Lab.5: Determination of Protein Concentration by Bradford Assay (Coomassie Dye-based Protein Assay)

Protein quantitation is often necessary prior to handling protein samples for isolation and characterization.
Use of Coomassie G-250 Dye in a colorimetric reagent for the detection and quantitation of total protein was first described by Dr. Marion Bradford in 1976.Bradford Assay has been used to measure “high molecular weight proteins” during fermentation in the beer brewing industry.
The Bradford assay is a dye-binding assay used to measure the protein concentration of a solution.

Advantages of Bradford Assay

- It is the fastest and easiest to perform of all protein assays.
- It is performed at room temperature and no special equipment is required.
- It is not specific for any particular protein.
- It is compatible with most salts, solvents, buffers, thiols, reducing substances and metal chelating agents encountered in protein samples.

Disadvantages of Bradford Assay

- Incompatibility with surfactants.
- Since the Coomassie dye reagent is highly acidic, a small number of proteins cannot be assayed with this reagent due to their poor solubility in the acidic reagent.
- A standard curve must be generated each time you perform the assay.
- Coomassie dye stains the glass or quartz cuvettes used to hold the solution in the spectrophotometer.

Solutions and Reagents for Protein Determination

A. Coomassie Brilliant Blue G-250

1. Dissolve 100 mg of Coomassie Brilliant Blue G-250 in 50 ml of 95% ethanol.
2. Add 100 ml of 85% (w/v) phosphoric acid to solution above.
3. Complete its volume to 1000 ml by distilled water.
4. Filter through Whatman No.1 filter papers.
5. Store this reagent in dark and clean bottle at 4°C.

B. Bovine Serum Albumin (BSA) Stock Solution

1. Dissolve 0.01 gm of BSA in 10 ml of the distilled water.
2. Prepare 20,40,60,80 and 100 µg/ml concentrations from the solution above.
3. Use these concentrations to determine the standard curve of protein.

C. 0.15M Tris-HCl Stock Solution

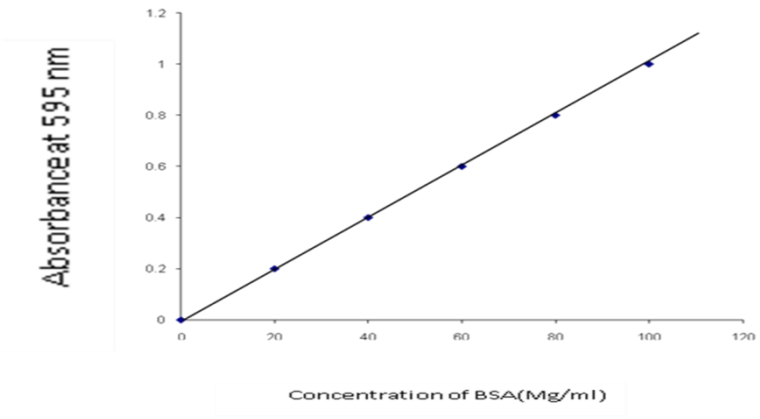
1. Dissolve 0.3 gm of Tris-HCl in 80 ml of the distilled water.
2. Adjust pH to 7.5.
3. Complete its volume to 100 ml by distilled water.

D. Reagent Blank

Mix 0.1ml of 0.15M Tris-HCl (pH 7.5) with 5ml of Coomassie Brilliant Blue G-250.

Procedure:

1. Add 0.1ml of each concentration of BSA to 5ml of Coomassie Brilliant Blue G-250 and mix by inversion.
2. Measure the absorbance at 595nm after 2 minutes against a reagent blank.
3. Plot standard curve of BSA between absorbance at 595nm and concentration of BSA.
4. Determine the concentration of protein in the sample at the same manner (by aid of standard curve of BSA).



In the acidic environment of the reagent, protein binds to the Coomassie dye. This results in a spectral shift from the reddish/brown form of the dye (absorbance maximum at 465 nm) to the blue form of the dye (absorbance maximum at 610 nm).The difference between the two forms of the dye is greatest at 595 nm, so that is the optimal wavelength to measure the blue color form of the Coomassie dye-protein complex. If desired, the blue color can be measured at any wavelength between 575 nm and 615 nm. At the two extremes (575 nm and 615 nm) there is a loss of about 10% in the measured amount of color (absorbance) compared to that obtained at 595 nm.