SHIMADZU

Application News

Spectrophotometric Analysis No.A420

Quantitation of Nucleic Acid Using BioSpec-nano

The BioSpec-nano has 2 optical pathlengths (0.2 mm, 0.7 mm), allowing quantitation of nucleic acid with sample volumes of 1 μL and 2 μL, respectively. In addition, if the optional cell (sample volume: 2 mL) having a 5 mm pathlength is used, measurement can be conducted using the cell (cuvette).
Here we conducted measurement of dsDNA at various concentrations at each optical pathlength, and we report the results on the linearity of photometric values (OD) obtained from nucleic acid quantitation, as well as the measurement repeatability as CV (%), and accuracy of the photometric values (OD). In addition, we also investigated the effect of the automatic wiping performance conducted during nucleic acid quantitation.

■ Analytical Conditions

The sample consisted of purified dsDNA dissolved in Tris-EDTA (TE) buffer solution, and the individual samples were prepared in the concentration ranges listed at right for each pathlength. Next, 10 successive measurements were conducted using each of the pathlengths and concentrations using the BioSpec-nano, and the OD (Optical Density,absorbance corresponding to 10 mm pathlength) at 260 nm was determined. The Y-axis values (Measured OD260) in Fig. 1, 2, and 3 correspond to BioSpec-nano measurement values.
The standard value (Corrected OD260, X-axis in each figure) for determining the accuracy was obtained using the Shimadzu UV-2450 Ultraviolet-Visible spectrophotometer, an appropriately diluted sample and a 1 mm pathlength cell. The linearities of Fig. 1, 2 and 3 indicate the linearity of the standard values, and the deviation from each of the straight lines correspond to OD error.

1) Pathlength 0.7 mm
   Sample concentration: 0.3-21 OD (15-1000 ng/μL dsDNA)
   Sample volume : 2 μL
2) Pathlength 0.2 mm
   Sample concentration: 1-75 OD (50-3700 ng/μL dsDNA)
   Sample volume : 1 μL
3) Pathlength cell
   Sample concentration: 0.04-3 OD (2-150 ng/μL dsDNA)
   Sample volume : 2 mL

■ Analysis Results with 0.7 mm Pathlength

0.999 was obtained for the coefficient of correlation of the measured value (Measured OD260) with respect to the standard value (Corrected OD260).
When the OD value was greater than 1.4 (70 ng/μL dsDNA), the measurement repeatability as CV (%) was less than 1.4 %, and the OD error (%) was from -8.6 % to 4.4 %. The data are shown in Fig. 1.

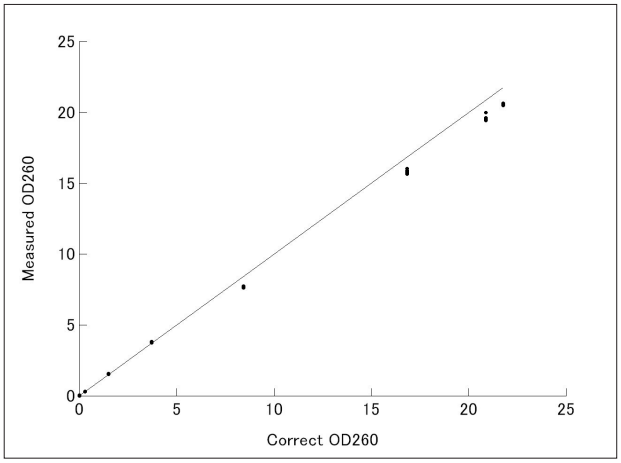

Fig. 1 Analysis Results with 0.7 mm Pathlength

■ Analysis Results with 0.2 mm Pathlength

0.999 was obtained for the coefficient of correlation of the measured value (Measured OD260) with respect to the standard value (Corrected OD260).
When the OD value was greater than 5 (250 ng/μL dsDNA), the measurement repeatability as CV (%) was less than 1.4 %, and the OD error (%) was from -5.4 % to 2.8 %. The data are shown in Fig. 2.

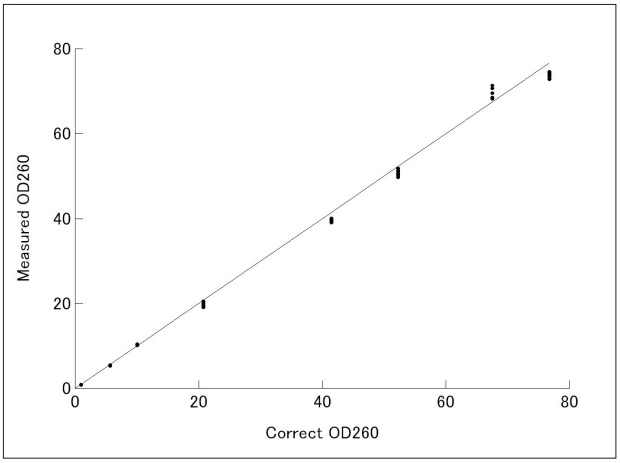

Fig. 2 Analysis Results with 0.2 mm Pathlength

■ Analysis Results with Optional 5 mm Pathlength Cell

0.999 was obtained for the coefficient of correlation of the measured value (Measured OD260) with respect to the standard value (Corrected OD260).
When the OD value was greater than 0.2 (10 ng/µL dsDNA), the measurement repeatability as CV (%) was less than 0.6 %, and the OD error (%) was from -1.6 % to 3.6 %. The data are shown in Fig. 3.

