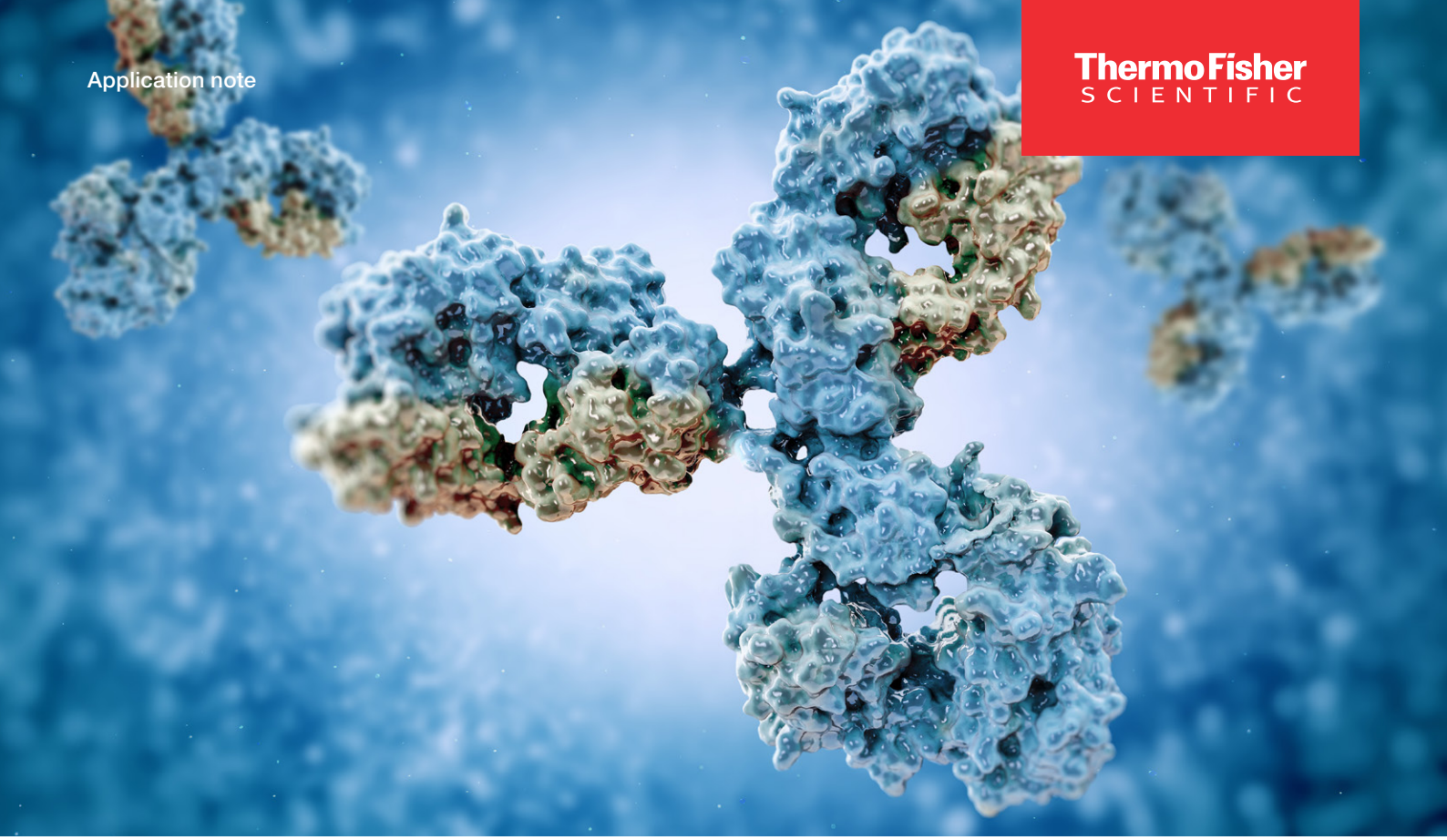
Application note



NanoDrop Ultra Spectrophotometer and Fluorometer Protein A280 Performance Data

Introduction

The Thermo Scientific™ NanoDrop™ Ultra Microvolume UV-Vis Spectrophotometers and Fluorometers provide fast and simple protein analysis. With several pre-programmed protein applications and a microvolume pedestal, proteins can be easily quantified using only 1.0 – 2.0 µL sample volumes, without the need for error-prone dilutions. The typical reproducibility for bovine serum albumin (BSA) is a standard deviation of ± 0.03 mg/mL for concentrations between 0.03 – 10 mg/mL; above 10 mg/mL, the typical reproducibility is ± 2% coefficient of variance (%CV). Proteins can be quantified with a NanoDrop Ultra spectrophotometer accurately and with high reproducibility across a wide measurement range of 0.03 mg/mL to 820 mg/mL (BSA).

