Absorbance-based methods for protein quantification on BMG LABTECH instruments

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- **Protein quantification with BMG LABTECH instruments using A_{280}, Bradford-, BCA- and Lowry-assay**
- **Predefined protocols support easy and quick measurements**
- **One-Click-calculation of protein concentrations using the MARS Analysis Software**

Introduction

Measuring the protein concentration of liquid samples is a routine analysis in many life science laboratories. Accurate quantification is often a critical step for subsequent analyses such as protein characterization or western blots. Absorbance-based methods are well-established, easy to handle and cheap. They can be performed in microplates, allowing for high sample numbers processed at a time and low reagent volumes. Spectrometer-based BMG LABTECH readers capture absorbance spectra or absorbance at discrete wavelengths from 220-1000 nm in less than a second per well and therefore can easily read these assays. The most common methods absorbance at 280 nm, Bradford, Bicin-Choninic Acid (BCA) and Lowry assay are presented here.

Materials & Methods

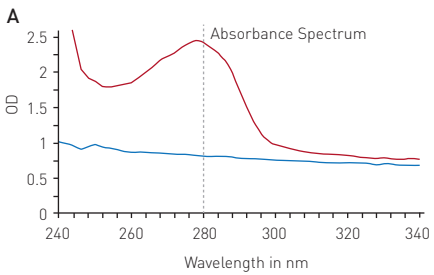
- Reagents were obtained from Sigma Aldrich
- Clear 96 well plates from Greiner
- SPECTROstar® *Nano*, FLUOstar® Omega, PHERAstar® *FSX* and CLARIOstar® plate readers from BMG LABTECH with rapid and precise default settings
- The protein standard Bovine Serum Albumin (BSA) was dissolved in ddH_2O to a stock of 2 mg/ml

Results & Discussion

Absorbance at 280 nm

The aromatic residues of tryptophan and tyrosine amino acids absorb UV-light at a wavelength of 280 nm (Fig. 1A) which reflects the protein concentration. As it does not require addition of further reagents, it is a quick and simple quantification method. The concentration range of 125-1000 µg/ml BSA was reliably detected using a volume as low as 3 µl and the BMG LABTECH LVis plate (Fig. 1B).

- 3 µl (BSA in ddH_2O) on LVis plate in duplicates
- Absorbance spectrum 240-400 nm, analysis of A_{280}



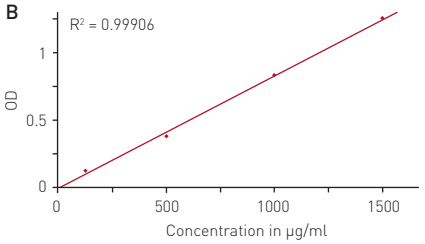


Fig. 1: Total protein quantitation by absorbance at 280 nm (A_{280}) **A)** absorbance spectrum of water (blue) and BSA (2 mg/ml in ddH_2O) **B)** Protein standard curve of BSA.

Bradford assay

The Bradford assay exploits an absorbance shift of Coomassie Brilliant blue G-250 from 460 nm in its free state, to 595 nm if complexed with proteins (Fig. 2A). The absorbance of unknown protein samples is related to samples with defined protein (e.g. BSA) concentrations that is measured in parallel. The assay provided a linear signal in the range of 62.5-1000 µg/ml BSA (Fig. 2B).

- 5 µl sample + 250 µl Bradford solution → Shake for 30 s
- Incubate in the dark for 5-45 min → Measure absorbance 400-700 nm (analysis at 595 nm)

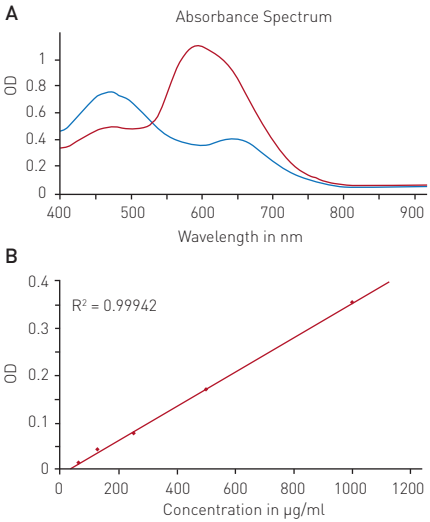


Fig. 2: Bradford protein quantification assay. **A)** Absorbance spectrum of Coomassie Brilliant Blue G 250 (without protein – blue, in presence of BSA – red) **B)** Protein standard curve of BSA.

