Labrox® Application Note #005

Labrox® multimode plate reader:

Protein determination by the Bradford method using the Labrox multimode reader

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Introduction

The Bradford method (1) is one of the most used methods for protein determination. The Coomassie Brilliant Blue G-250 dye used in this method binds selectively to positively charged residues (lysine, arginine and histidine) and aromatic residues. The binding to proteins is accompanied by a shift in absorbance maximum from 465 nm to 595 nm. This assay is fast, inexpensive and sensitive, and tolerates a wide range of buffers. The Bradford assay is recommended for general use and in particular for determining protein content of cell fractions and assessing protein concentrations for gel electrophoresis.

Labrox multimode plate readers are developed for several applications. 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 concentration with accuracy over a wide range of concentrations, using the Bradford Method.

Figure 1. Labrox multimode plate reader.

Materials

- BSA (Albumin Bovine Fraction V) Biotechnology Grade from AMRESCO
- Lonza AccuGENE™ Molecular Biology Water cat. BE51200
- Bradford Reagent (proteonic grade) from AMRESCO
- Sodium Chloride (NaCl) Molecular Biology Grade from Fisher Bioreagents.
- Micropipettes and tubes
- 96 well UV transparent plates (Greiner bio-one, 96 well UV-Star®, F-bottom, chimney well, µClear®)
- Labrox reader

Methods

BSA 0.5 mg/ml and NaCl 0.15 M solutions were prepared and from these stock solutions a series of BSA standard dilutions was prepared according to Table 1.

Table 1. Preparation of the BSA standard curve

Standard	0.5 mg/ml BSA (µl)	0.15 M NaCl (µl)	standard/ well (µl)	BSA/well (µg)
Blank	0	100	20	0
S1	5	95	20	0,5
S2	10	90	20	1
S3	15	85	20	1,50
S4	20	80	20	2

Three replicates of each standard sample (20 µl) were pipetted into the appropriate wells and 200 µl of Bradford Reagent were then added to each well and mixed with the samples by pipetting. Two samples of unknown concentration (U1 and U2) were also measured.

After 2 minutes of incubation at room temperature, the plate was read at 595 nm using the Labrox reader absorbance mode.

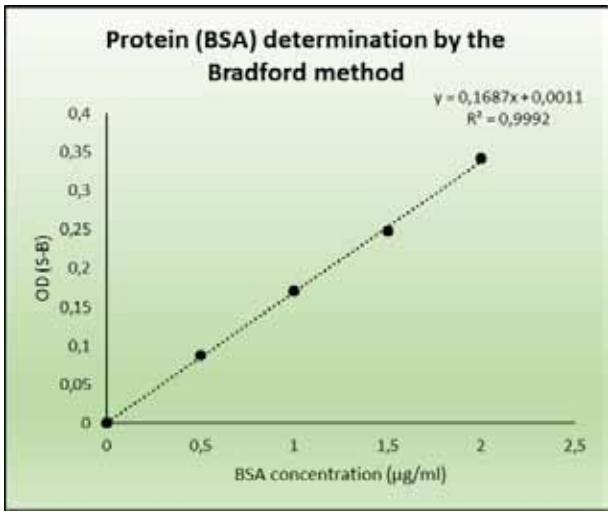

Figure 1. OD versus BSA concentration linearity.

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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