

Bradford Assay Performed on BMG LABTECH's FLUOstar Omega with new Evaluation Software

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- Fast and homogeneous assay to determine the protein concentration of samples
- Dye shift can be followed with the spectrometer tool in the new FLUOstar Omega
- New easy to use Omega evaluation software is introduced

Introduction

Determining the protein concentration of samples is a necessary and often used method in biochemistry. Different colorimetric protein assays have been developed. The most commonly used methods are the Bradford assay, the Lowry assay and the BCA assay. In this application note we demonstrate how to determine the protein concentration of samples by using the Bradford assay and the new FLUOstar Omega. The Bradford assay is based on the binding of protein to a dye, leading to a shift in the absorbance maximum of the dye¹. After creating a standard curve of protein solutions with known concentrations, the protein concentration of unknown samples can be calculated. The dye used for the Bradford assay is Coomassie® Brilliant Blue G-250 (Figure 1).

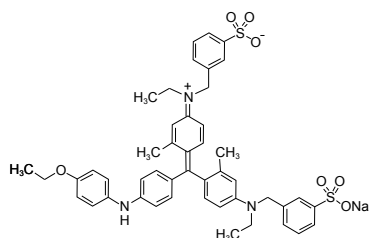


Fig. 1: Chemical structure of Coomassie® Brilliant Blue G-250

The acidic solution of this dye has an absorbance maximum at 465 nm. After the addition of protein, hydrophobic amino acid residues and arginine residues bind to the dye. As a result, the absorbance maximum of the dye shifts from 465 nm to 595 nm (Figure 2).

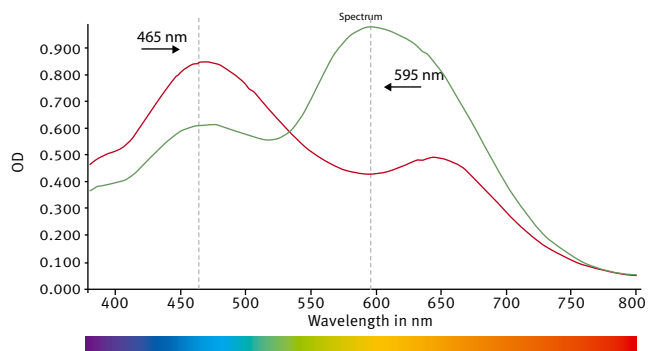


Fig. 2: The spectrum from unbound (red line) and protein bound (green line) Coomassie® Brilliant Blue. After binding the absorbance maximum of the dye shifts from 465 nm to 595 nm.

The new FLUOstar Omega features high speed full spectrum absorbance. The spectrometer tool allows measuring the whole spectrum of a sample from 220-850 nm with selectable resolution in about 1 sec per well. In case the optimal wavelengths are already known, it is also possible to measure up to 8 pre-selected wavelengths at once.

Materials and Methods

- 96 well transparent microplates from Greiner, Frickenhausen, Germany
- FLUOstar Omega, BMG LABTECH, Offenburg, Germany
- Bovine Serum Albumin (BSA, Cat. No. A-9647) from Sigma-Aldrich, Taufkirchen, Germany
- Bradford Reagent (Cat. No. B6919) from Sigma-Aldrich, Taufkirchen, Germany

The Bradford Reagent was bought ready to use. A stock solution of bovine serum albumin in distilled water (10 mg/ml) was prepared as a protein standard. For the measurements, a dilution of bovine serum albumin was done starting with 1 mg/ml. Bradford reagent, 290 µl, was pipetted into a transparent 96 well microplate. 10 µl of the protein dilution was added followed by mixing in the wells. After 5 min of incubation at room temperature, the plate was read at 595 nm or in spectrum mode in the FLUOstar Omega.

Instrument settings

Number of flashes: 20
Wave-length range: 380-800 nm (or discrete wavelength at 595 nm)
Wave-length step width: 1 nm

The progress of the measurement can be followed using the Current State Window (Figure 3).

