

Analysis Performance of Protein Measurement Using the NanoQ Microvolume Spectrophotometer System

Technical Note
#T230002082

Abstract

This technical note demonstrates the protein analysis performance of the microvolume spectrophotometers in the NanoQ series (NanoQ Plus, NanoQ) for bio-related protein experiments.

This assesses the accuracy, linearity, and precision of measurements in the BSA measurement mode of the Protein UV tab on the NanoQ system. The performance equivalence was demonstrated based on comparative data with the microvolume spectrophotometers in the NanoQ series and NanoDrop One.

Introduction

In bio-related experiments, protein concentration determination is frequently conducted, and protein concentration is a crucial variable. Previously, methods involving the reaction of proteins with reagents like Bradford analysis, Lowry analysis, etc., were used to quantify concentrations by correlating absorbance with concentration after colorimetric reactions. However, these methods posed challenges such as requiring significant effort and sample quantities.

With the development of ultra-trace spectrophotometer technology, it was confirmed that proteins exhibit distinctive absorbance proportional to their concentration at 280 nm. Utilizing the BSA standard factor value of 1.5, absorbance at 280 nm can be calculated, allowing for the quantification of protein samples.

This technical note demonstrates the capability of the NanoQ series to measure the concentration of proteins and nucleic acids using low sample volumes, such as 1 ~ 2 μ L. It proves the accuracy, linearity, and precision of the microvolume spectrophotometers in the NanoQ series for trace analysis of proteins, focusing on protein measurements.

This technical note compares the performance of the NanoQ series microvolume spectrophotometers with the NanoDrop One product. It presents accurate measurement results to demonstrate the analytical performance of the NanoQ series.

Sample and Method

The ***COA concentration** of the sample was 204 mg/mL. Using this as a reference, the sample was diluted by half 8 times to prepare 9 protein samples with varying concentrations. Consequently, the expected concentrations of the prepared samples are as follows.

Initial sample: 204 mg/mL
First dilution: $204 \text{ mg/mL} \times 1/2 = 102 \text{ mg/mL}$
Second dilution: $102 \text{ mg/mL} \times 1/2 = 51 \text{ mg/mL}$
Third dilution: $51 \text{ mg/mL} \times 1/2 = 25.5 \text{ mg/mL}$
...
Eighth dilution: $1.59375 \text{ mg/mL} \times 1/2 = 0.796875 \text{ mg/mL}$

Following the dilution process described above, samples with protein concentrations of 204, 102, 51, 25.5, 12.8, 6.4, 3.2, 1.6, and 0.8 mg/mL were prepared. To minimize pipetting errors, measurements were performed using the same pipette, dispensing 2 μL of each sample, repeated five times. Additionally, to ensure homogeneity, vortexing was completed for all samples before the measurements.

In this experiment, a sample of Sigma's **Protein Standard-analytical standard (Cat: P5369, Lot: SLCQ0935)** at a concentration of 200 mg/mL was used.

***COA concentration** stands for Certificate of Analysis (COA), which represents information obtained from a Certificate of Analysis. It is a document provided by manufacturers or suppliers to confirm the precise concentration of compounds or substances used in the laboratory experiments.

[Table 1] Precision and Accuracy of Protein Concentration Measurements

Expected(mg/mL)	NanoQ			NanoDrop		
	Measured(mg/mL)	%CV	%Error	Measured(mg/mL)	%CV	%Error
204.0	187.2	0.9	8.2	178.6	0.5	12.5
102.0	92.5	0.3	9.3	86.3	0.7	15.4
51.0	47.1	0.6	7.6	48.8	0.5	4.3
25.5	22.4	0.5	12.3	23.8	0.7	6.8
12.8	11.4	0.4	10.3	11.3	0.6	11.3
6.4	5.5	0.5	13.9	5.4	1.4	15.5
3.2	2.7	0.1	14.2	2.6	0.8	17.5
1.6	1.4	0.7	15.3	1.3	1.6	19.4
0.8	0.7	1.0	17.1	0.6	0.6	23.4

In this table, the **NanoQ Plus** product from K LAB Co., Ltd. and **NanoDrop One** product from Thermo Fisher Scientific, Inc. were used.

