BIORAD ASSAY PROTEIN CONCENTRATION DETERMINATION

Bio-Rad Protein Assay Dye Reagent Concentrate is a colormetric assay for protein concentration. Similar to the Lowry assay, but with the following improvements: the reaction reaches 90% of it's maximum color development within 15 minutes and the color changes not more than 5% in 1 hour. The assay is based on the reaction of proteins with an alkaline copper tartrate solution and Folin reagent. A standard curve should be prepared each time the assay is preformed.

Materials:
Bio-Rad Protein Assay Dye Reagent Concentrate (cat#500-0006)
BSA standard (2µg/µl)
Disposable cuvettes (BioRad #223-9955)
Spectrophotometer (we use a Hitachi U-2000)

Procedure:
1) Dilute the BioRad assay dye 1:5 and mix well. Make enough for 1ml/sample plus 7ml for the standards. (note: it is useful to make enough for 2ml for each unknown sample plus a few extra mls in case any samples need to re-checked).

   10ml
   2 ml BioRad dye
   8 ml dH_2O

2) Prepare standards (for best results, the standards should always be prepared in the same buffer as the sample).
   • Dispense 1 ml aliquots of the diluted dye into 7 tubes.
   • Add the appropriate amount of BSA standard to each tube: (0, 0, 2, 4, 6, 8, 10µg)
   • Mix each tube well; there should be a gradient of blue color ranging from the lowest to highest amount of BSA added.

3) Prepare sample:
   • Dispense 1ml of the diluted dye to the number of tubes needed for each sample.
   • For each sample, add 1µl and 2µl respectively to 2 separate tubes.
   • Mix each sample well.

4) Transfer each sample to a disposable plastic cuvette.

5) Read each sample in the spectrophotometer (595l):

   1. Press the "Main Menu" button on the spec.
   2. Select #6 (Test Menu). ENTER
   3. Select #1 (Load Test). ENTER.
   4. Select #15 (BioRad AS). ENTER.
   5. Read the BSA Standards first.

• Place one of the “0” cuvettes in the “blank” chamber and place the other “0” cuvette in the “sample” chamber. Press “Auto Zero”. To begin reading, press “Start”.
• Remove the “0” cuvette from the sample chamber and place the “2 µg” sample there. Press “Start”.
• Repeat this with the 4, 6, 8, and 10µg sample.
• A graph will immediately print out following the 10µg sample. If your standard curve should be 0.995 or better to be considered accurate.

6. Place the first unknown sample in the sample chamber. Press “start”. The concentration will immediately appear; the unit of the sample will be ___µg/ how many µl of unknown sample you used.