NanoDrop Ultra Spectrophotometers and Fluorometers Nucleic Acid Performance Data

Introduction

The Thermo Scientific™ NanoDrop™ Ultra Microvolume UV-Vis Spectrophotometer and Fluorometer is a fundamental analytical instrument in life science laboratories studying nucleic acids. After DNA or RNA is extracted from tissue samples, saliva, plants, or other sources, the NanoDrop Ultra spectrophotometer enables a valuable step in determining nucleic acid purity and concentration ahead of downstream experiments. The auto-ranging pathlength technology implemented in the microvolume pedestal accommodates a broad concentration range to eliminate the need for error-prone and time-consuming dilutions. Table 1 outlines the concentration ranges and typical reproducibility for the dsDNA, ssDNA, and RNA applications using the microvolume pedestal.

Method

Dilutions of calf thymus DNA solution (Invitrogen, 15633019) were prepared using tris-EDTA buffer (TE pH 7.5) ranging from 5.0 ng/µL to 10,000 ng/µL and totaling eight samples. Additionally, green food coloring, which resembles the spectral shape of dsDNA, was prepared to mimic a high concentration of about 25,000 ng/µL and served as the ninth sample.

The dsDNA application was utilized on a NanoDrop Ultra instrument and a Thermo Scientific™ NanoDrop™ One^{C} Spectrophotometer, which served as the reference. Each sample was measured in replicates of ten using 2.0 µL sample sizes on the pedestals on both NanoDrop instruments.

Five samples in the low concentration range of 1.0 ng/µL to 50 ng/µL were also measured to verify reproducibility on the NanoDrop Ultra instrument using a 2.0 µL sample size for five replicate measurements. The Thermo Scientific™ Evolution™ One Plus Spectrophotometer (a cuvette-based instrument) served as the reference. For the Evolution One Plus instrument, a 2.0 mL sample size was used in a 1.0 cm quartz cuvette and three replicate measurements were made.

The concentrations from both dilution series were calculated using the absorbance measured at 260 nm, and the dsDNA factor 50 ng-cm/µL applied to Beer's Law. The average concentrations were determined along with the standard deviation and coefficient of variation (%CV). The measurements made on the NanoDrop Ultra instrument were compared with those measured on the NanoDrop One and Evolution One Plus spectrophotometers.

Application	Lower Detection Limit (ng/µL)	Upper Detection Limit (ng/µL)	Typical Reproducibility*
dsDNA	1.0	27,500	± 1.0 ng/µL for sample concentrations 1.0 - 100 ng/µL ± 2% for samples > 100 ng/µL
ssDNA	0.66	18,150	± 0.66 ng/µL for sample concentrations 0.66 - 100 ng/µL ± 2% for samples > 100 ng/µL
RNA	0.8	22,000	± 0.8 ng/µL for sample concentrations 0.8 - 100 ng/µL ± 2% for samples > 100 ng/µL

Table 1. Pedestal concentration detection ranges for the dsDNA, ssDNA, and RNA applications. Typical reproducibility is defined for the specific range provided for each application. *Unit ng/µL indicates standard deviation; percentage indicates coefficient of variation (%CV).

	NanoDrop One Spectrophotometer			NanoDrop Ultra Spectrophotometer		
Sample Name	**Concentration (ng/µL)**	**Standard Deviation (ng/µL)**	**%CV**	**Concentration (ng/µL)**	**Standard Deviation (ng/µL)**	**%CV**
Sample 1	4.4	0.5	11.62	4.2	0.4	8.60
Sample 2	9.9	0.3	3.05	9.6	0.3	2.73
Sample 3	50.7	0.7	1.46	51.7	0.8	1.48
Sample 4	102.5	0.3	0.25	103.0	0.9	0.91
Sample 5	500	1	0.28	503	1	0.20
Sample 6	1008	4	0.39	991	5	0.51
Sample 7	4830	40	0.80	4760	20	0.36
Sample 8	10030	20	0.20	9780	40	0.36
Sample 9	25370	60	0.24	24500	200	0.75

Table 2. Average concentrations of nine serial dilutions of dsDNA measured on a NanoDrop One spectrophotometer and a NanoDrop Ultra spectrophotometer. Each sample was averaged from replicates of ten and the standard deviation (ng/µL) and %CV are both reported.

