

Quantify protein and peptide preparations at 205 nm

NanoDrop One Spectrophotometer

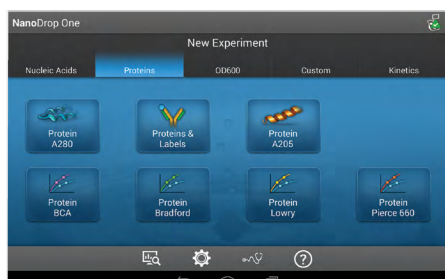


Figure 1. NanoDrop One Proteins Home screen showing available preprogrammed applications for protein quantitation.



Abstract

Life scientists can quantify peptide and protein samples on the Thermo Scientific™ NanoDrop™ One/One^c Microvolume UV-Vis Spectrophotometers using the A205 preprogrammed direct absorbance application. The new A205 application offers a choice of methods for peptides that contain Tryptophan and Tyrosine residues in their sequence as well as peptides that completely lack aromatic amino acids. The A205 application offers enhanced sensitivity for peptide quantification in seconds from only 2 μ L of sample.

Introduction

Researchers have always needed ways to quickly quantify various biomolecules (e.g., protein and nucleic acid preparations) as a routine part of their workflows. This information helps them make informed decisions before proceeding with downstream experiments. There are many protein quantification methods to choose from including gravimetric approaches, colorimetric assays, direct spectrophotometric UV measurements (such as A280), and amino acid analysis. All of these methods have their strengths and weaknesses. Direct spectrophotometric microvolume UV measurements are a popular choice for researchers because they are simple to perform, require no reagents or standards, and consume very little sample. The NanoDrop One Spectrophotometer has preprogrammed applications (Figure 1) for direct quantification of proteins using absorbance measurements at 280 nm and 205 nm. This application note specifically describes how to use the Protein A205 application to quantify protein samples.

A protein's peptide backbone absorbs light in the deep UV region (190 nm-220 nm), and this absorbance can be used for protein sample quantitation. The A205 protein quantitation method has several advantages over the direct A280 protein method such as lower protein-to-protein variability (because A205 extinction coefficients are not based on amino acid composition) and higher sensitivity (because of the high molar absorptivity proteins have at 205 nm). However, technical limitations made it difficult to obtain these measurements in the past. Spectrometers' stray light performance, deep UV linearity, and protein buffers containing UV-absorbing components have all added to the challenge of obtaining A205 data. The NanoDrop One patented sample-rentention technology and low stray light performance have simplified quantification of small amounts of protein by A205 methods.

In this application note, we discuss the three A205 measurement options included in the NanoDrop One Protein A205 application and present performance data for each option.

A205 extinction coefficients for peptide and protein measurements

The NanoDrop One Protein A205 application allows customers to choose from three different options (Figure 2). The selected option will automatically determine the extinction coefficient that will be used to calculate the protein concentration based on the sample absorbance at 205 nm.

- $\epsilon_{205}=31$ method
- Scopes method²
- Other = custom method $\epsilon_{205}^{1\text{mg/mL}}$

Previous studies showed that most protein solutions at 1 mg/mL have extinction coefficients ($\epsilon_{205}^{1\text{mg/mL}}$) ranging from 30 to 35². The ϵ_{205} of 31 mL mg⁻¹cm⁻¹ is an extinction coefficient often used for peptides lacking tryptophan and tyrosine residues¹. The Scopes method gives a more accurate ϵ_{205} , especially for proteins containing a significant amount of tryptophan (Trp) and tyrosine (Tyr) residues. The increased accuracy of this method takes into account the significant absorbance at 205 nm contributed by the aromatic side chains of Trp and Tyr. This method uses an A280/A205 ratio in its equation to correct for Trp and Tyr side-chain absorbance³. Recently, Anthis and Clore proposed the use of a sequence-specific ϵ_{205} calculation (e.g., custom/Other method), which is suitable for a wide range of proteins and peptides¹. This method is appropriate for pure preparations of proteins or peptides whose amino acid sequences are known.

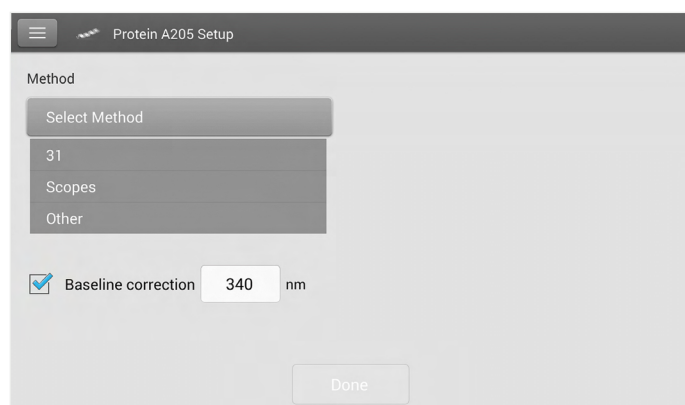


Figure 2. NanoDrop One Protein A205 methods selection screen.

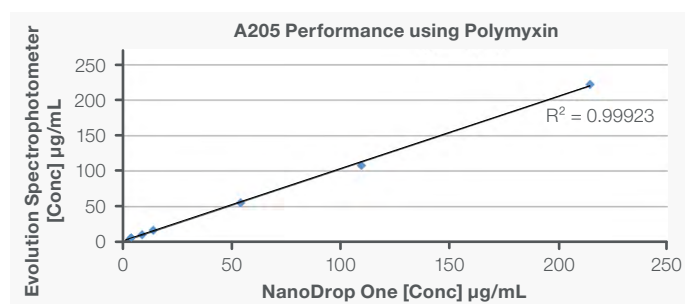


Figure 3. Polymyxin concentrations calculated with the Evolution Spectrophotometer and NanoDrop One instruments were plotted. Regression line shows that protein concentration measurements on the NanoDrop One instrument are in good agreement to those obtained on a traditional high end UV-Vis spectrophotometer.

