TECHNICAL NOTE

No. TN53448

Thermo Scientific
NanoDrop Eight Spectrophotometer nucleic acid performance data

Abstract

The Thermo Scientific™ NanoDrop™ Eight 8-channel Microvolume UV-Vis Spectrophotometer has several preprogrammed nucleic acid applications including dsDNA and RNA. This technical note illustrates the performance of the NanoDrop Eight Spectrophotometer when measuring dilutions with the dsDNA application compared to the same application with the Thermo Scientific™ NanoDrop™ 8000 Spectrophotometer.

Introduction

The NanoDrop Eight Spectrophotometer accurately measures a wide concentration range of nucleic acid 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 dsDNA dilutions measured on the NanoDrop Eight Spectrophotometer with the NanoDrop 8000 Spectrophotometer as a reference. Reproducibility for each dilution was calculated from concentration measured on the NanoDrop Eight instrument.

NanoDrop Eight 8-channel Microvolume UV-Vis Spectrophotometer

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Method

A dilution series of calf thymus DNA solution (Invitrogen, 15633019) ranging from 3000 ng/µL to 2.9 ng/µL was prepared with tris-EDTA buffer (TE, pH 7.6) as the diluent, totaling 11 samples. Each sample was measured using the dsDNA 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 TE as the reference, the dsDNA concentration was automatically calculated from absorbance at 260 nm by the NanoDrop Eight Software and the NanoDrop 8000 Software using a modified Beer's Law equation with a mass extinction coefficient of 50 ng/µL cm^{-1}.

The average concentration and 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.

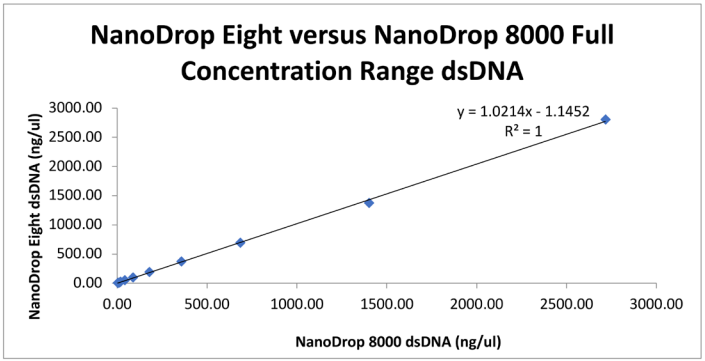

Figure 1: A linearity comparison between the NanoDrop Eight instrument versus the NanoDrop 8000 instrument across the entire concentration range for the sample dilutions. The regression line (R^2 = 1) demonstrates that the NanoDrop Eight dsDNA concentration results were well aligned with the values obtained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

NanoDrop 8000 Spectrophotometer			NanoDrop Eight Spectrophotometer		
Target [Conc] (ng/µL)	[Conc] dsDNA (ng/µL)	%CV	[Conc] dsDNA (ng/µL)	Standard Deviation	%CV
3000	2715.95	1.05	2801.73	51.66	1.84
1500	1400.70	0.68	1372.94	7.65	0.56
750	685.36	3.16	693.59	4.81	0.69
375	356.90	0.97	371.39	1.76	0.47
187.50	179.14	1.83	186.94	1.62	0.86
93.75	87.44	2.97	93.31	1.24	1.32
46.88	41.29	6.18	46.39	0.73	1.58
23.44	18.38	10.42	22.93	0.71	3.09
11.72	10.98	4.47	11.65	0.53	4.53
5.85	5.30	8.00	5.46	0.58	10.59
2.9	2.34	19.14	2.58	0.35	13.60

Table 1: Average concentration values from eleven serial dilutions of dsDNA were measured on a NanoDrop 8000 and a NanoDrop Eight Spectrophotometer. Ten replicates were measured per sample on the NanoDrop Eight instrument and 5 replicates were measured per sample on the NanoDrop 8000 instrument.

Sample	3000 ng/ul	1500 ng/ul	750 ng/ul	375 ng/ul	187.50 ng/ul	93.75 ng/ul	46.88 ng/ul	23.44 ng/ul	11.72 ng/ul	5.85 ng/ul	2.9 ng/ul
Replicate 1	2791.46	1370.44	693.00	370.46	187.78	93.37	46.41	23.02	11.74	5.81	2.63
Replicate 2	2833.28	1370.32	693.05	370.99	186.54	93.18	46.50	22.91	11.56	5.36	2.54
Replicate 3	2787.66	1373.02	694.54	370.96	186.49	92.98	46.33	23.40	11.78	5.35	2.72
Replicate 4	2790.90	1373.15	694.32	370.78	187.54	93.25	46.22	22.59	11.75	5.47	2.49
Replicate 5	2815.06	1372.39	691.84	370.88	187.11	92.97	46.19	22.86	11.83	5.26	2.54
Replicate 6	2801.80	1373.53	691.70	372.34	186.34	93.60	46.57	22.83	11.64	5.37	2.41
Replicate 7	2789.80	1373.50	695.16	371.13	186.54	93.74	46.76	22.84	11.53	5.43	2.40
Replicate 8	2832.49	1374.30	691.85	373.74	187.64	93.59	46.41	23.05	11.47	5.57	2.77
Replicate 9	2787.14	1375.18	694.35	371.40	187.21	93.23	46.19	23.07	11.67	5.27	2.65
Replicate 10	2789.79	1373.51	695.81	371.22	186.26	93.22	46.29	22.73	11.53	5.66	2.56
Average	**2801.73**	**1372.94**	**693.59**	**371.39**	**186.94**	**93.31**	**46.39**	**22.93**	**11.65**	**5.46**	**2.58**
Standard Deviation	**51.66**	**7.65**	**4.81**	**1.76**	**1.62**	**1.24**	**0.73**	**0.71**	**0.53**	**0.58**	**0.35**
%CV	**1.84**	**0.56**	**0.69**	**0.47**	**0.86**	**1.32**	**1.58**	**3.09**	**4.53**	**10.59**	**13.60**

Table 2: Measurement reproducibility was assessed on a NanoDrop Eight Spectrophotometer using 10 replicates of each dsDNA dilution. Each replicate is averaged from one 8-channel measurement. The average concentration across all replicates, the standard deviation and %CV were calculated for each dilution.

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Conclusion

The NanoDrop Eight Spectrophotometer exhibits a high degree of accuracy and linearity across various dsDNA 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 dsDNA concentration accuracy and reproducibility of the NanoDrop Eight Spectrophotometer show its effective nature of measuring nucleic acids 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.

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