

Quantitation of dsDNA Using the Micro-Volume BioSpec-nano Spectrophotometer

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The Shimadzu BioSpec-nano is a low-maintenance micro-volume spectrophotometer designed for the modern life science laboratory. It offers superior detection limits, up to 10 times better compared to the competition, making it the perfect instrument for quantitation of DNA, RNA, Protein analysis, and photometric measurements. The “Drop and Click” design combined with easy sample mounting and

automated cleaning offers a rapid 3 second analysis time and a 10 second cycle time between samples. The instrument features 3 different path length settings of 0.2, 0.7, and 5 mm with linear range as shown in **Table 2**. This communication presents data on the detection limits of the BioSpec-nano for analyzing dsDNA.

Path Length	5 mm	0.7 mm	0.2 mm
Detection Limit, ng/μL	0.15	0.40	0.89

Table 1: Detection Limits of BioSpec-nano by Path Length

Path Length	5 mm	0.7 mm	0.2 mm
Linear Dynamic Range, ng/μL	0.63-20	6.58-842.5	13.16-3370

Table 2: Linear Dynamic Range of Shimadzu BioSpec-nano by Path Length

¹ For extended linear range, see the brochure for specifications.



Experimental Conditions

All measurements were carried out using double stranded calf thymus DNA (Sigma, USA, D3664-5X-1MG). A series of dilutions was carried out on the DNA standards providing concentrations in the range of 2 to 3500 ng/μL, and were measured using the 0.2, 0.7, and the optional 5 mm path length cells. Data analysis was carried out using the software provided with the instrument. The formula that was used for determining the concentration was: sample concentration = dilution factor X (OD260-OD320) X

Nucleic acid concentration factor (50), where OD is the Optical Density. The nucleic acid concentration factor is set in accordance with the analyte selected. The automatic wiper feature was used to clean the target surface, thus eliminating the need for time-consuming manual cleaning techniques used by other manufacturers. All measurements were repeated 3 times or more. The detection limit was determined with an 85% confidence limit from the following equation²:

$$\Delta\bar{x}_{lim} > t_{\alpha,\varphi} s_{xb} \sqrt{\frac{1}{n_s} + \frac{1}{n_b}}$$

where $\Delta\bar{x}_{lim}$ is the detection limit, $t_{\alpha,\varphi}$ is the confidence limit based off of the number of degrees of freedom, s_{xb} is the standard deviation of the

blank, n_s and n_b are the number of observations on the sample and blank, respectively.

Results

Figure 1 is a plot of the acquired data using the BioSpec-nano after measuring dsDNA with the 0.7 mm path length setting. As seen from the plot, the

instrument demonstrates a linear dynamic range from 6.58 to 842.50 ng/μL.

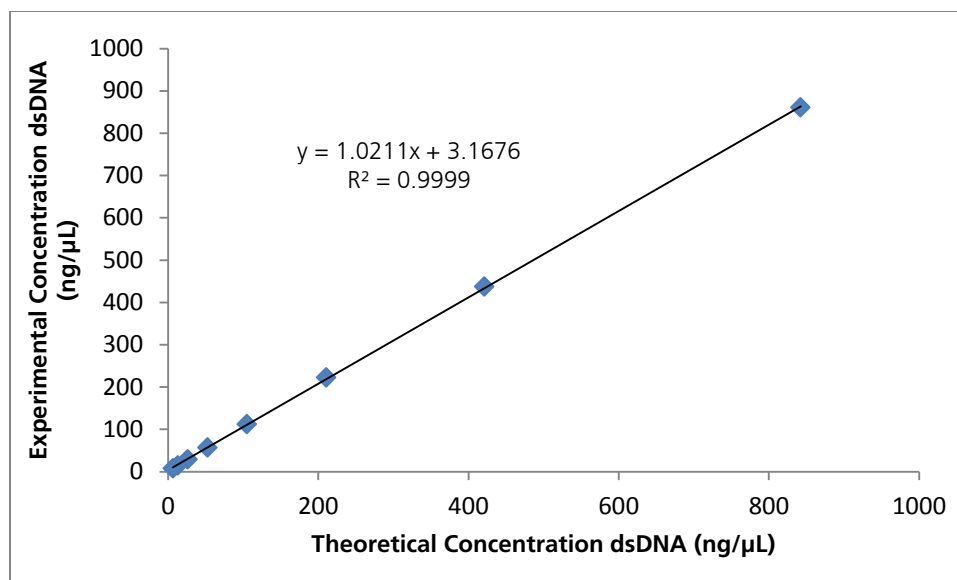


Figure 1: Graph of the experimental concentrations plotted as a function of the theoretical values acquired using the 0.7 mm path length.

Theoretical Concentration	Experimental Concentration				
	Trial 1 ng/μL	Trial 2 ng/μL	Trial 3 ng/μL	Average ng/μL	Standard Deviation ng/μL
842.50	862.38	858.09	861.12	860.53	2.21
421.25	438.78	435.46	436.94	437.06	1.66
210.63	222.92	221.53	221.70	222.05	0.76
105.31	111.68	112.01	113.28	112.32	0.84
52.66	57.29	55.37	56.91	56.52	1.02
26.33	28.07	29.18	28.64	28.63	0.56
13.16	15.48	14.84	13.48	14.6	1.02
6.58	7.52	7.37	7.49	7.46	0.08

Table 3: Average dsDNA concentration values obtained from the BioSpec-nano using a 0.7 mm path length.

Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10	Average	Standard Deviation
0.41	0.55	0.19	1.66	1.6	0.9	0.85	0.32	0.03	0.64	0.72	0.55

Table 4: Blank values recorded by the BioSpec-nano in ng/μL for the 0.7 mm path length.

Summary

The BioSpec-nano demonstrates detection limits of 0.15, 0.40, and 0.89 ng/μL for the 5 mm, 0.7 mm, and 0.2 mm pathlengths, respectively. The adjustable path length capability of the BioSpec-nano allowed for measurement of samples across the full

concentration dynamic range. Concentrations were calculated by the software in the defined concentration range for each respective path length setting.

References

1. BioSpec-nano Instruction Manual, 206-97213C.
2. Peters, et. al. "Chemical Separations and Measurements-Theory and Practice of Analytical Chemistry," pg. 29.



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