Quantitative Protein Analysis Using the Bradford Assay

Background

The Bradford Assay, developed by Marion M. Bradford in 1976, is a colorimetric protein assay widely used for quantifying the concentration of proteins in a sample. This assay relies on the binding of Coomassie Brilliant Blue G-250 dye to proteins. When the dye binds to protein molecules, primarily through arginine residues, the absorbance maximum of the dye shifts from 465 nm to 595 nm. This shift corresponds to a change in colour from brown to blue, which can be measured using a UV-Vis spectrophotometer. The intensity of the blue colour is directly proportional to the protein concentration, allowing for accurate and rapid quantification.

The Bradford Assay provides a simple, quick, and sensitive method for protein determination, and is particularly valued for its compatibility with a wide range of protein samples and its minimal interference from other substances commonly found in biological samples. This assay is extensively used in protein purification, enzyme kinetics, and various other research applications, making it a fundamental tool in the laboratory for protein analysis.

Instrument and measurement conditions

In this experiment, UV-1900i UV-Vis Spectrophotometer (shown in Fig. 1) is recommended for analysis. The measurement conditions are pre-installed in the CBB method for rapid and effortless analysis.

Fig. 1 UV-1900i UV-Vis Spectrophotometer

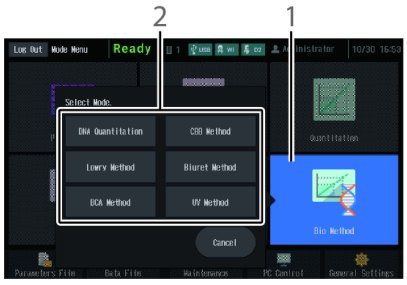

Figure 2 Preinstalled common quantitation methods for rapid and effortless analysis

Materials

- Bradford reagent
- Bovine Serum Albumin (BSA) or Bovine Gamma-Globulin
- Spectrophotometer
- Cuvettes

A) Reagent and sample preparation

Solutions can be prepared following the method detailed in Bradford, M.M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of protein-dye Binding. *Analytical Biochemistry*, 72(1-2), pp.248–254. Available at: https://doi.org/10.1016/0003-2697(76)90527-3

1) Prepare the Bradford reagent according to the manufacturer's instructions. For example, the Bradford reagent can be prepared by dissolving 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol. To this solution, add 100 ml 85% (w/v) phosphoric acid. Dilute the resulting solution to a final volume of 1 L with deionised H_2O.
2) Prepare 5 standards using Bovine Serum Albumin (BSA) (linear range 125-1,000 µg/ml) or Bovine Gamma-Globulin (linear range 125-1,500 µg/ml) using deionised H_2O as the diluent. Prepare a blank standard of deionised H_2O and Bradford reagent (1:50).
3) Dilute down the unknown samples if necessary (preferably with the same diluent as the standards). The concentration of the protein sample should be within the linear range of the calibration curve.
4) Add the Bradford reagent to each standard or unknown sample in 1:50 (standard or unknown sample: Bradford reagent).
5) Mix well by inverting several times. Leave to incubate at room temperature for 10 minutes.
6) Transfer the solutions to cuvettes ready for analysis.

B) Analysis procedure

1) From the start page select [Bio Method], then select [CBB Method].

Hint It is recommended to measure the standard and unknown solutions in duplicate or triplicate. This is easily performed by setting the repetitions value in [Parameters] - [3. Measurement Settings].

2) Click [Attachments] button in the bottom right of the display.
3) Click [Std. Table] button in the bottom right of the display.
4) Enter the concentrations of the standards into the table.
5) Select [Measure Cell 1] as the Abs. input method.
6) Lift the cover, set the reference sample (deionised H_2O) in the instrument's front cell holder (leaving the rear cell holder vacant) and close the cover.
7) Click 

(Auto Zero)
8) Replace the reference sample with the first standard (blank) and press 

(start).
9) Repeat for the remaining standards.
10) The calibration curve will be automatically generated. To view the calibration curve, click the [Calib. Curve] button in the bottom right of the display.

11) On the [Calib. Curve] page, set the reference sample (deionised H_2O) in the instrument's front cell holder (leaving the rear holder vacant) and close the lid.

12) Click 

(Auto Zero)

13) Replace the reference sample with the first unknown sample and press 

(start).

14) Repeat for all unknown samples. The Abs. and concentration values will be displayed in the table.

15) Save the results and calibration table by selecting 

(Save Table).