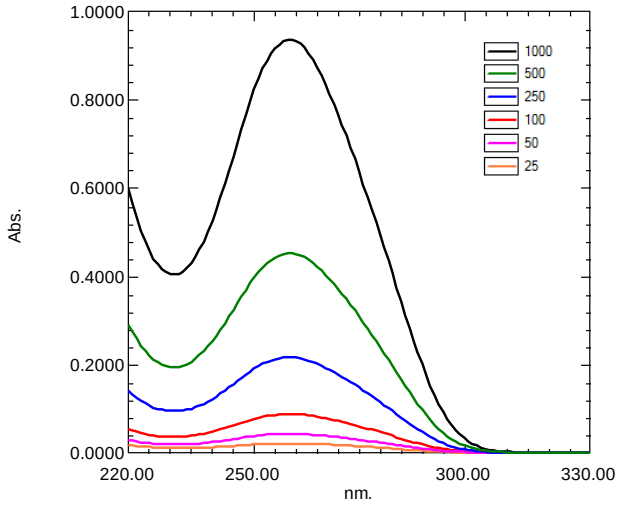

Figure 4: Absorbance spectra of DNA at different concentrations

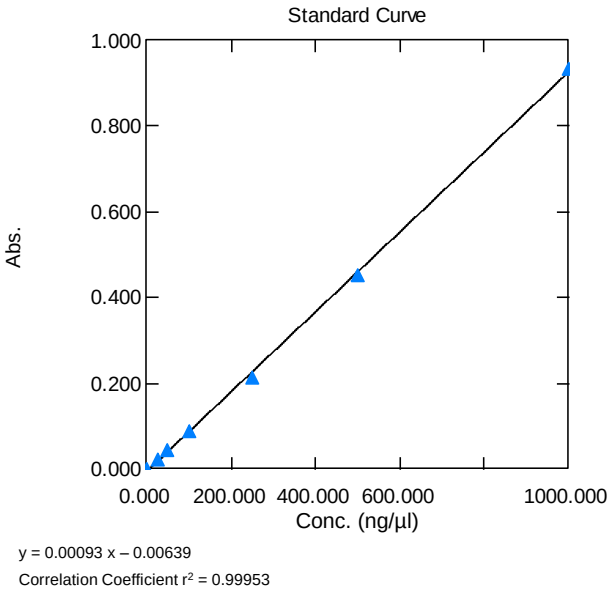

Figure 5: Calibration curve of DNA standards

Table 2. Absorbance of 500 ng/µl measured ten times at 260 nm

	Absorbance at 260 nm
1	0.461
2	0.459
3	0.450
4	0.447
5	0.443
6	0.445
7	0.448
8	0.450
9	0.456
10	0.449
Average	0.451
SD	0.006
RSD (%)	1.32

Table 2 shows the absorbance values, standard deviation (SD), relative standard deviation (RSD) for ten replicate measurements at 260 nm. A low SD of 0.006 and good RSD of 1.32 % were obtained.

❑ Conclusions

The SINCO Nano Stick together with UV-1280 Spectrophotometer provides a system that enables easy measurement of micro volume of nucleic acids with good repeatability.

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