APPLICATION NOTE

Quantification of proteins by UV-Vis absorbance using VICTOR Nivo with microvolume plates.

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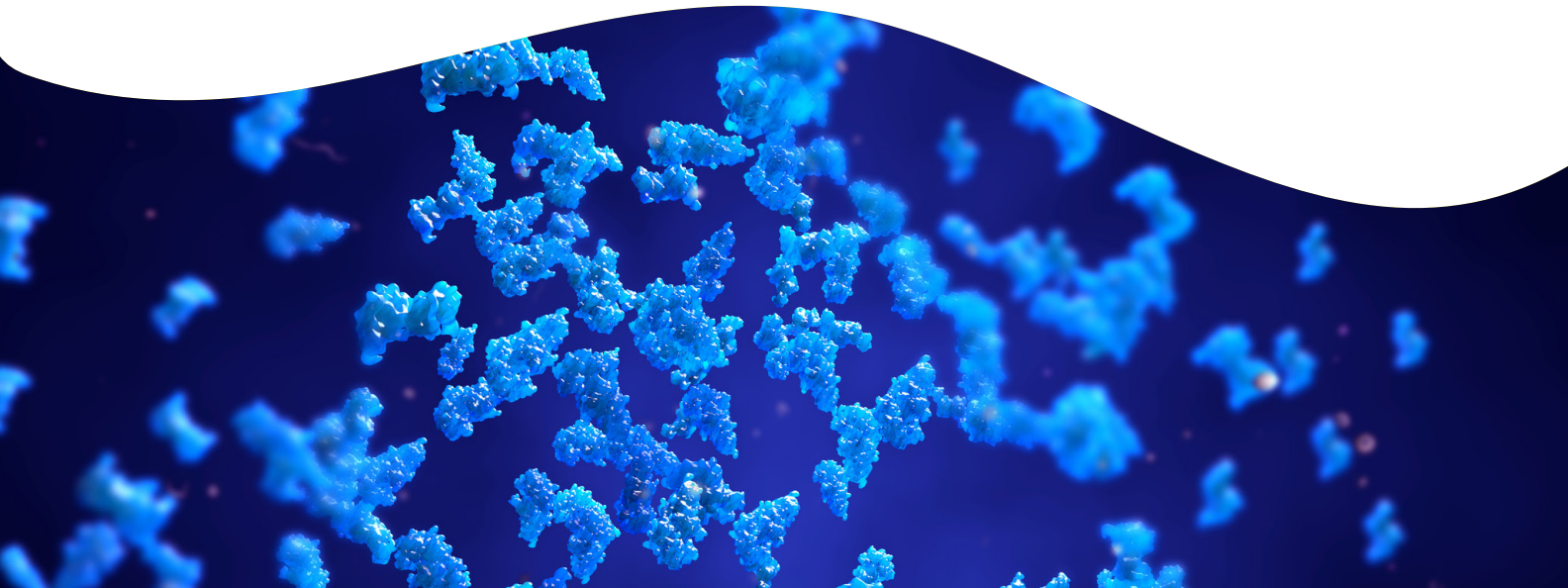
Introduction

Knowing the protein concentration is commonly required in studies of protein biochemistry and molecular biology. Protein samples display a characteristic absorption spectrum at 280 nm, so a direct method of measuring protein concentration is to determine the absorbance of a sample at this wavelength. This UV based approach depends strongly on the purity and primary sequence of a protein. Aromatic amino acid residues such as Tryptophan (Trp), Tyrosine (Tyr) and Phenylalanine (Phe) absorb UV-light at 280 nm which allows recalculation of the protein content. This method enables scientists to rapidly determine the concentration of protein, relative to a standard, or using an assigned extinction coefficient applying the Lambert-Beer law: $A280 = c * \varepsilon * b$ (c: protein concentration, ε: wavelength- dependent protein extinction coefficient, b: pathlength). Each pure protein has a unique extinction coefficient.

The advantages of this method are that the procedure is simple to carry out, it is nondestructive, no special reagents or standard curves are required, and it consumes very little sample. However, this UV based approach depends strongly on the purity and primary sequence as the exact extinction coefficient for the protein or protein mixture quantified is needed. Additionally, some substances which are commonly present in protein samples (e.g. nucleic acids or detergents) show strong UV absorbance, which should be corrected for or avoided altogether (not assessed in this application note). In this study, we describe how to perform protein quantification in a re-useable microvolume plate (µDrop™ Plate) on a plate reader (VICTOR Nivo).



