

NanoDrop Lite Plus Performance Data: Nucleic Acid and Protein A280

Introduction

Quantifying nucleic acids and proteins with the Thermo Scientific™ NanoDrop™ Lite Plus Microvolume UV Spectrophotometer is quick and simple. In five seconds or less, concentration, absorbance, and purity ratios are reported, reducing the time spent for quality and quantity identification prior to downstream applications. With automatically adjusting pathlengths, samples measured on the NanoDrop Lite Plus instrument do not require dilutions, eliminating common errors due to pipetting.

The NanoDrop Lite Plus Spectrophotometer is a standalone instrument that includes preprogrammed applications for dsDNA, ssDNA, RNA, and a variety of protein sample types. The concentration detection limits for dsDNA are 2.0 ng/μL – 1,500 ng/μL and 0.06 mg/mL – 45 mg/mL for bovine serum albumin (BSA), using as little as 1 to 2 μL of sample. This technical note outlines the performance of the NanoDrop Lite Plus Spectrophotometer for measuring a wide range of dsDNA and BSA concentrations across the instrument's dynamic range (0.04A – 30A).

Method

To test the NanoDrop Lite Plus Spectrophotometer performance for measuring dsDNA, calf thymus DNA (Invitrogen™, 15633019) was diluted in Tris-EDTA buffer pH 7.6 (Fisher BioReagents, BP2474500) to yield a range of concentrations from 2.0 ng/μL – 1,500 ng/μL. To test protein

performance, BSA (Sigma-Aldrich®, A7284) was diluted in phosphate buffered saline (PBS) solution (Fisher BioReagents, BP24384) to yield a concentration range of 0.06 mg/mL – 45 mg/mL. The concentrations for dsDNA and BSA were confirmed with a NanoDrop One Spectrophotometer as a reference using the dsDNA and Protein A280 application, respectively.

Using a modified Beer's Law equation to calculate the concentration of dsDNA, the NanoDrop Lite Plus Spectrophotometer measures absorbance at 260 nm and uses the mass extinction coefficient of 50 ng/μL cm⁻¹ for dsDNA. Applying Beer's Law to calculate BSA concentration, the NanoDrop Lite Plus instrument measures absorbance at 280 nm and uses the E1% of 6.7 L/gm-cm for a 1% (10 mg/mL) solution of BSA.

Results

The average concentration values from the NanoDrop One Spectrophotometer and the NanoDrop Lite Plus Spectrophotometer (Tables 1 and 2) were plotted (Figures 2 and 3) and analyzed. The R² results for both dsDNA (R² = 0.9999) and BSA (R² = 0.9999) demonstrate the strong measurement correlation between the two spectrophotometers.

Published dsDNA reproducibility specifications for the NanoDrop Lite Plus Spectrophotometer are a standard deviation of ±2 ng/μL for sample concentrations between 2.0 and 100 ng/μL, and ±2% CV for samples >100 ng/μL. BSA reproducibility specifications for the NanoDrop Lite Plus Spectrophotometer are a standard deviation of ±0.10 mg/mL for sample concentrations between 0.10 – 10 mg/mL, and ±2% CV for samples >10 mg/mL. The standard deviation and %CV data provided in Tables 1 and 2 indicate the NanoDrop Lite Plus Spectrophotometer performs well within the published reproducibility specifications.

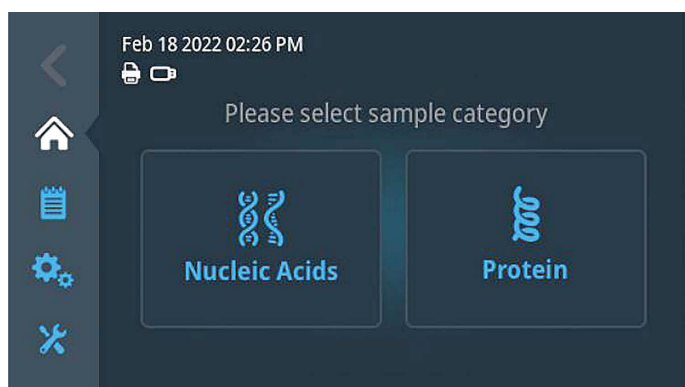


Figure 1: NanoDrop Lite Plus Spectrophotometer home screen.