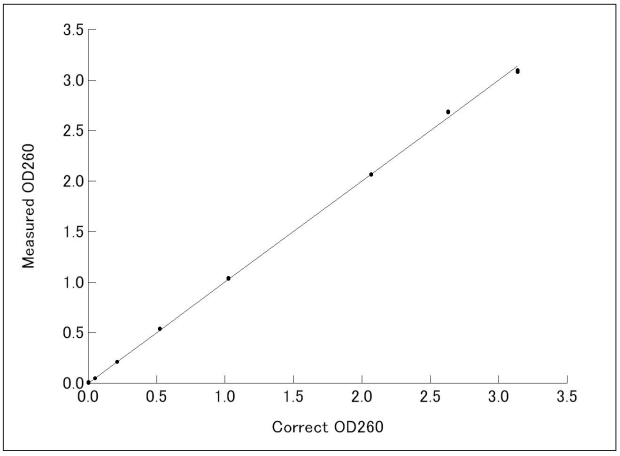

Fig. 3 Analysis Results with Optional 5 mm Pathlength Cell

■ Performance of Automatic Wiping in Nucleic Acid Quantitation

We alternated measurement of purified dsDNA (11.7 OD, 578 ng/µL) and TE buffer solution using a 0.7 mm pathlength, 3 µL sample volume, and 1 wipe operation between measurements.
Carryover (%) of dsDNA to the TE buffer solution was used as an index of the automatic wiping performance. The measurement cycle is shown below.

Round 1

dsDNA measurement, wipe once
TE buffer solution measurement, wipe once

Round 2

dsDNA measurement, wipe once
TE buffer solution measurement, wipe once
Etc.

Thus, automatic wiping is performed twice in one round. Carryover (%) of each round was obtained using the following expression.

$$\text{Carryover (\%)} = 100 \times \frac{[\text{(Nucleic acid concentration in TE measurement)}]}{[\text{(Nucleic acid concentration in dsDNA measurement)}]}$$

As shown in Fig. 4, carryover (%) was held below 0.3 % over 60 rounds (120 wiping operations), demonstrating that carryover is suppressed to extremely low levels when automatic wiping is performed.

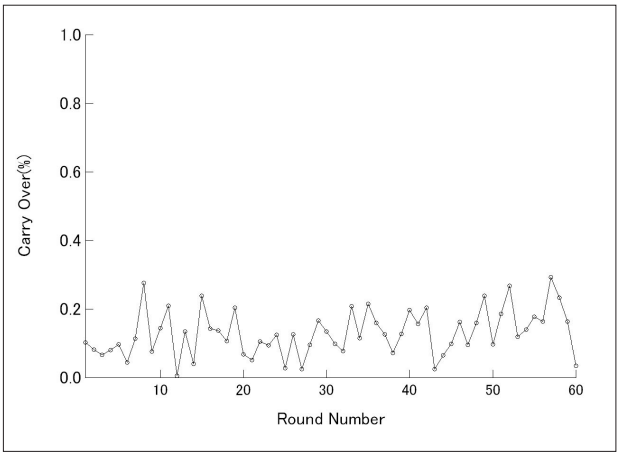

Fig. 4 Performance of Automatic Wiping in Nucleic Acid Quantitation

■ Summary

When conducting nucleic acid quantitation with sample volumes in the order of µL using a cell (cuvette)-free type of spectrophotometer, the formation state of the sample droplet will have great effects on the analysis results. With the BioSpec-nano, a well-formed droplet is stably obtained with the adoption of an automatic mounting mechanism which automatically forms the pathlength.
We conducted nucleic acid quantitation verification using 1 µL and 2 µL samples of purified dsDNA with pathlengths of 0.2 mm and 0.7 mm, respectively, and obtained excellent results for photometric linearity, measurement repeatability, and accuracy of photometric values.
The automatic wiping mechanism is an epoch-making feature that saves the operator the trouble of performing repetitive, time-consuming cleaning of liquid-contact parts. In verifying the performance of automatic wiping using a purified dsDNA sample, data indicating less than 0.3 % carryover was obtained over 120 continuous wiping operations.

SHIMADZU

SHIMADZU CORPORATION. International Marketing Division
3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan Phone: 81(3)3219-5641 Fax. 81(3)3219-5710
Cable Add.:SHIMADZU TOKYO

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