

APPLICATION NOTE

Low-volume, high-throughput DNA and protein detection on the SpectraMax ABS Plus Microplate Reader

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Introduction

Nucleic acid and protein quantitation are essential measurements upstream of many sophisticated assays in genetics and molecular biology. Various methods have been developed to quantitate these biological components, however, the most commonly used technique is still ultra-violet (UV) spectrophotometry. The basis of spectrophotometry is that every molecule absorbs or transmits light over a certain wavelength range, and their concentrations can be calculated by using the Beer-Lambert law (Equation 1) with preceding knowledge of the sample's molar extinction coefficient and the measurement pathlength.

$$A = \epsilon cL$$

Equation 1: The Beer Lambert law states that absorbance (**A**) is equal to the measured molecule's molar coefficient (**ϵ**) multiplied by the concentration (**c**) and pathlength (**L**) used to measure said molecule. Re-arranging the equation allows us to use absorbance to calculate concentration.

Nucleic acid quantitation is a very well-established technique, and its basic principles have not been modified much since its inception. To calculate nucleic acid concentration, samples are measured at 260 nm, and auxiliary measurements are taken at 230 nm and 280 nm wavelengths to check for sample purity.

Protein samples can be quantitated using UV spectrophotometry as well, however, there are more accurate colorimetric assays available. The UV spectrophotometric method utilizes

tryptophan's aromatic properties to absorb light at 280 nm, but calculated protein concentrations can be skewed by varying amounts of tryptophan residues in the amino acid sequence. Alternatively, the Bicinchoninic Acid (BCA) protein assay can measure protein concentrations independent of amino acid sequence and length. The assay utilizes the copper-based Biuret reaction where the amino acid backbone forms a color-chelate complex with copper molecules in an alkaline environment.

The SpectraMax® ABS Plus is a compact UV-Vis absorbance microplate reader ideal for these types of quantitative assays. Here, we demonstrate several different ways the ABS Plus reader, in combination with the SpectraDrop™ Micro-Volume Microplate and SoftMax® Pro Software, can quantitate double-stranded DNA and bovine serum albumin (BSA) protein.

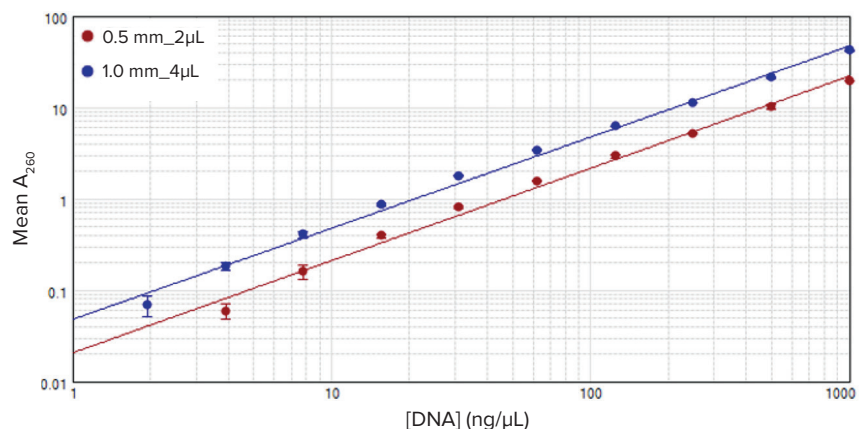


Figure 1. dsDNA quantitation on the SpectraDrop Micro-Volume Microplate. The ABS Plus reader with the SpectraDrop microplate could detect down to 2 ng/μL and 4 ng/μL of DNA using the 1.0 mm (blue) and 0.5 mm (red) coverslips respectively.

Benefits

- Widen the range of quantitative experiments with a 190-1000 nm wavelength range
- Minimize sample volume while retaining sensitivity with the SpectraDrop Micro-Volume Microplate
- Simplify and expedite experiments with SoftMax Pro Software's preconfigured protocols

Materials

- SpectraMax ABS Plus Microplate Reader (Molecular Devices cat. #ABS Plus)
- SpectraDrop Micro-Volume Microplate (Molecular Devices cat. #O200-6262)
- 96-well, clear, flat-bottom polystyrene microplate (Greiner Bio-One cat. #655101)
- UltraPure™ Calf Thymus DNA Solution (ThermoFisher Scientific cat. #15633019)
- Pierce BCA Protein Assay Kit (ThermoFisher Scientific cat. #23225)
- Pierce™ Bovine Serum Albumin Standard Ampules, 2 mg/mL (ThermoFisher Scientific cat. #23209)
- UV-Star® 96-well microplates (Greiner Bio-One cat. #655801)

