

Estimation of Protein by Bradford method

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Aim: To estimate the protein using Bradford method.

Principle: The assay is based on the ability of protein to bind coomassie brilliant blue G250 and form a complex whose *extinction coefficient* is much greater than that of the free dye.

Reagents Required:

1. Dye Concentrate: Dissolve 100 mg of coomassie brilliant blue G250 in 50 ml of 95 % ethanol. Add 100 ml of concentrated orthophosphoric acid. Add distilled water to a final volume of 200 ml. store refrigerated in amber bottles; the solution is stable at least 6 months.

- Mix 1 volume of concentrated dye solution with 4 volumes of distilled water for use. Filter with Whatman No. 1 paper.

2. Protein Standard: 100 µg/ml in PBS.

Apparatus and Glass wares required: Test tubes, Pipettes, Colorimeter, etc.,

Procedure:

1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes. Also, Pipette out 1 ml of the given sample in another test tube.
2. Make up the volume to 1 ml in all the test tubes with PBS. A tube with 1 ml of distilled water serves as the blank.
3. Now add 5 ml of diluted dye solution to all the test tubes including the test tubes labeled 'blank' and 'unknown'.
4. Mix the contents of the tubes by vortexing / shaking the tubes and allow the colour to develop for at least 5 min but not more than 30 min. The red dye turns blue when it binds protein. Now record the absorbance at 595 nm against blank.
5. Then plot the standard curve by taking concentration of protein along X-axis and absorbance at 595 nm along Y-axis.
6. Then from this standard curve calculate the concentration of protein in the given sample.

Result: The given unknown sample contains ----µg protein/ml.

Observations and Calculations

| Volume of standard BSA (ml) | Volume of distilled water (ml) | Concentration of Protein (µg) | Volume of Biuret reagent (ml) | Allow to develop colour from 5 30 min | A595 |
|-----------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------------------------|------|
| 0.0 | 1.0 | 00 | 5 | | 0.00 |
| 0.2 | 0.8 | 1 | 5 | | |
| 0.4 | 0.6 | 2 | 5 | | |
| 0.6 | 0.4 | 3 | 5 | | |
| 0.8 | 0.2 | 4 | 5 | | |
| 1.0 | 0.0 | 5 | 5 | | |
| 1.0 UK | 0.0 | To be estimated | 5 | | |