Labrox® Application Note #006

Labrox® multimode plate reader:

Protein quantification by absorbance using the Labrox multimode reader

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Introduction

There are several methods for determining the total concentration of proteins in solution, each of which exploits properties that are general to all proteins. One widely used method is the measurement of UV (ultraviolet) absorbance, at 280 nm, since proteins in solution absorb ultraviolet light with absorbance maxima at 280 nm. Quantifying protein by directly measuring absorbance is fast and convenient, since no additional reagents or incubations are required and the procedure does not consume the protein (1).

Labrox multimode plate readers, developed for several applications, are versatile and easy to use. They include various detection modes, among them Absorbance (ABS). In this application note, we demonstrate that Labrox readers in ABS mode can measure protein at 280 nm with accuracy over a wide range of concentrations.

Figure 1. Labrox multimode plate reader.

Materials

- BSA (Albumin Bovine Fraction V) Biotechnology Grade from AMRESCO
- Lonza AccuGENE™ Molecular Biology Water cat. BE51200
- 96 well UV transparent plates (Greiner bio-one, 96 well UV-Star®)
- Micropipettes and tubes
- Labrox reader

Methods

A BSA dilution series (Table 1) was prepared to obtain the standard curve.

Two BSA samples of unknown concentration were also measured (U1 and U2)

Three replicates (200 µl) of each standard sample and each unknown sample were added to the appropriate wells of a UV transparent microplate and the plate was read at 280 nm.

Results

The results are presented in Table 1 and Figure 2.

Table 1. OD at 280 nm of the different measured samples (averages, standard deviation and signal to background values are presented).

BSA (mg/ml)	aver. OD	S-B	SD %
0	0,0317	0	6
0,01	0,0368	0,0051	4
0,05	0,0525	0,0208	6
0,1	0,0711	0,0394	2
0,25	0,1272	0,0955	3
0,5	0,2166	0,1849	1
1	0,3931	0,3614	1
U1	**0,0691**	**0,0374**	**4**
U2	**0,0421**	**0,0104**	**3**

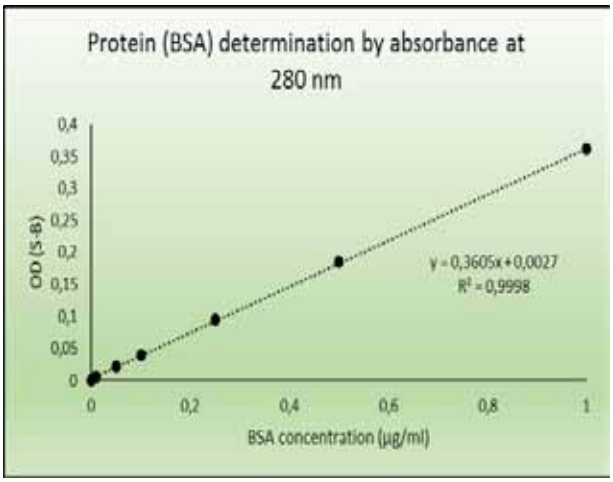

Figure 2. OD versus BSA concentration linearity.

Using the standard curve equation (y=0,3605x+0,0027), U1 and U2 concentrations were determined:

U1- 0,0374- 0,0027= 0,3605 x; x= 0,096 mg/ml = **96 µg/ml**

U2- 0,0104- 0,0027= 0,3605x; x= 0,021 mg/ml = **21 µg/ml**

Conclusions

Labrox reader, in addition to its many other measurement technologies, is appropriate to measure protein concentration accurately by absorbance measurement at 280 nm. Perfectly linear standard curves can be obtained and by using those curves, it is possible to determine the protein concentration of unknown samples.

References

1. “Strategies for Protein Quantitation” J. Proteome Res. 8, 787–797

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