1.0mm_4uL										
Sample	Well	KnownConcentration ng/μL	A260	Average A260	STDev A260	A280	A230	260:280	260:230	Co
01	H7	1000.000	41.445	41.776	0.889	21.457	17.843	1.931	2.323	
	I7		41.351			21.470	17.785	1.926	2.325	
	J7		41.207			21.322	17.663	1.933	2.333	
	K7		43.101			27.930	24.539	1.543	1.756	
02	H8	500.000	21.129	21.102	0.103	10.872	8.875	1.944	2.361	
	I8		21.029			10.808	8.813	1.946	2.386	
	J8		21.015			10.801	8.817	1.946	2.383	
	K8		21.237			11.183	9.203	1.899	2.308	
03	H9	250.000	11.329	11.282	0.041	5.859	4.691	1.933	2.415	
	I9		11.257			5.808	4.641	1.938	2.426	
	J9		11.259			5.816	4.639	1.936	2.427	
	K9		Masked			Masked	Masked	Masked	Masked	
04	H10	125.000	6.335	6.296	0.035	3.367	2.567	1.881	2.468	
	I10		6.283			3.321	2.505	1.892	2.508	
	J10		6.269			3.320	2.509	1.889	2.499	
	K10		Masked			Masked	Masked	Masked	Masked	
05	H11	62.500	3.449	3.425	0.031	1.914	1.349	1.802	2.557	
	I11		3.395			1.868	1.291	1.818	2.630	
	J11		3.401			1.884	1.311	1.806	2.594	
	K11		3.453			1.909	1.331	1.808	2.594	

Figure 2. SoftMax Pro Software preconfigured data table. The software's preconfigured protocols simplify DNA quantitation experiments by automatically calculating absorbance ratios and concentrations of unknown samples.

Methods

DNA quantitation

A 2-fold dilution series of dsDNA starting at 1000 ng/μL was prepared from UltraPure calf thymus DNA diluted in 1x PBS. Either 2 μL or 4 μL of sample was pipetted onto a 64-well SpectraDrop microplate, and a 0.5 mm or a 1.0 mm coverslip was used to cover the dsDNA sample respectively. The preconfigured protocol, "SpectraDrop DNA Quantitation", was opened in SoftMax Pro Software, and the plate was read using the provided settings. A log-log curve fit was applied to the data, and a standard curve was generated using SoftMax Pro Software. Additionally, a spectral scan ranging from 220 nm to 350 nm with 4 nm steps was performed on all concentrations to assess sample purity.

DNA quantitation in a cuvette or a microplate format was also compared. A 2-fold dilution series from 250 ng/μL to 0.5 ng/μL was transferred to a UV-transparent 96-well microplate at a volume of 200 μL per well or several UV-Vis cuvettes at 1000 μL. Both formats were read on the SpectraMax ABS Plus Microplate reader at 260 nm. A log-log curve fit was applied to each data set, and the standard curves were generated using SoftMax Pro Software.

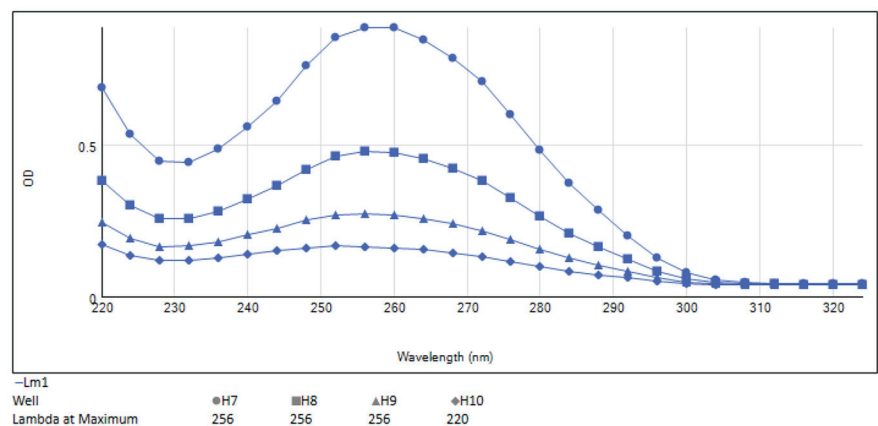


Figure 3. SoftMax Pro Software preconfigured data table. Spectral scans were performed to assess sample purity. Four representative concentrations of DNA were scanned and only a peak at 260 nm was identified, indicating pure dsDNA.