Sample	1	2	3	4	5	6	7	8	9
Replicate 1	4.23	9.44	50.5	103.26	503.35	992.96	4736.13	9759.56	24587.9
Replicate 2	4.08	9.65	52.12	100.92	502.98	994.69	4749.87	9783.59	24567.79
Replicate 3	4.37	10.02	51.27	102.46	503.1	988.46	4746.05	9822.47	24678.29
Replicate 4	4.2	9.5	51.62	102.8	500.33	991.65	4729.65	9811.16	24654.37
Replicate 5	3.81	9.35	52.23	102.99	502.65	979.28	4785.64	9794.18	24392.66
Replicate 6	3.82	9.11	51.83	104.21	503.81	989.11	4769.5	9806.24	24721.79
Replicate 7	3.81	9.79	51.46	102.92	502.9	998.07	4767.01	9786.22	24659.08
Replicate 8	4.94	9.49	52.38	102.98	501.47	988.15	4766.19	9698.29	24277.88
Replicate 9	3.88	9.82	52.81	103	502.75	989.7	4756.15	9787.07	24170.11
Replicate 10	4.37	9.48	50.52	101.38	502.19	993.84	4744.43	9763.92	24520.56
Average (ng/µL)	4.2	9.6	51.7	102.7	503	991	4760	9780	24500
Standard Deviation (ng/µL)	0.4	0.3	0.8	0.9	1	5	20	40	200
%CV	8.60	2.73	1.48	0.91	0.20	0.51	0.36	0.36	0.75

Table 3. Concentration results for each replicate measurement displayed in ng/µL for the nine samples measured on the NanoDrop Ultra spectrophotometer. At the bottom of the table, the average concentration, standard deviation, and %CV are listed for each sample.

Results

The average concentrations of the nine prepared samples are outlined in Table 2 along with the standard deviation and %CV. Table 3 contains the concentration for each replicate measurement from the NanoDrop Ultra spectrophotometer. For samples below 100 ng/µL, the standard deviation specification is ± 1.0 ng/µL and all samples were below the specification limit, with the highest deviation being 0.76 ng/µL. For samples above 100 ng/µL the specification limit is 2% CV, where 0.91% was the highest for the NanoDrop Ultra spectrophotometer.

A linearity curve comparing the concentration calculated on the NanoDrop One spectrophotometer versus the concentration calculated on the NanoDrop Ultra spectrophotometer is displayed in Figure 1. The R^2 of the regression line was 1.000, which demonstrates excellent alignment in concentration values reported by both instruments.

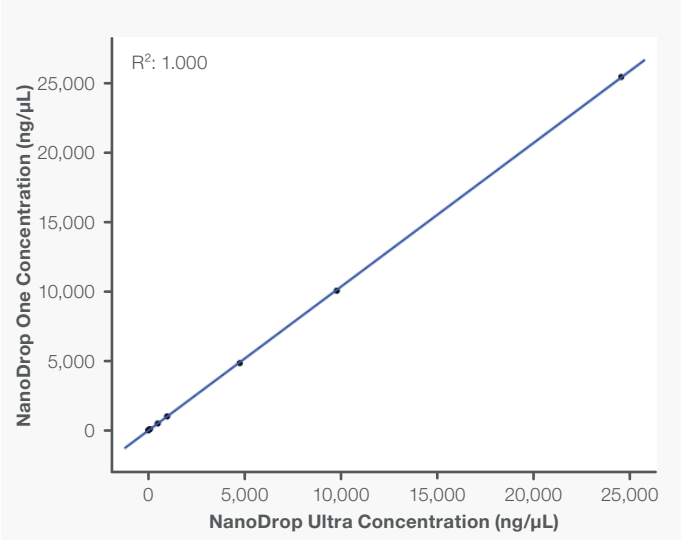

Figure 1. Linearity comparison between the NanoDrop One spectrophotometer versus the NanoDrop Ultra spectrophotometer. The regression line (R^2 = 1.000) indicates strong correlation between sample measurements.

