Bradford Protein Assay

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[Abstract] The Bradford protein assay is used to measure the concentration of total protein in a sample. The principle of this assay is that the binding of protein molecules to Coomassie dye under acidic conditions results in a color change from brown to blue. This method actually measures the presence of the basic amino acid residues, arginine, lysine and histidine, which contributes to formation of the protein-dye complex. Unlike the BCA assay, reducing agents (*i.e.,* DTT and beta—mercaptoethanol) and metal chelators (*i.e.,* EDTA, EGTA) at low concentration do not cause interference. However, the presence of SDS even at low concentrations can interfere with protein-dye binding. This technique was invented by Bradford (1976).

Materials and Reagents

1. Bovine Serum Abumin (BSA) (Sigma-Aldrich)
2. Coomassie Brilliant Blue G-250 (Sigma-Aldrich, catalog number: 27815)
3. Methanol
4. Phosphoric acid (H_3PO_4)
5. Bradford reagent (see Recipes)

Equipment

1. Spectrophotometer (Tecan)
2. Whatman #1 paper (Whatman)

Procedure

A. Standard assay procedure (for sample with 5-100 µg ml^{-1} protein)

1. Prepare five to eight dilutions of a protein (usually BSA) standard with a range of 5 to 100 µg protein.
2. Dilute unknown protein samples to obtain 5-100 µg protein/30 µl.
3. Add 30 µl each of standard solution or unknown protein sample to an appropriately labeled test tube.
4. Set two blank tubes. For the standard curve, add 30 µl H_2O instead of the standard solution. For the unknown protein samples, add 30 µl protein preparation buffer instead. Protein solutions are normally assayed in duplicate or triplicate.

5. Add 1.5 ml of Bradford reagent to each tube and mix well.
6. Incubate at room temperature (RT) for at least 5 min. Absorbance will increase over time; samples should incubate at RT for no more than 1 h.
7. Measure absorbance at 595 nm.

B. Microassay procedure (<50 µg ml^{-1} protein):

1. Prepare five standard solutions (1 ml each) containing 0, 10, 20, 30, 40 and 50 µg ml^{-1} BSA.
2. Pipet 800 µl of each standard and sample solution (containing for <50 µg ml^{-1} protein) into a clean, dry test tube. Protein solutions are normally assayed in duplicate or triplicate.
3. Add 200 µl of dye reagent concentrate to each tube and vortex.
4. Follow the procedure described above for the standard assay procedure.

Recipes

1. Bradford reagent
   Dissolve 50 mg of Coomassie Brilliant Blue G-250 in 50 ml of methanol and add 100 ml 85% (w/v) phosphoric acid (H_3PO_4).
   Add the acid solution mixture slowly into 850 ml of H_2O and let the dye dissolve completely (*note: Do not add H_2O into the acid solution*).
   Filter using Whatman #1 paper to remove the precipitates just before use.
   Store in a dark bottle at 4 °C.

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References

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2. Stoscheck, C. M. (1990). Quantitation of protein. *Methods Enzymol* 182: 50-68.