Method

The NanoDrop Ultra instrument's protein specifications were evaluated and confirmed against a Thermo Scientific™ NanoDrop™ One Spectrophotometer and a cuvette-based Thermo Scientific™ Evolution™ One Plus Spectrophotometer, which served as reference instruments. A dilution series of BSA (Sigma Aldrich, A7284) was prepared to yield a concentration range of 0.75 mg/mL to 15 mg/mL using phosphate buffered saline (PBS) as the diluent (samples 1-4).

Another dilution series of 70 mg/mL to 300 mg/mL was made using potassium hydrogen phthalate (KHP) (Thermo Scientific Chemicals, 424061000), as the UV spectrum mimics that of purified proteins with a peak at 280 nm (samples 5-7). KHP was used for this concentration range because BSA becomes highly viscous at high concentrations, which makes accurate pipetting difficult. Replicates of ten, 2.0 µL samples were measured on both the NanoDrop Ultra and NanoDrop One spectrophotometers.

A low concentration range of 0.03 mg/mL to 1.5 mg/mL was also tested and compared against the Evolution One Plus reference instrument. Replicates of ten, 2.0 µL samples were measured on the NanoDrop Ultra instrument and triplicates of 2.0 mL were measured on the Evolution One Plus instrument using a 1.0 cm quartz cuvette.

For each dilution series, samples on the NanoDrop instruments were measured using the Protein A280 application and selecting "BSA" as the sample type to apply the correct E1% of 6.7 for use in Beer's Law. For the Evolution One Plus instrument, absorbance was measured using the Fixed application with a 280 nm analysis wavelength, 1.0 nm bandwidth, and 1.0 second integration time. The average, standard deviation, and %CV were then calculated for each sample.

	NanoDrop One Spectrophotometer			NanoDrop Ultra Spectrophotometer		
Sample Name	**Concentration (mg/mL)**	**Standard Deviation (mg/mL)**	**%CV**	**Concentration (mg/mL)**	**Standard Deviation (mg/mL)**	**%CV**
Sample 1	0.74	0.01	1.574	0.75	0.01	1.893
Sample 2	1.48	0.02	1.411	1.52	0.02	1.050
Sample 3	7.42	0.01	0.185	7.49	0.01	0.151
Sample 4	15.13	0.08	0.493	15.23	0.03	0.205
Sample 5	71.6	0.3	0.488	70.4	0.2	0.323
Sample 6	147.8	0.7	0.447	144.9	0.4	0.309
Sample 7	299.8	0.8	0.274	293	1	0.416

Table 1. Average concentrations of serial dilutions of BSA (samples 1-4) and KHP (samples 5-7) measured on the NanoDrop One and the NanoDrop Ultra spectrophotometers. Each sample was averaged from replicates of ten and the standard deviation (mg/mL) and %CV are both reported.

Sample	1	2	3	4	5	6	7
Replicate 1	0.736	1.529	7.479	15.208	70.342	145.366	293.311
Replicate 2	0.753	1.521	7.498	15.210	70.326	144.473	294.268
Replicate 3	0.773	1.522	7.505	15.204	70.444	144.788	292.169
Replicate 4	0.754	1.511	7.483	15.208	70.169	144.702	293.911
Replicate 5	0.766	1.495	7.468	15.262	70.756	145.025	290.744
Replicate 6	0.761	1.498	7.494	15.192	70.433	144.483	291.915
Replicate 7	0.759	1.520	7.491	15.209	70.745	144.592	294.352
Replicate 8	0.725	1.493	7.500	15.277	70.138	145.575	292.672
Replicate 9	0.758	1.541	7.500	15.248	70.353	144.411	293.628
Replicate 10	0.748	1.523	7.491	15.267	70.104	145.516	291.687
Average (mg/mL)	0.75	1.52	7.49	15.23	70.4	144.9	293
Standard Deviation (mg/mL)	0.01	0.02	0.01	0.03	0.2	0.4	1
%CV	1.893	1.050	0.151	0.205	0.323	0.309	0.416

Table 2. Reported concentration for each replicate measurement from the NanoDrop Ultra spectrophotometer. Provided at the bottom of the table are the average, standard deviation, and %CV for each sample.

Results

The average, standard deviation, and %CV for samples 1-7 of the BSA and KHP dilution series are outlined in Table 1. Each sample replicate measured on the NanoDrop Ultra spectrophotometer is shown in Table 2. For samples 1-3, the standard deviations are well below the specification limit of 0.03 mg/mL, where 0.016 mg/mL was the highest amount of deviation. The samples with concentrations greater than 10 mg/mL (samples 4-7) also displayed %CV below the 2% limit, with the greatest being 0.42%. The linearity curve in Figure 1 confirms a strong correlation in reported sample concentrations by the NanoDrop One and the NanoDrop Ultra spectrophotometers, as evidenced by an R^2 of 1.000.

