Bradford protein assay

Considerations for use

The Bradford assay is very fast and uses about the same amount of protein as the Lowry assay. It is fairly accurate and samples that are out of range can be retested within minutes.
The Bradford is recommended for general use, especially for determining protein content of cell fractions and assesing protein concentrations for gel electrophoresis.
Assay materials including color reagent, protein standard, and instruction booklet are available from Bio-Rad Corporation. The method described below is for a 100 µl sample volume using 5 ml color reagent. It is sensitive to about 5 to 200 micrograms protein, depending on the dye quality. In assays using 5 ml color reagent prepared in lab, the sensitive range is closer to 5 to 100 µg protein. Scale down the volume for the "microassay procedure," which uses 1 ml cuvettes. Protocols, including use of microtiter plates are described in the flyer that comes with the Bio-Rad kit.

Principle

The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible color change. The assay is useful since the extinction coefficient of a dye-albumin complex solution is constant over a 10-fold concentration range.

Equipment

In addition to standard liquid handling supplies a visible light spectrophotometer is needed, with maximum transmission in the region of 595 nm, on the border of the visible spectrum (no special lamp or filter usually needed). Glass or polystyrene (cheap) cuvettes may be used, however the color reagent stains both. Disposable cuvettes are recommended.

Procedure

Reagents

1. Bradford reagent: Dissolve 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol, add 100 ml 85% (w/v) phosphoric acid. Dilute to 1 liter when the dye has completely dissolved, and filter through Whatman #1 paper just before use.
2. (Optional) 1 M NaOH (to be used if samples are not readily soluble in the color reagent).

The Bradford reagent should be a light brown in color. Filtration may have to be repeated to rid the reagent of blue components. The Bio-Rad concentrate is expensive, but the lots of dye used have apparently been screened for maximum effectiveness. "Homemade" reagent works quite well but is usually not as sensitive as the Bio-Rad product.

Assay

1. Warm up the spectrophotometer before use.
2. Dilute unknowns if necessary to obtain between 5 and 100 µg protein in at least one assay tube containing 100 µl sample
3. If desired, add an equal volume of 1 M NaOH to each sample and vortex (see Comments below). Add NaOH to standards as well if this option is used.
4. Prepare standards containing a range of 5 to 100 micrograms protein (albumin or gamma globulin are recommended) in 100 µl volume. See how to set up an assay for suggestions as to setting up the standards.
5. Add 5 ml dye reagent and incubate 5 min.
6. Measure the absorbance at 595 nm.

Analysis

Prepare a standard curve of absorbance versus micrograms protein and determine amounts from the curve. Determine concentrations of original samples from the amount protein, volume/sample, and dilution factor, if any.

Comments

The dye reagent reacts primarily with arginine residues and less so with histidine, lysine, tyrosine, tryptophan, and phenylalanine residues. Obviously, the assay is less accurate for basic or acidic proteins. The Bradford assay is rather sensitive to bovine serum albumin, more so than "average" proteins, by about a factor of two. Immunoglogin G (IgG - gamma globulin) is the preferred protein standard. The addition of 1 M NaOH was suggested by Stoscheck (1990) to allow the solubilization of membrane proteins and reduce the protein-to-protein variation in color yield.

References

- Bradford, MM. A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry 72:* 248-254. 1976.
- Stoscheck, CM. Quantitation of Protein. *Methods in Enzymology 182:* 50-69 (1990).

Bicinchoninic Acid (BCA) Protein Assay (Smith)

Considerations for use

The bicinchoninic acid (BCA) assay is available in kit form from Pierce (Rockford, Ill.). This procedure is very applicable to microtiter plate methods. The BCA is used for the same reasons the Lowry is used. Stoscheck (1990) has suggested that the BCA assay will replace the Lowry because it requires a single step, and the color reagent is stable under alkaline conditions. Both a standard assay for concentrated proteins and a micro assay for dilute protein solutions are described below.

Principle

BCA serves the purpose of the Folin reagent in the Lowry assay, namely to react with complexes between copper ions and peptide bonds to produce a purple end product. The advantage of BCA is that the reagent is fairly stable under alkaline conditions, and can be included in the copper solution to allow a one step procedure. A molybdenum/tungsten blue product is produced as with the Lowry.

Equipment

In addition to standard liquid handling supplies a visible light spectrophotometer is needed with transmission set to 562 nm. Glass or polystyrene (cheap) cuvettes may be used.

Procedure 1 (standard assay)

Reagents

1. Reagent A: 1 gm sodium bicinchoninate (BCA), 2 gm sodium carbonate, 0.16 gm sodium tartrate, 0.4 gm NaOH, and 0.95 gm sodium bicarbonate, brought to 100 ml with distilled water. Adjust the pH to 11.25 with 10 M NaOH.
2. Reagent B: 0.4 gm cupric sulfate (5 x hydrated) in 10 ml distilled water.
3. Standard working solution (SWR): Mix 100 volumes reagent A with 2 volumes reagent B.
4. The stock solutions are stable. The working solution is stable for 1 week and should be green.

Assay

1. Prepare samples containing 0.2 to 50 micrograms protein in microliters.
2. Add 1 ml SWR to each 20 microliters sample and mix. Incubate 30 min. at 60 degrees C.
3. Cool the samples and read at 562 nm. Color will be stable for at least one hour.

Procedure 2 (micro assay)

Reagents

1. Reagent A: 8 gm sodium carbonate monohydrate, 1.6 gm sodium tartrate, brought to 100 ml with distilled water. Adjust the pH to 11.25 with 10 M NaOH.
2. Reagent B: 4 gm BCA in 100 ml distilled water.
3. Reagent C: 0.4 gm cupric sulfate (5 x hydrated) in 10 ml water.
4. Working solution: Mix 1 volume reagent C with 25 volumes reagent B, then add 26 volumes reagent A to the C/B mixture.

Assay

1. Prepare samples containing 0.2 to 50 micrograms protein in 500 microliters.
2. Add 500 microliters working solution to each 500 microliters sample and mix. Incubate 60 min. at 60 degrees C.
3.
4. Cool the samples and read at 562 nm.

Analysis
Prepare a standard curve of absorbance versus micrograms protein (or *vice versa*), and determine amounts from the curve. Determine concentrations of original samples from the amount protein, volume/sample, and dilution factor, if any. If you are unfamiliar with how to obtain a protein concentration for a diluted sample from a standard curve, see how to prepare and use a protein standard curve.

Comments
A longer incubation increases the sensitivity of the assay. The heating can be stopped earlier to prevent the color from becoming too dark. The assay can be performed at room temperature, but there is greater variability among proteins and the assay is less sensitive.

Reference
Stoscheck, CM. Quantitation of Protein. *Methods in Enzymology 182:* 50-69 (1990).