	Evolution One Plus Spectrophotometer		NanoDrop Ultra Spectrophotometer	
Sample Name	**Average Concentration (ng/µL)**	**Standard Deviation (ng/µL)**	**Average Concentration (ng/µL)**	**Standard Deviation (ng/µL)**
Sample 1	0.99	0.01	1.3	0.2
Sample 2	5.09	0.02	5.3	0.4
Sample 3	9.43	0.01	9.0	0.5
Sample 4	24.22	0.00	24.4	0.3
Sample 5	48.46	0.02	48.9	0.6

Table 4. Reproducibility of the lower detection range on the NanoDrop Ultra spectrophotometer. The average concentrations and standard deviations are based on replicates of five for the NanoDrop Ultra instrument and triplicates on the Evolution One Plus instrument.

The low concentration range (1.0 ng/µL to 50 ng/µL) on the NanoDrop Ultra spectrophotometer was compared to the concentrations determined by the cuvette-based Evolution One Plus spectrophotometer. The average concentrations and standard deviations for each of the five samples tested are shown in Table 4. The high reproducibility of the sample measurements was confirmed by the standard deviations remaining below 0.6 ng/µL. In addition, the regression line displayed in Figure 2 exhibits strong linearity at the low end of the NanoDrop Ultra instrument's detection range with an R^2 of 1.000.

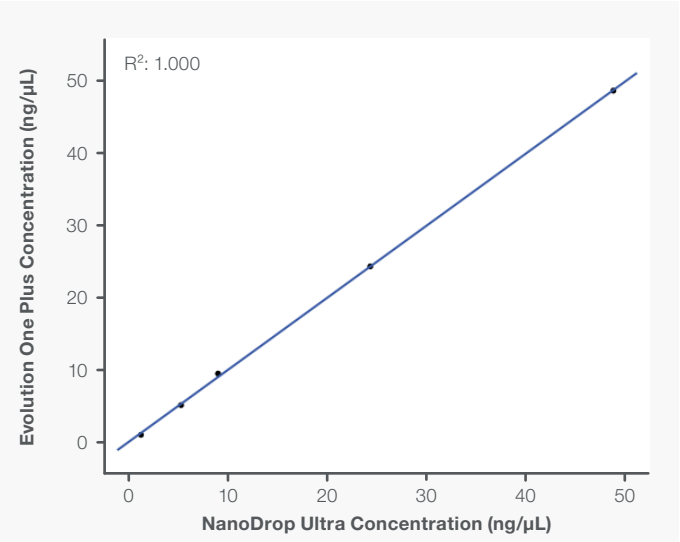

Figure 2. Linearity comparison between the Evolution One Plus spectrophotometer versus the NanoDrop Ultra spectrophotometer for the low concentration range. The strong correlation in concentration values reported by each instrument is confirmed by the regression line (R^2 = 1.000).

Conclusions

Across the entire dsDNA dynamic range, 1.0 ng/µL to 27,500 ng/µL, sample concentrations were reported by the NanoDrop Ultra spectrophotometer with high reproducibility and accuracy. Upon closer examination of the lower detection range, 1.0 ng/µL to 50 ng/µL, the NanoDrop Ultra spectrophotometer has shown excellent reproducibility and linearity when compared to a reference spectrophotometer. For both concentration ranges that were tested, the reproducibility was well within the instrument's specification limits. The average concentrations reported by the NanoDrop Ultra spectrophotometer also correlated well with those from the reference instruments as evidenced by the R^2 of 1.000 for both concentration ranges.

With the microvolume pedestal surface and the auto-ranging pathlength technology, nucleic acid samples can be quantified and qualified using only 1.0 – 2.0 µL sample sizes without the need for error-prone dilutions. The pre-configured software built into the NanoDrop Ultra instrument makes UV-Vis spectrophotometry a simple and quick technique for any life science laboratory.

Learn more at **thermofisher.com/nanodrop**