TECHNICAL NOTE

Thermo Scientific

NanoDrop Eight Spectrophotometer protein performance data

Abstract

The Thermo Scientific™ NanoDrop™ Eight 8-channel Microvolume UV-Vis Spectrophotometer has several preprogrammed protein applications that allow users to quantify their protein samples. This technical note illustrates the performance of the NanoDrop Eight Spectrophotometer when measuring bovine serum albumin (BSA) dilutions with the Protein A280 application compared to the same application with the Thermo Scientific™ NanoDrop™ 8000 Spectrophotometer.

Introduction

The NanoDrop Eight Spectrophotometer accurately measures a wide concentration range for protein samples using a 1 to 2 µL sample size. The auto-ranging pathlength technology on the NanoDrop Eight instrument pedestals allows life scientists to measure samples in an expanded concentration range, eliminating the need for error-prone dilutions. The 8-channel pedestal system on the NanoDrop Eight Spectrophotometer allows for simple implementation in high throughput workflows and includes a quick measurement time of about 15 seconds. The accuracy was evaluated by comparing several protein dilutions measured on the NanoDrop Eight Spectrophotometer with the NanoDrop 8000 Spectrophotometer as a reference. Reproducibility for each protein dilution was calculated from concentration data obtained by the NanoDrop Eight instrument.

NanoDrop Eight 8-channel Microvolume UV-Vis Spectrophotometer

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Method

A dilution series of BSA (Sigma Aldrich, A7284) ranging from 100 mg/ml to 0.195 mg/ml was prepared with phosphate buffered saline (PBS) as the diluent, totaling ten samples. Each sample was measured using the Protein A280 application on the NanoDrop 8000 instrument in five replicates and ten replicates on the NanoDrop Eight instrument in 2 µL sample aliquots per pedestal. With PBS as the reference, the protein concentration was automatically calculated from absorbance at 280 nm by the NanoDrop Eight Software and the NanoDrop 8000 Software using a modified Beer's Law equation with an E1% of 6.7.

The average concentration and the coefficient of variance (%CV) were calculated for the NanoDrop Eight and NanoDrop 8000 instruments as shown in Table 1 and concentration accuracy was plotted in Figure 1.

Reproducibility data for the NanoDrop Eight instrument is shown in Table 2.
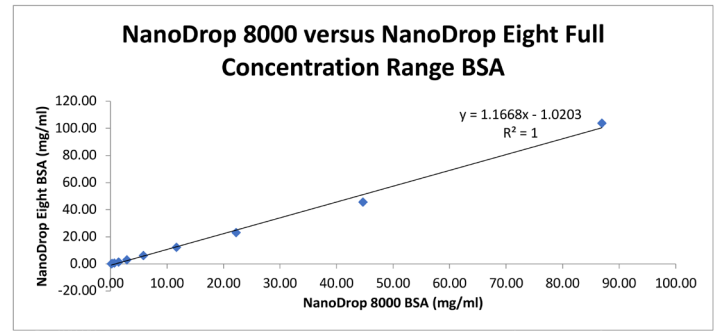

Figure 1: A linearity comparison between the NanoDrop Eight instrument versus the NanoDrop 8000 instrument across the entire concentration range for the sample dilutions. The regression line ($R^2 = 1$) demonstrates that the NanoDrop Eight BSA concentration results were well aligned with the values obtained on the NanoDrop 8000 Spectrophotometer.

NanoDrop 8000 Spectrophotometer			NanoDrop Eight Spectrophotometer		
Target [Conc] (mg/ml)	[Conc] BSA (mg/ml)	%CV	[Conc] BSA (mg/ml)	Standard Deviation	%CV
100	86.94	0.88	103.72	1.39	1.34
50	44.69	1.62	45.67	0.49	1.08
25	22.24	1.83	22.99	0.35	1.54
12.5	11.69	0.68	12.15	0.18	1.48
6.25	5.84	0.58	6.01	0.07	1.16
3.125	2.92	0.97	3.04	0.09	2.99
1.56	1.44	1.75	1.47	0.04	2.83
.78	0.70	2.94	0.70	0.02	2.31
.39	0.33	7.25	0.33	0.02	4.86
.195	0.15	13.15	0.16	0.01	9.24

Table 1: Average concentration values from ten serial dilutions of BSA that were measured on a NanoDrop 8000 and a NanoDrop Eight Spectrophotometer. Ten replicates were measured per sample on the NanoDrop Eight instrument and five replicates were measured per sample on the NanoDrop 8000 instrument.

Sample	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml	1.56 mg/ml	.78 mg/ml	.39 mg/ml	.195 mg/ml
Replicate 1	103.24	45.46	22.97	12.53	6.07	3.08	1.48	0.70	0.33	0.16
Replicate 2	103.35	45.63	22.85	12.08	5.96	3.05	1.48	0.70	0.33	0.15
Replicate 3	102.89	45.56	23.00	12.12	5.98	3.04	1.47	0.70	0.33	0.15
Replicate 4	103.72	45.45	22.99	12.05	5.97	3.02	1.46	0.70	0.33	0.15
Replicate 5	103.77	45.54	22.99	12.15	5.97	3.07	1.47	0.70	0.33	0.16
Replicate 6	104.52	45.62	23.09	12.12	6.06	3.03	1.47	0.70	0.34	0.17
Replicate 7	103.48	45.78	23.12	12.15	6.12	3.04	1.46	0.70	0.33	0.15
Replicate 8	104.61	46.14	22.88	12.10	5.99	3.03	1.46	0.68	0.33	0.16
Replicate 9	103.75	45.90	23.05	12.13	6.04	3.02	1.47	0.69	0.33	0.15
Replicate 10	103.75	45.62	22.98	12.11	5.98	3.02	1.47	0.69	0.33	0.15
Average	103.72	45.67	22.99	12.15	6.01	3.04	1.47	0.70	0.33	0.16
Standard Deviation	1.39	0.49	0.35	0.18	0.07	0.09	0.04	0.02	0.02	0.01
%CV	1.34	1.08	1.54	1.48	1.16	2.99	2.83	2.31	4.86	9.24

Table 2: Measurement reproducibility was assessed on a NanoDrop Eight Spectrophotometer using ten replicates of each BSA dilution. Each replicate was averaged from one 8-channel measurement. The average concentration across all replicates, the standard deviation and %CV were calculated for each dilution.

Conclusion

The NanoDrop Eight Spectrophotometer exhibits a high degree of accuracy and linearity across various protein dilutions. In this study, excellent reproducibility and accuracy were demonstrated when compared to the NanoDrop 8000 reference instrument. The calculated R^2 value for the concentration range tested supports a strong correlation with the concentrations measured on the NanoDrop Eight and NanoDrop 8000 instruments. The protein concentration accuracy and reproducibility of the NanoDrop Eight Spectrophotometer shows its effective nature of measuring proteins quickly and efficiently.

In addition to accurate and reproducible measurements, users can preserve their precious samples and support high-throughput analysis with the 8-channel microvolume pedestal system on the NanoDrop Eight instrument. The quick and simple system allows the user to implement the NanoDrop Eight Spectrophotometer into their workflow with ease, saving time and valuable resources across a wide dynamic measurement range.

Find out more at **thermofisher.com/nanodrop**

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