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TECHNICAL NOTE

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Nucleic acid measurements at 260 nm

NanoDrop One performance data

Authors

Sean Loughrey, Jessica Mannion, and Brian Matlock
Thermo Fisher Scientific, Wilmington, DE

Abstract

Life scientists can quantify nucleic acid samples on the Thermo Scientific™ NanoDrop™ One and One^{C} Microvolume UV-Vis Spectrophotometers using the preprogrammed applications for dsDNA, ssDNA, RNA, oligo DNA and oligo RNA (Figure 1). This technical note illustrates NanoDrop One Spectrophotometer performance across the instrument's dynamic range using dsDNA.

Figure 1: NanoDrop One software, nucleic acids Home screen

Introduction

The NanoDrop One Spectrophotometer is capable of accurately measuring samples ranging in concentration from 2 to 27,500 ng/µL dsDNA (0.04–550A) using as little as 1 to 2 µL of sample. The patented* sample-retention system and auto-ranging pathlength technology of the NanoDrop One/One^{C} instruments allow users to measure samples spanning a wide concentration range, thus eliminating the need for dilutions or consumables.

With the NanoDrop One instrument, no prior knowledge of sample concentration is needed to ensure measurement accuracy because sample concentration is essentially always within the dynamic range of the instrument. The NanoDrop One instrument was evaluated for accuracy across this dynamic range by comparing nucleic acid sample results to those obtained using the cuvette-based, benchtop Thermo Scientific™ Evolution™ 300 UV-Vis Spectrophotometer. The data presented here demonstrates that the NanoDrop One/One^{C} instruments accurately measure dsDNA samples as dilute as 3 ng/µL and as concentrated as 28,000 ng/µL (and up to 18,150 ng/µL ssDNA and 22,000 ng/µL RNA).

NOTE: the NanoDrop One^{C} instrument contains both a pedestal and a cuvette measuring position. The data presented here was collected on a NanoDrop One instrument and reflects the accuracy of pedestal measurements on both NanoDrop One and NanoDrop One^{C} instruments.

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Method

A series of solutions, ranging from 3 to 28,000 ng/µL, were prepared by diluting dsDNA sodium salt from salmon testes (Sigma Aldrich®, #D1626) in HPLC grade ddH_2O (Acros Organics™, #268300040). The initial concentration of the prepared dsDNA stock was validated spectrophotometrically at 260 nm using the Evolution 300 Spectrophotometer as a reference. Samples that fell outside of the absorbance detection limits of the reference spectrophotometer were diluted to fall within the working range of the reference instrument. The concentration values obtained for the diluted samples on the Evolution 300 instrument were used to manually calculate the original concentration of the dsDNA samples; the appropriate factors were applied to account for dilutions and pathlength differences as needed. The absorbance of each solution was then measured on a NanoDrop One instrument using the dsDNA application program. Sample sizes used to measure concentrations for each instrument were; 1.) 1 µL for the NanoDrop One instrument, 2.) 3 mL in a 10 mm quartz cuvette for the Evolution 300 instrument, and 3.) 1 µL in the Hellma® Analytics TrayCell cuvette-like device for the Evolution 300 instrument.

To calculate the concentration of each sample, the NanoDrop One software uses the measured absorbance at 260 nm, the mass extinction coefficient for dsDNA (50 ng/µL cm^{-1}) and Beer's Law. The average concentration values acquired from both the NanoDrop One and the Evolution 300 (Table 1) Spectrophotometers were plotted (Figures 2 and 3) and analyzed. These results demonstrate the high degree of measurement agreement between the two spectrophotometers.

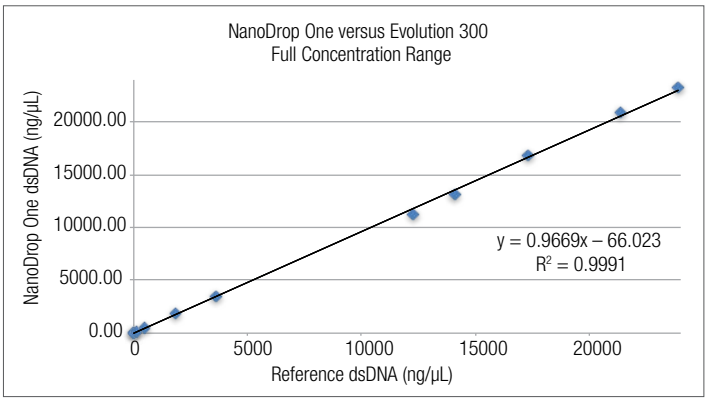

Figure 2: A linearity comparison between the NanoDrop One versus Evolution 300 Spectrophotometers across the entire instrument concentration range was plotted. The regression line demonstrates that the NanoDrop One dsDNA concentration results were well aligned with the values obtained on the Evolution 300 Spectrophotometer.

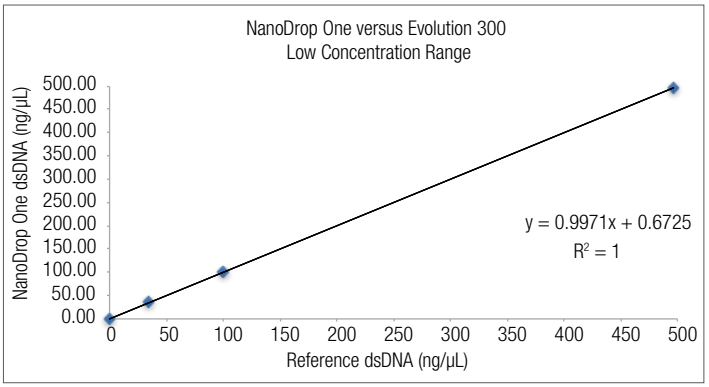

Figure 3: A closer look at the linearity comparison between the NanoDrop One and Evolution 300 Spectrophotometers at the low concentration range was also plotted (from 35 to 495 ng/µL). The regression line shows a close correlation to the Evolution 300 results and superb linearity at the lower end of the detection range.