Instrumentation

The VICTOR® Nivo™ is a high-performance, compact, and light-weight multimode plate reader designed for life science research laboratories. Its software has a modern, workflow-oriented user interface which is easy to learn and use and includes pre-written application protocols to get users productive quickly. In addition, MyAssays® Desktop Standard software is provided for data analysis. For absorbance measurements using the VICTOR Nivo multimode plate reader, there is a choice of either a filter- or a spectrometer-based system. With the spectrometer-based plate reader, full spectrum absorbance measurements from 230-1000 nm are ultrafast with a measurement time of less than one second per well. Alternatively, up to eight discrete wavelength bands can be selected in a single operation with no slowdown in speed or wavelength switching. The bandwidths can be 2, 5 or 10 nm and the spectrometer- based VICTOR Nivo system also allows the detection of a wide range of dyes or measurement of samples with unknown absorbance spectra.

21 CFR part 11 compliance

Microplate readers are often used in regulated GxP environments, mainly those where compliance with GLP (Good Laboratory Practice) or GMP (Good Manufacturing Practice) is needed. Since the readers are producing and maintaining electronic data, they also need to enable customers to reach compliance with 21 CFR Part 11, EU Annex 11, or likewise regulations.

Figure 1: VICTOR Nivo multimode plate reader.

The VICTOR Nivo features an Enhanced Security software option that fulfills this need by adding additional features like advanced user management, audit trail, electronic signatures, a secure data file transfer to 3rd party analysis tools, and more.

Materials and methods

The µDrop™ Plate (Thermo Scientific™; Cat. No. N12391) is made of aluminum and quartz glass similar to microscope glasses. The low volume area is partly covered with Teflon (PTFE). The pathlength is reduced to 0.5 mm compared to 10 mm in a standard cuvette.This enables not only the measurement of volumes as small as 2-5 µl, but also the measurement of higher concentrations, since the absorbance is directly dependent on the pathlength.

BSA solution at 200 mg/mL (Sigma-Aldrich, Product Nr P5369-10ML; LOT#SLCB4049) in PBS was used. Dilutions of the stock solution in Molecular Biology Grade UltraPure Water (Rockland) were prepared in order to evaluate a large concentration range (50 – 0.1 mg/mL). Each BSA sample was measured 7-fold using 2 µL sample size on the VICTOR Nivo multimode plate reader using the µDrop™ Plate. All data were normalized to a 10mm path length and are shown in Table 1. The data demonstrate that measurements done with the VICTOR Nivo multimode plate reader show high linearity as the calculated coefficient of determination (R2) is 0.9999 (Figure 2). Analysis of the replicates (n=7) shows a high precision within the used concentration range, the %CV within the higher OD range is ≤ 2 % while in the lowest OD range the %CV is ≤ 7% (Table 1).

The accuracy of the VICTOR Nivo multimode plate reader was evaluated by measuring a certified BSA solution in 150 mM NaCl, 0.1 % NaN3 (Sigma-Aldrich, Product Nr P5369-10ML; LOT#SLCB4049). The certified stock concentration of 200 mg/mL BSA (by Biuret method) was used in a 1:2 dilution series in Molecular Biology Grade UltraPure Water (Rockland). Each dilution was measured seven times with a µDrop™ Plate (Thermo Scientific™; Cat. No. N12391) to determine the precision and the accuracy.

Proteins show the propensity to adsorb passively to interfaces via hydrophobic interactions. The amount adsorbed depends on many factors such as the protein itself, the ambient conditions and the surface material. To measure the amount of protein adsorbing to the quartz glass, the BSA sample at 50 mg/mL was stored overnight

at room temperature in the µDrop™ plate. In the morning, the µDrop™ plate was cleaned according to the standard procedure and afterwards, Molecular Biology Grade UltraPure Water (Rockland) was added. All sample wells (n=14) were measured and no adsorption was detected as the measured OD values were identical to the blanks (n=2) (Data not shown). The experiments were repeated with an antibody named Pertuzumab and similar results were produced. Data is available upon request.

Results

Table 1: Absorbance values measured at A280 with VICTOR Nivo for a BSA dilution series (no blanks were subtracted).