A205 performance on the NanoDrop One

Preparations of polymyxin, a cationic detergent antibiotic with a peptide backbone, but no Trp or Tyr residues, were made in 0.01% Brij® 35 buffer and measured on the NanoDrop One and the Thermo Scientific™ Evolution™ UV-Vis Spectrophotometers. To ensure the validity of the measurements taken with the Evolution Spectrophotometer instrument, the polymyxin preparations were diluted in 0.01% Brij buffer to ensure that the measurements taken were within the linear range of the detector. For measurements on the NanoDrop One instrument 2 µL of sample were pipetted directly on the sample pedestal, while a 10 mm quartz cuvette was used for measurements on the Evolution Spectrophotometer. The polymyxin concentration data obtained on both instruments (Table 1) were plotted (Figure 3). Regression line shows that protein concentration results from the NanoDrop One instrument are in good agreement to the results obtained on a traditional high end UV-Vis spectrophotometer using a cuvette.

Target [Conc] µg/mL	NanoDrop One			Evolution
	A205	Std. Dev.	[Conc] µg/mL	[Conc] µg/mL
0	-0.01	0.04	-0.18	-0.02
5	0.11	0.01	3.60	5.05
10	0.27	0.01	8.84	10.53
15	0.44	0.02	14.08	17.09
50	1.68	0.01	54.14	55.32
100	3.39	0.01	109.44	108.48
200	6.64	0.03	214.16	222.50

Table 1. Various preparations of Polymyxin were measured on the NanoDrop One and Evolution Spectrophotometers. Five(5) replicates of each solution were measured on the NanoDrop One instrument using the 205=31 application. Solutions with absorbance over 1.0A were diluted and measured in triplicate on the Evolution Spectrophotometer instrument.

Protein Preparation	# of Trp of Tyr					
	Trp	Tyr	A205	STDV	[Concentration] $\epsilon_{205}=31$ ($\mu\text{g/mL}$)	[Concentration] Scopes Method ($\mu\text{g/mL}$)
BSA 1	3	21	3.960	0.013	127.73	131.80
BSA 2	3	21	37.271	0.218	1202.30	1261.71
BSA 3	3	21	70.044	0.239	2259.48	2387.91
BSA 4	3	21	129.170	1.458	4166.77	4345.20
BSA 5	3	21	271.027	0.851	8742.81	9198.13
Lysozyme 1	6	3	29.069	0.169	937.71	795.95
Lysozyme 2	6	3	53.651	0.545	1730.68	1459.05
Lysozyme 3	6	3	102.713	0.668	3313.32	2814.79
Polymyxin 1	0	0	0.112	0.015	3.60	3.12
Polymyxin 2	0	0	0.274	0.014	8.84	10.12
Polymyxin 3	0	0	0.437	0.021	14.08	16.03
Polymyxin 4	0	0	1.678	0.014	54.14	60.99
Polymyxin 5	0	0	3.393	0.014	109.44	125.16
Polymyxin 6	0	0	6.639	0.034	214.16	244.87

Table 2. Comparison of different A205 methods for various protein and peptide preparations on the NanoDrop One Spectrophotometer.

To assess the effect that the extinction coefficients used at 205 nm (i.e., Scopes and $\epsilon_{205}=31$ methods) would have on the result, we prepared dilutions of three different proteins with varied amounts of aromatic residues: bovine serum albumin (BSA, 3 Trp and 21 Tyr residues), lysozyme (6 Trp and 3 Tyr residues) and polymyxin (no Trp, no Tyr). These preparations were measured on the NanoDrop One instrument using the $\epsilon_{205}=31$ and Scopes methods (Table 2).

Conclusion

To assess NanoDrop One Spectrophotometer performance at A205, we compared polymyxin concentration results obtained with the NanoDrop One and the Evolution benchtop Spectrophotometers, which have excellent stray light performance. Table 1 shows that the NanoDrop One instrument provided very consistent results between replicate measurements at 205 nm with standard deviations below 0.04A. In addition, the results obtained with both instruments were comparable (Figure 3). Comparison between the A205 methods (Scopes and $\epsilon_{205}=31$ methods) offered in the NanoDrop One A205 application shows that the number of tryptophan and tyrosine residues has a large effect on the calculated concentration (Table 2). This is because tryptophan is the largest contributor to A280 absorbance, and the Scopes method uses the A280/A205 ratio to correct for aromatic side-chain absorbance at A205.

Our results show that A205 quantification using the $\epsilon_{205}=31$ method gives comparable results when proteins have only a few tryptophan residues.

One limitation of the A205 method is that many of protein buffers commonly used have absorbance at 205 nm. Before using this technique, we recommend checking the protein buffer for any contribution to the absorbance at 205 nm.

References

1. Anthis, NJ and Clore, GM 2013. Sequence-specific determination of protein and peptide concentrations by absorbance at 205 nm. *Protein Science* 22:851-858.
2. Goldfarb, AR, Saidel, LJ, Mosovich E 1951. The ultraviolet absorption spectra of proteins. *Journal of Biological Chemistry* 193(1):397-404.
3. Scopes, RK 1974. Measurement of protein by spectrophotometry a 205 nm. *Analytical Biochemistry* 59:277-282.

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