Evolution 300 Spectrophotometer		NanoDrop One Spectrophotometer		
dsDNA [Conc] (ng/µL)	% CV	dsDNA [Conc] (ng/µL)	Standard Deviation	% CV
3.4	0.43	3.9	0.5	N/A
34.3	0.29	35.8	0.3	N/A
100.1	0.29	100.1	0.6	0.1
496.2	0.23	495.5	0.6	0.0
1844.5	0.33	1815.8	7.3	0.0
3606.4	0.25	3389.0	9.5	0.0
12259.8	0.10	11170.1	70.6	0.1
14095.8	6.81	13108.7	57.8	0.0
17289.4	1.97	16789.2	115.1	0.1
21338.6	3.74	20896.0	122.6	0.1
23887.3	3.24	23217.9	199.0	0.1

Table 1: Average concentration values from various preparations of dsDNA were measured both on a NanoDrop One and an Evolution 300 Spectrophotometer. Ten separate replicates of each solution were measured directly on the pedestal of the NanoDrop One instrument without further dilution. Solutions were diluted as necessary and measured in triplicate on the Evolution 300 Spectrophotometer fitted with a 10 mm quartz cuvette (Starna Cells, Inc., 1-Q-10) and a Hellma Analytics TrayCell micro-cell with a 0.2 mm cap.

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Published nucleic acid reproducibility specifications for the NanoDrop One 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 measured on the pedestal. Table 2 presents reproducibility data from 10 replicates of dsDNA samples ranging in concentration from 3 to 28,000 ng/µL. The standard deviation data makes abundantly clear the superior measurement repeatability of the NanoDrop One Spectrophotometer. Measurement reproducibility exceeded specifications across the sample concentration range (Table 2).

Conclusion

The NanoDrop One Microvolume Spectrophotometer demonstrates excellent linearity across the complete dynamic range of the instrument (Figure 2) and superior linearity in the low concentration range (Figure 3). The calculated R^2 values in both cases show a close correlation between the concentrations obtained from the two spectrophotometers, the reference cuvette-based instrument and the NanoDrop One. The NanoDrop One instrument can produce this high level of reliability due to its patented*, pedestal sample-retention technology and automatic pathlength selection, so critical to managing microvolume measurements.

In addition to a high degree of accuracy across the concentration range tested, the NanoDrop One Spectrophotometer saves valuable time and money with its ease of operation and sample size requirement of only 1–2 µL. The NanoDrop One Spectrophotometer eliminates the need for sample dilutions and costly consumables such as quartz cuvettes. With pre-configured applications specifically designed for life science labs and an integrated high-resolution, touchscreen interface, the NanoDrop One instrument is fast and simple to use while being accurate and reliable.

**Patents US6628382 and US6809826*

Sample	1	2	3	4	5	6	7	8	9	10	11
Replicate 1	4.4	36.1	101.1	495.2	1799.5	3400.6	11005.2	13132.6	17004.4	21042.0	23155.9
Replicate 2	4.6	35.8	100.0	495.8	1820.5	3394.6	11121.2	13022.4	16873.8	21110.6	23159.4
Replicate 3	3.8	35.4	99.5	496.0	1821.6	3386.3	11159.4	12995.4	16817.8	20925.5	23332.2
Replicate 4	3.3	35.9	99.5	494.4	1818.6	3390.5	11169.7	13111.4	16750.4	20706.0	23276.3
Replicate 5	3.5	35.4	100.3	495.5	1812.8	3372.5	11170.5	13123.3	16765.5	20820.3	23519.3
Replicate 6	3.6	36.3	100.1	495.2	1824.9	3378.8	11126.0	13156.9	16781.7	20896.9	23337.1
Replicate 7	4.4	35.5	100.1	496.5	1814.0	3387.6	11200.5	13167.0	16829.4	20895.1	22991.7
Replicate 8	3.5	35.8	100.7	495.4	1810.1	3391.3	11251.0	13070.1	16557.3	20875.5	23439.8
Replicate 9	4.1	35.9	100.0	495.3	1817.6	3376.3	11217.0	13043.1	16703.5	20790.7	22890.0
Replicate 10	4.5	35.6	99.3	495.7	1810.6	3399.2	11231.8	13106.1	16796.8	21019.0	23077.6
Average	4.0	35.8	100.1	495.5	1815.0	3387.8	11165.2	13092.8	16788.0	20908.1	23217.9
Std Dev	0.5	0.3	0.6	0.6	7.3	9.5	70.6	57.8	115.1	122.6	199.0
% CV	N/A	N/A	0.06	0.01	0.04	0.03	0.06	0.04	0.07	0.06	0.09

Table 2: Measurement reproducibility was assessed on a NanoDrop One instrument using 10 separate aliquots of each concentration of dsDNA sodium salt from salmon testes. The average concentration, standard deviation, and % CV were calculated for each preparation. The measurement reproducibility across the dynamic range of the instrument meets and exceeds specification.

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