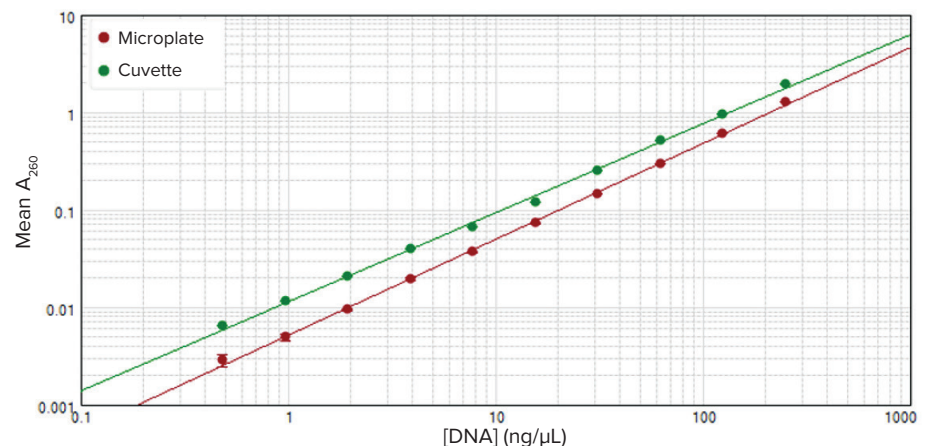


Figure 4. dsDNA quantitation in a 96-well microplate or cuvette. The dsDNA dilution series was detectable from 250 ng/μL to 0.50 ng/μL in both cuvette and microplate formats. Additionally, both dilution series demonstrated high linearity.

Protein quantitation

The BCA assay standard curve was prepared by diluting provided BSA according to the assay kit's protocol. 25 μL of protein standards and 200 μL of the BCA working reagent was transferred to a 96-well clear microplate and incubated for 30 minutes at 37°C. The "BCA" preconfigured protocol was opened in SoftMax Pro Software, and the plate was read at 562 nm using the settings provided. A quadratic curve fit was applied to the data, and a standard curve was generated using SoftMax Pro Software.

Results

The ABS Plus Microplate reader was able to quantitate dsDNA concentration using both low-volume and standard microplate methods. Using the SpectraDrop plate and pre-configured protocol, the ABS Plus microplate reader could detect down to 2 ng/ μL using as little as 4 μL of sample, and could automatically measure and calculate relevant parameters such as A_{260}/A_{280} and A_{260}/A_{230} ratios (Figure 1 and Figure 2). A spectral scan further demonstrated that the samples were contaminant-free (Figure 3).

Alternatively, the ABS Plus microplate reader can quantitate dsDNA in a microplate format. Using a UV-transparent microplate, the reader could measure as little as 0.5 ng/ μL dsDNA (Figure 4).

Finally, the SpectraMax ABS Plus Microplate Reader can be used to quantitate protein samples with the aid of the BCA assay. Following the provided protocol, BSA standards were measured, and a standard curve was generated comparable to the curve in the assay kit's literature (Figure 5).

Conclusion

The SpectraMax ABS Plus Microplate Reader is a compact and adaptive microplate reader capable of quantitating nucleic acid and protein samples in various formats such as cuvettes or microplates. When combined with the SpectraDrop Micro-Volume Microplate, the reader can quantitate up to 64 samples with as little as 2 μL sample volume in a single read. Additionally, SoftMax Pro Software's preconfigured protocols allow for optimized experimentation and faster time to results.

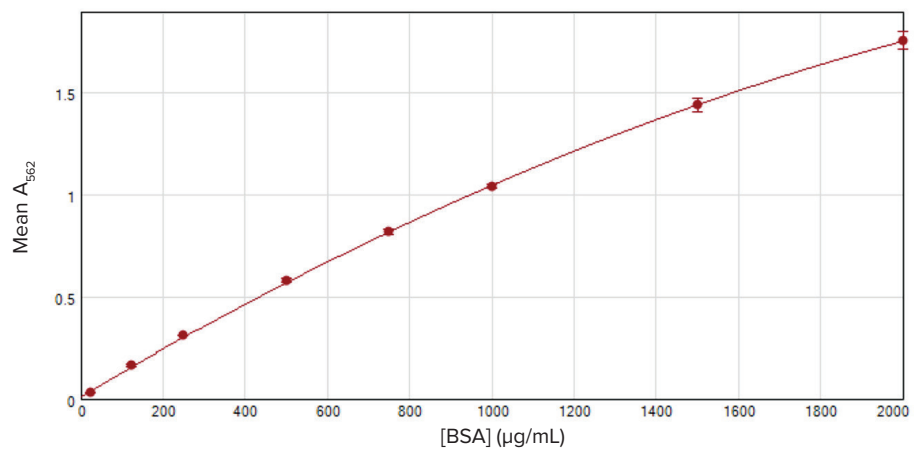


Figure 5. BCA Assay standard curve. The ABS Plus microplate reader was able to detect the BSA standards from the BCA assay. SoftMax Pro Software was used to apply a quadratic curve fit to the data points, and a robust standard curve ($r^2 = 1.000$) was generated.

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