NanoDrop One Spectrophotometer			NanoDrop Lite Plus Spectrophotometer		
dsDNA Concentration (ng/μL)	Standard Deviation	% CV	dsDNA Concentration (ng/μL)	Standard Deviation	% CV
1.8	0.29	N/A	1.6	0.52	N/A
7.6	0.24	N/A	7.6	0.45	N/A
247.3	0.52	0.21	246.7	1.74	0.71
701.9	3.72	0.53	692.1	3.58	0.52
1160.0	8.07	0.70	1148.9	3.43	0.30
1402.6	5.42	0.39	1398.3	7.31	0.52

Table 1: Average concentration, standard deviation, and coefficient of variation (%CV) for various dsDNA concentrations. N/A indicates not applicable for dilute samples. Ten replicates of each sample were measured on the NanoDrop One Spectrophotometer and the NanoDrop Lite Plus Spectrophotometer.

NanoDrop One Spectrophotometer			NanoDrop Lite Plus Spectrophotometer		
BSA Concentration (mg/mL)	Standard Deviation	% CV	BSA Concentration (mg/mL)	Standard Deviation	% CV
0.05	0.01	N/A	0.06	0.01	N/A
0.15	0.01	N/A	0.15	0.01	N/A
12.20	0.16	1.35	12.27	0.07	0.58
22.17	0.27	1.22	22.26	0.26	1.17
46.85	0.71	1.53	46.83	0.55	1.17

Table 2: Average concentration, standard deviation, and coefficient of variation (%CV) for various BSA concentrations. N/A indicates not applicable for dilute samples. Ten replicates of each sample were measured on the NanoDrop One Spectrophotometer and the NanoDrop Lite Plus Spectrophotometer.

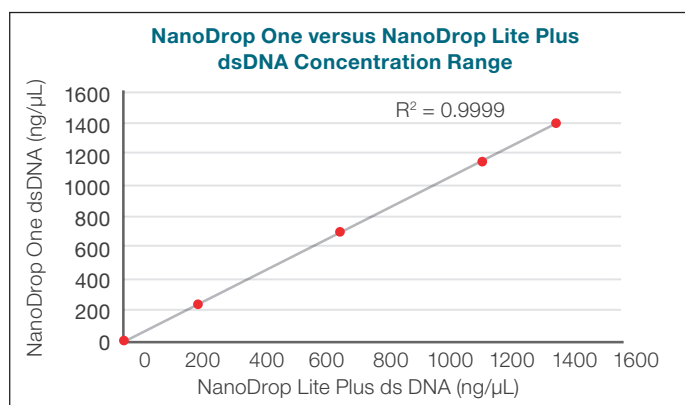


Figure 2: A linearity comparison between the NanoDrop One Spectrophotometer and the NanoDrop Lite Plus Spectrophotometer for dsDNA across the full concentration range. $R^2 = 0.9999$, indicating strong correlation between the two instruments.

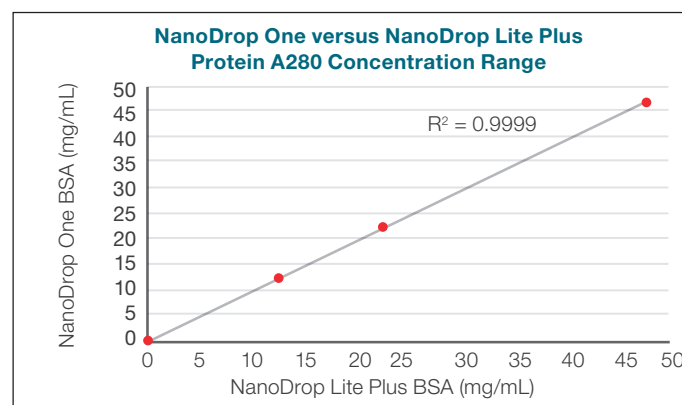


Figure 3: A linearity comparison between the NanoDrop One Spectrophotometer and the NanoDrop Lite Plus Spectrophotometer for BSA across the full concentration range. $R^2 = 0.9999$, indicating strong correlation between the two instruments.

Conclusion

The NanoDrop Lite Plus Spectrophotometer is a simple and convenient tool for measuring the concentration, absorbance, and purity ratios of purified nucleic acids and proteins with a high degree of accuracy and reproducibility. When compared with the NanoDrop One Spectrophotometer, the concentrations measured on the NanoDrop Lite Plus Spectrophotometer show excellent correlation and accuracy with $R^2 = 0.9999$ for BSA and dsDNA measurements. The small, 1 – 2 μL sample size required for pedestal measurements allows for the conservation of precious sample volume required for downstream applications, and the 5-second measurement time provides seamless integration into life science labs.

Liste des appareils

Nous contacter