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Chapter 31

Quantitative protein estimation using the Bradford assay: Principles, protocols, and applications

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1. Introduction:

In biochemical study, the Bradford method is a frequently used method for measuring protein concentration. This test, named for the scientist Marion M. Bradford who first presented it in 1976, is based on the absorbance spectrum of Coomassie Brilliant Blue dye shifting when it binds to proteins in an acidic environment (Bradford, 1976; Sambrook, and Russell, 2001; Yadav et al., 2016). The blue hue produced rises in intensity with protein concentration; it can be quantified spectrophotometrically at 595 nm (Voet et al., 2018; Yadav et al., 2021). Laboratory protein measurement often uses this fast, sensitive, and comparatively easy technique (Srivastava et al., 2022). It is important to remember, although, that detergents, reducing agents, and some interfering chemicals might affect the Bradford assay, hence appropriate sample preparation and controls are necessary. All things considered, the Bradford method provides an invaluable instrument for precisely estimating the amounts of proteins in different biological materials (Kruger, 2009).

2. Materials Required:

1. Protein samples
2. Bradford protein assay reagent (e.g., Coomassie Brilliant Blue G-250)
3. Bovine Serum Albumin – BSA (Stanard protein)
4. Microplate or cuvette suitable for spectrophotometric measurements
5. Spectrophotometer capable of measuring absorbance at 595 nm
6. Microcentrifuge tubes or cuvettes for dilution and mixing
7. Distilled water
8. Micropipettes and tips

3. Procedure:

1. Preparation of reagents:
   a. Dissolve 100 mg of Coomassie Brilliant Blue G250 in 50 ml of 95% ethanol.
   b. Add 100 ml of 85% phosphoric acid and dilute the solution to 600 ml with distilled water.
   c. Filter the solution and supplement with 100 ml of glycerol, then adjust the volume to 1000 ml.
   d. Allow the solution to mature for 24 hours before use.
   e. The solution is suitable for use with BSA.
2. Prepare a range of standard protein solutions by transferring varying volumes from the stock solution (e.g., 0.2, 0.4, 0.6, 0.8, and 1.0 ml) into a series of test tubes and adjusting the volume to 1 ml.
3. Pipette 0.2 ml of the sample into two additional test tubes and adjust the volume to 1 ml.
4. Use a test tube containing 1 ml of water as a blank reference.
5. Add 5.0 ml of Coomassie Brilliant Blue to each tube and thoroughly mix by vortexing or inversion.
6. Allow the solutions to incubate for 10-30 minutes, then measure the absorbance of each standard and sample at 595 nm.
7. Construct a standard curve by plotting the absorbance of the standards against their known concentrations.
8. Create a graph showing the relationship between optical density and concentration. Use this graph to determine the protein content in the unknown sample.

Table 1: Observation table

S.No	Vol. of BSA (ml)	Conc. of BSA (mg/ml)	Vol. of Distilled water (ml)	Vol. of Bradford reagent (ml)	Incubation for 10 min	OD at 595 nm
1.						
2.						
3.						
4.						
5.						

4. Precautions:

1. Ensure all spectrophotometric equipment is calibrated and maintained according to manufacturer specifications.
2. Use high-purity chemicals and reagents to prepare Bradford reagent and protein samples.
3. Handle protein samples carefully to avoid contamination and denaturation.

4. Mix Bradford reagent and protein samples thoroughly but gently to ensure uniform reaction.
5. Incubate samples at the appropriate temperature and duration to allow sufficient color development.
6. Perform blank measurements using the Bradford reagent alone to correct for background absorbance.
7. Use a spectrophotometer to measure absorbance at the specific wavelength recommended for the Bradford assay.
8. Prepare a standard curve using known concentrations of protein standards to correlate absorbance with protein concentration.
9. Ensure protein standards cover the expected range of concentrations in your samples for accurate estimation.
10. Validate the linearity of the standard curve to ensure the assay adheres to the principles of the Bradford method.
11. Perform protein estimation in triplicate or more to ensure reproducibility and reliability of results.
12. Dispose of used reagents, protein samples, and contaminated materials according to laboratory waste disposal guidelines.

5. References:

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