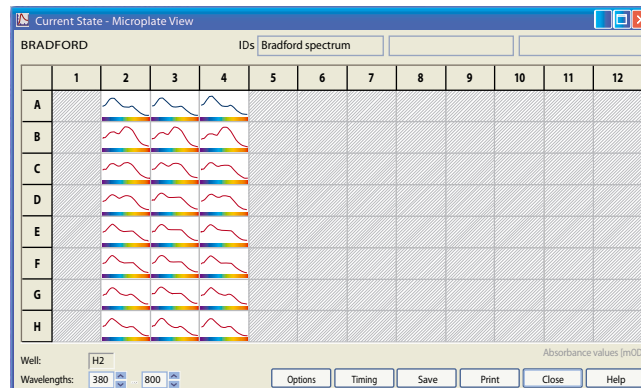


Fig. 3: Current State Window of Bradford measurements monitoring spectra from 380 to 800 nm. Standards are indicated as red lines, blanks are indicated as blue lines. Samples were run in triplicates.

Furthermore, during the measurement, it is possible to magnify a selected well and get information about the measured values over the spectral range (Figure 4).

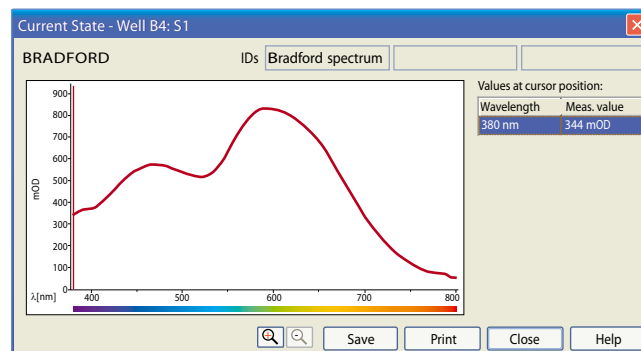


Fig. 4: Magnified current state picture of one selected well. The spectrum is taken from 380 to 800 nm. The cursor can be set to any wavelength for checking OD values during the measurement.

Results and Discussion

After measurements are taken, the data is transferred to the evaluation software. Pre-defined templates can be used to do the calculations needed instantaneously, i.e. average of raw data, blank correction, performing curve fits and much more.

For the Bradford assay the blank corrected values are used for the standard curve (Figure 5).

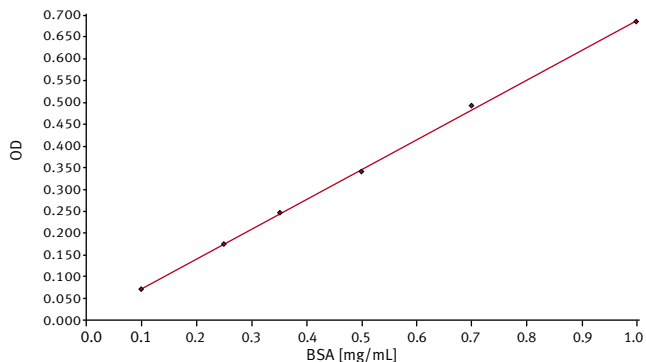


Fig. 5: BSA standard curve (linear regression fit performed with the new Omega Evaluation Software)

With the help of the standard curve the software calculates the protein concentration for unknown samples automatically. If the option “path length correction” is used, the measured data is multiplied by a factor that depends on the type of microplate and volume used. With the help of this calculation, the data is normalized to a path length of 1 cm, thereby allowing a comparison to be made between absolute data obtained from a microplate reader with data obtained from a cuvette-based spectrometer.

Conclusion

The Bradford assay was successfully performed on the FLUOstar Omega (Fig. 6). According to the manufacturers protocol² this protein assay is linear in the range of 0.1 – 1.4 mg/ml. Because of its homogeneous and fast nature, the assay is a preferred method to determine the protein concentration of samples.



Fig. 6: BMG LABTECH's FLUOstar Omega

The FLUOstar Omega offers entirely new possibilities with its spectrometer tool. A whole absorbance spectrum can be read in about 1 sec per well. Furthermore, the new Omega Evaluation Software allows for the absorbance maximum or minimum to be recognized at once after clicking on the spectral curve. Any wavelength can be selected to give the values for the optical density in any well. The speed of the spectrometer and the easy work-up in the software provide users with unmatched flexibility that can be used to optimize absorbance settings for all experiments.

References

- 1 Bradford, MM. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye-binding. *Anal Biochem.* **72**, 248-254.
- 2 www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/B6916

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