BSA	A280									
Concentration (mg/mL)	A	B	C	D	E	F	G	AVG	STDEV	%CV
50.000	1.670	1.626	1.608	1.598	1.589	1.589	1.591	1.610	0.030	1.840
25.000	0.874	0.859	0.844	0.833	0.829	0.831	0.831	0.843	0.017	2.060
12500	0.451	0.440	0.434	0.429	0.427	0.434	0.429	0.435	0.008	1.920
6.250	0.239	0.232	0.228	0.232	0.228	0.230	0.227	0.231	0.004	1.780
3.125	0.137	0.132	0.130	0.131	0.131	0.134	0.135	0.133	0.003	1.920
1.563	0.084	0.084	0.084	0.083	0.083	0.086	0.082	0.084	0.001	1.500
0.781	0.065	0.059	0.058	0.058	0.060	0.064	0.063	0.061	0.003	4.830
0.391	0.047	0.049	0.045	0.047	0.046	0.051	0.047	0.047	0.002	4.190
0.195	0.049	0.043	0.042	0.042	0.042	0.048	0.047	0.045	0.003	7.040
0.098	0.039	0.040	0.043	0.041	0.040	0.043	0.039	0.041	0.002	4.190

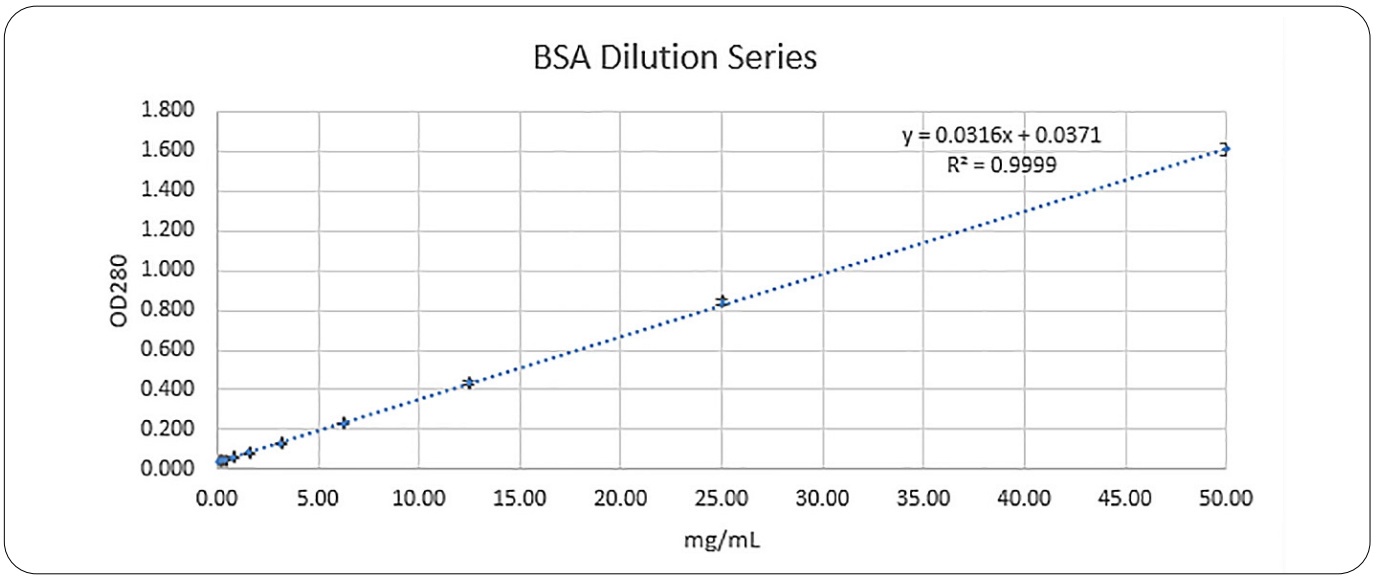


Figure 2: Linearity of A280 BSA quantification with VICTOR Nivo.

Conclusions

The VICTOR Nivo multimode plate reader is an appropriate tool for UV/Vis analysis of proteins with a µDrop™ Plate. The VICTOR Nivo multimode plate reader in combination with a µDrop™ Plate provides robust and fast read-out of a single sample or up to 16 reads at once. Combining small sample consumption (2-5 µL) with a large linear range eliminates pipetting dilution series and thus keeping the integrity and production conditions unchanged as preferred by regulatory authorities.

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