Section 2
Instructions

2.1 Reconstituting the Standard

To reconstitute the lyophilized bovine gamma globulin and bovine serum albumin standards, add 20 ml of deionized water and mix until dissolved. If the standard will not be used within 60 days, it should be aliquoted and frozen at -20 °C.

Note: The standards contain buffer salts required for solubilizing the protein.

2.2 Standard Procedure

1. Prepare dye reagent by diluting 1 part Dye Reagent Concentrate with 4 parts distilled, deionized (DDI) water. Filter through Whatman #1 filter (or equivalent) to remove particulates. This diluted reagent may be used for approximately 2 weeks when kept at room temperature.

2. Prepare three to five dilutions of a protein standard, which is representative of the protein solution to be tested. The linear range of the assay for BSA is 0.2 to 0.9 mg/ml, whereas with IgG the linear range is 0.2 to 1.5 mg/ml. (See Common Questions, question 4, for more information.)

3. Pipet 100 µl of each standard and sample solution into a clean, dry test tube. Protein solutions are normally assayed in duplicate or triplicate.

4. Add 5.0 ml of diluted dye reagent to each tube and vortex.

5. Incubate at room temperature for at least 5 minutes. Absorbance will increase over time; samples should incubate at room temperature for no more than 1 hour.

6. Measure absorbance at 595 nm.

![Graph showing standard curves for BSA and IgG]

Fig. 1. Typical standard curve for the Bio-Rad Protein Assay, bovine gamma globulin (standard I), bovine serum albumin (standard II). O.D. at 595 nm corrected for blank: 200-1,400 µg/ml x 0.1 ml = 20-140 µg protein.

2.3 Microassay Procedure

1. Prepare three to five dilutions of a protein standard which is representative of the protein solution to be tested. The linear range of the assay for BSA is 1.2 to 10.0 µg/ml, whereas with IgG the linear range is 1.2 to 25 µg/ml. (See Common Questions, question 4, for more information.)

2. Pipet 800 µl of each standard and sample solution 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. Incubate at room temperature for at least 5 minutes. Absorbance will increase over time; samples should incubate at room temperature for no more than 1 hour.

5. Measure absorbance at 595 nm.