Introducing the new FLUOstar Omega: Performing the Bradford Assay

BMG LABTECH

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Introduction

The new FLUOstar Omega is a multi-detection microplate reader for research and life science laboratories which offers the following four detection modes:

- Fluorescence Intensity – including FRET
- Time-Resolved Fluorescence – including DELFIA
- Luminescence (flash and glow) – including BRET
- UV/Vis Absorbance – measurement of complete spectra or individual wavelengths

With full spectrum absorbance and three other detection modes the FLUOstar Omega provides users with unmatched flexibility and sensitivity to perform any assay needed.
In this poster we demonstrate the use of the full-range absorbance spectrometer for the colorimetric determination of the protein concentration by utilizing the Bradford assay.

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. The Bradford assay is based on the binding of protein to a dye leading to a shift in the absorbance maximum of the dye[1]. 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).

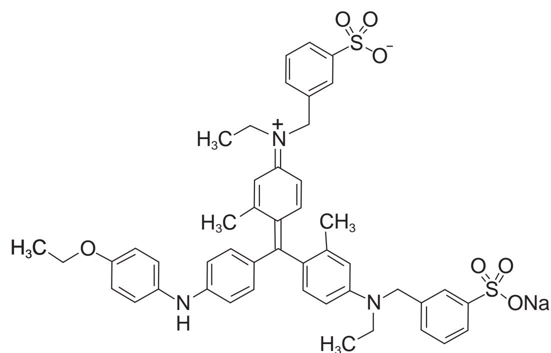

Fig. 1: Chemical structure of Coomassie® Brilliant Blue G-250

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).

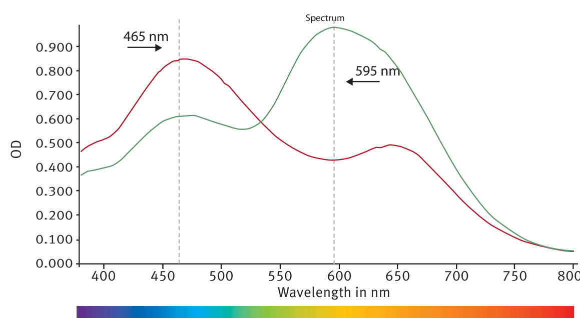

Fig. 2: The spectrum from unbound (red line) and protein bound (green line) Coomassie® Brilliant Blue. After binding the absorbance maximum of the dye is shifted from 465 nm to 595 nm.

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.

Material 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).

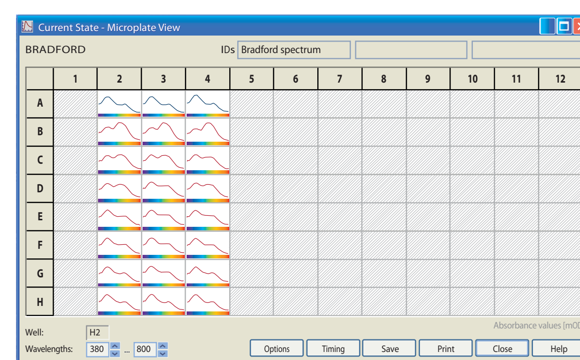

Fig. 3: Current State Window of Bradford measurements monitoring spectra from 380 to 800 nm. Standards are indicated as red lines, blanks are indicated as blue lines. Samples were run in triplicates.

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).

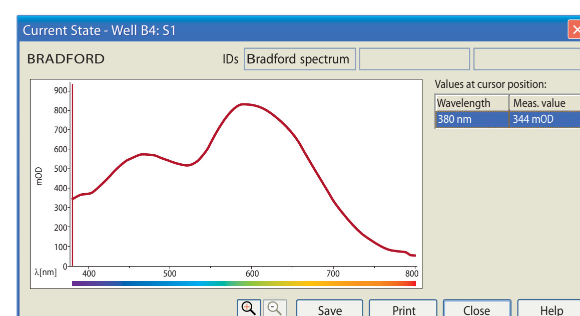

Fig. 4: Magnified a current state picture of one selected well. The spectrum is taken from 380 to 800 nm. The cursor can be set to any wavelength for checking OD values during the measurement.

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 at once, 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).

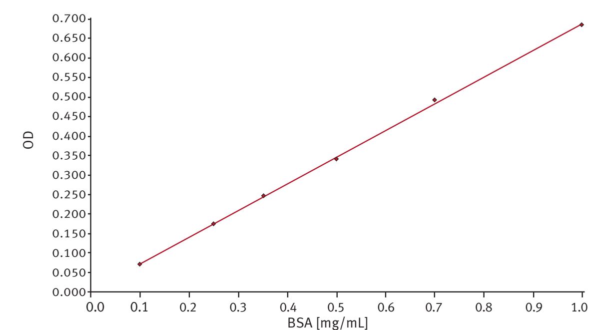

Fig. 5: BSA standard curve (linear regression fit performed with the new Omega Evaluation Software)

Conclusion

The Bradford assay was successfully performed on the FLUOstar Omega (Figure 6). According to the manufacturers protocol[2] 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

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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