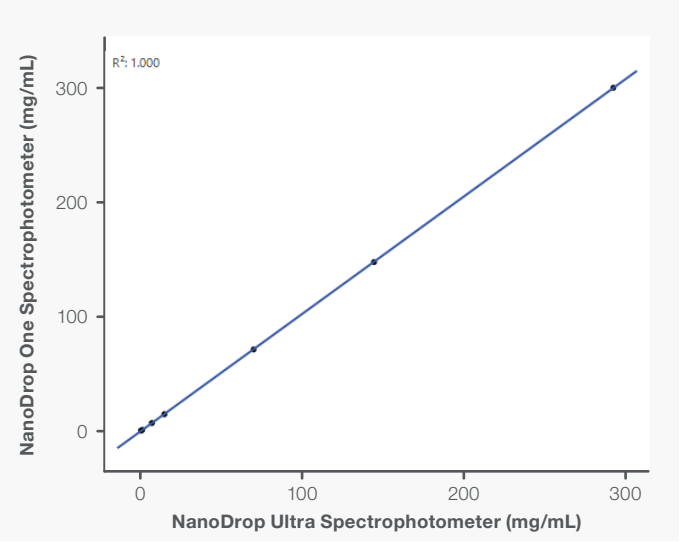


Figure 1. Linearity comparison between the NanoDrop One spectrophotometer versus the NanoDrop Ultra spectrophotometer. The regression line (R^2 = 1.000) indicates sample concentration measurements were well correlated.

	Evolution One Plus Spectrophotometer		NanoDrop Ultra Spectrophotometer	
Sample Name	**Concentration (mg/mL)**	**Standard Deviation (mg/mL)**	**Concentration (mg/mL)**	**Standard Deviation (mg/mL)**
Sample 1	1.565	0.000	1.56	0.01
Sample 2	1.180	0.001	1.1	0.02
Sample 3	0.766	0.000	0.770	0.007
Sample 4	0.143	0.000	0.14	0.01
Sample 5	0.026	0.000	0.031	0.008

Table 3. Average concentrations and standard deviations for each sample in the low concentration range (0.03 mg/mL to 1.5 mg/mL). The samples from the Evolution One Plus spectrophotometer were averaged from triplicates, while those from the NanoDrop Ultra spectrophotometer were averaged from replicates of ten.

The low concentration range (0.03 mg/mL to 1.5 mg/mL) measured on the NanoDrop Ultra instrument was compared to the concentrations measured on the Evolution One Plus instrument. The average and standard deviation for each sample are listed in Table 3. The highest standard deviation from the NanoDrop Ultra instrument was 0.02 mg/mL, which was below the specification limit of 0.03 mg/mL. A linearity curve comparing the two instruments is shown in Figure 2. The R^2 of the regression line (R^2 = 1.000) confirms excellent alignment between the concentrations reported by the Evolution One Plus and NanoDrop Ultra spectrophotometers.

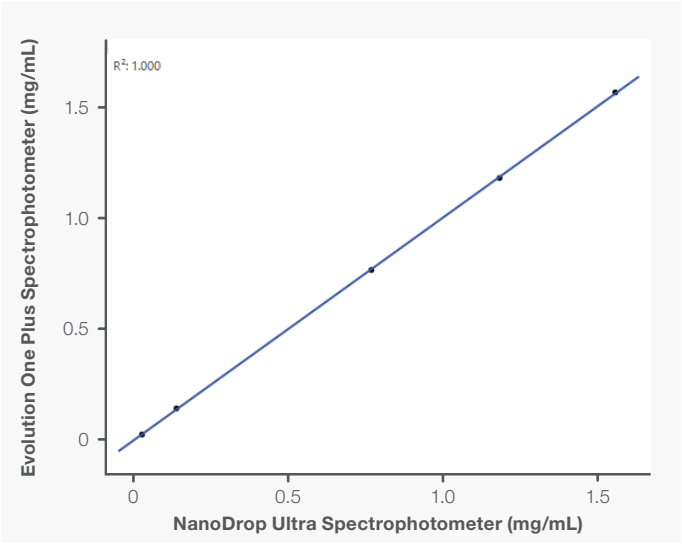


Figure 2. Linearity comparison of the Evolution One Plus and NanoDrop Ultra spectrophotometers. The R^2 value of the regression line (1.000) indicates excellent correlation between reported sample concentrations.

Conclusions

The NanoDrop Ultra spectrophotometer has demonstrated linearity and reproducibility for determining concentrations of purified proteins. When compared to reference spectrophotometers, the results provided by the NanoDrop Ultra instrument were well-aligned as evidenced by R^2 values of 1.000 for both the low- and full-range protein concentrations. The efficiency of quantifying protein samples on a NanoDrop Ultra spectrophotometer is proven by its fast measurement time, pre-programmed applications, and the absence of required dilutions. With a wide dynamic range of 0.03 mg/mL to 820 mg/mL for BSA proteins, dilutions that are typical for cuvette-based spectrophotometers are unnecessary. A NanoDrop Ultra instrument is easily implemented into the protein quantification workflow and will help save time and resources in the laboratory.

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