BCA assay (bicinchoninic acid)

The BCA assay is based on the capability of peptide bonds to reduce Cu (II) sulfate to Cu^{+} which in turn complexes with two molecules of BCA giving a chromophore with an absorbance maximum at 562 nm (Fig. 3A). This assay also relates the measurement of unknown protein samples to a calibration curve obtained with samples of known protein concentration. The BCA assay allows for quantification of a broad BSA concentration range from 15.63-1500 µg/ml using a hyperbola fit (Fig. 3B).

- 240 µl working reagent + 30 µl sample
- Incubate at 37 °C for 30 min → Measure absorbance 450-750 nm (analysis at 562 nm)

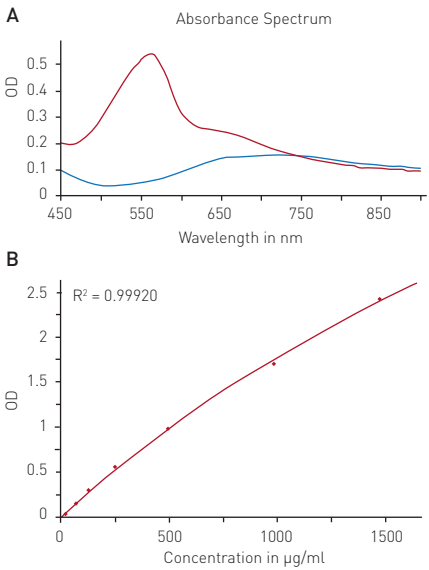


Fig. 3: BCA protein quantification assay. **A)** Absorbance spectrum of bicinchoninic acid (native – blue, BCA-Cu^{+}-complex in presence of BSA - red) **B)** Protein standard curve of BSA.

Lowry assay

The modified Lowry assay likewise depends on the reduction of Copper (II) sulfate by peptide bonds. A subsequent reaction of the Cu^{+} protein complex with Folin–Ciocalteu reagent leads to a chromophore with a broad absorption spectrum between 500 and 800 nm (Fig. 4A). This quantification assay requires a calibration curve to be determined in parallel. Reliable detection of BSA solution between 15.63 and 1000 µg/ml was possible with the modified Lowry assay and using a hyperbola fit (Fig. 4B).

- 100 µl sample + 100 µl Lowry reagent → Incubate 20 min
- Add 50 µl Folin & Ciocalteu's Phenol Regent and mix
- Incubate for 30 min → Measure 500-800 nm (analysis at 710 nm)

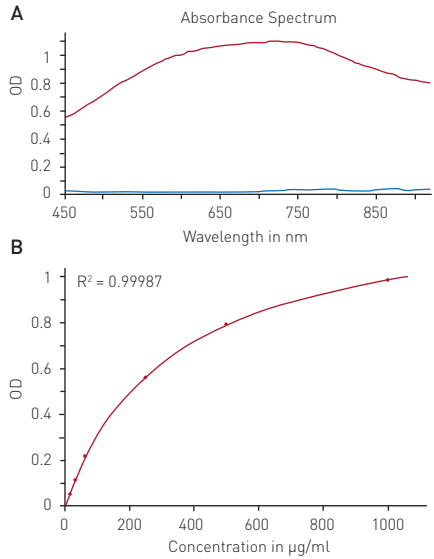


Fig. 4: Modified Lowry protein quantification assay. **A)** Absorbance spectrum of Lowry reaction (without protein - blue, in presence of BSA - red) **B)** Protein standard curve of BSA.

Tab. 1: Comparison of protein quantification methods.

Concentration range (µg/ml)	15.63	31.25	62.5	125	250	500	1000	1500	Time in min.	Volume	Minimum protein (µg) per reaction
A_{280}				■	■	■	■		1	2-3 µl	0.38
Bradford			■	■	■	■	■		5	5 µl	0.31
BCA	■	■	■	■	■	■	■	■	30	30 µl	0.47
Lowry	■	■	■	■	■	■	■		50	100 µl	1.56

Conclusion

The standard absorbance-based protein quantification assays were easily detected by BMG LABTECH readers. The protein quantification methods differed in regard to the covered concentration range, the volume required and the time of preparation and incubation (Table 1). Nevertheless, the appropriate quantification depends on additional factors such as detergents in the buffer which differently interfere with the assays.

References

1. Bradford, M.M. (1976) *Anal, Biochem.*, **72**,248-254.
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3. Smith PK. et al. (1985) *Anal. Biochem* .,**150**(1), 76-85.