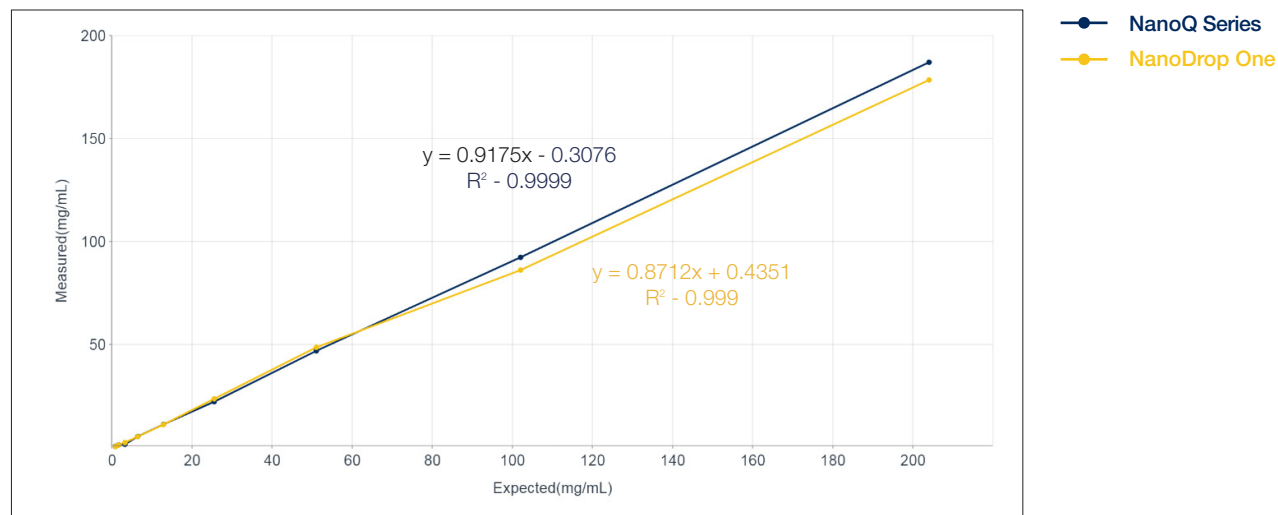
Precision and Accuracy Results

The precision and accuracy results can be precisely examined in **[Table 1]**. Linear range specifications indicate the operating range of the equipment, while precision and accuracy signify the reliability of the equipment.

%CV is an indicator used to evaluate the precision of the equipment, calculated by dividing the standard deviation from measurements repeated five times by the mean concentration and expressing it as a percentage. A lower %CV indicates better precision. In the NanoQ series, within the protein concentration range of approximately 0.8 to 200 mg/mL, %CV values are less than 1%, demonstrating excellent precision. This value is comparable to that of NanoDrop One.

%Error is a metric used to assess equipment accuracy, calculated as the absolute difference between expected and measured concentrations divided by the expected concentration, displayed as a percentage. Low %Error values indicate high accuracy. In the protein concentration range of approximately 0.8 to 200 mg/mL, NanoQ series %Error values closely match those of NanoDrop One, with exceptions at 25.5 mg/mL and 51 mg/mL, where NanoQ series %Error is lower. Both devices show %Error increase at low concentrations, especially below 6.4 mg/mL.

[Figure 1] Linearity of Protein Concentration Measurements



Linearity Results

The linearity between the expected protein concentrations (mg/mL) of the prepared protein samples and the measured concentrations (mg/mL) using the NanoQ series can be accurately verified in [Figure 1]. In the protein concentration range of approximately 0.8 to 200 mg/mL, the NanoQ series exhibits a correlation coefficient (R^2) of 0.9999, indicating a close relationship between the expected and measured concentrations of the samples. This confirms a strong linear correlation. When compared to the R^2 value of 0.999 for NanoDrop One, the NanoQ series demonstrates its linearity effectively.

Conclusion

The analysis of the accuracy, linearity, and precision of the microvolume spectrophotometer NanoQ series demonstrates the usability of NanoQ Plus in protein concentration quantification experiments within various biological experiments. These outstanding linearity and accuracy metrics serve as crucial indicators not only for protein concentration quantification experiments but also for a wide range of potential applications. The comparison data were based on performance equivalence between the microvolume spectrophotometer NanoQ series in BSA mode and NanoDrop One, aiming to establish the performance similarity.

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