

Determination of Protein Content:

Bradford reagent:

Dissolve 100 mg coomassie brilliant blue – G250 in 50 ml of 95% ethanol, add 100 ml of 85 % w/v phosphoric acid then diluted by adding dis.H₂O to complete the volume to 1 L.

(keep in dark bottle).

After the dye has completely dissolved, filter through whatman #1 filter paper just before use. The reagent should be light brown .Filtration may have to be repeated to get rid of all blue components.

Preparation of bovine serum albumin protein assay standard:

Warm up the spectrophotometer for 15 min. before use.

- 1- Prepare a 5-fold dilution of a 1 mg/ml BSA (extension coefficient of BSA is 0.667) sample by adding 200 μ l of 1 mg/ml BSA to 800 μ l of distilled water to make 200 μ g/ml BSA.

- 2- Generate test sample for the reference cell, blank, BSA standards and the protein sample to be tested according to Table 1 in disposable cuvettes.
- 3- Note that a dilution of the unknown protein sample may be required for the resulting absorbance to fall within the linear range of the assay.
- 4- Allow each sample to incubate at room temperature for 5 minutes.
- 5- Measure the absorbance of each sample at 595 nm.
- 6- Use excel to plot the absorbance of each BSA standard as a function of its theoretical concentration. Determine the best fit of the data to a straight line in the form of the equation " $y=mX+b$ ", where y = absorbance at 595 nm and X = protein concentration.
- 7- Use this equation to calculate the concentration of the protein sample based on the measured absorbance. The linear range for the assay is 0.2 -0.8 O.D. units).

Number	(μ l)	Water (μ l)	Bradford reagent (μ l)
Blank	0	200	800
BSA standard (4 μ g/ml)	20	180	800
BSA standard (8 μ g/ml)	40	160	800
BSA standard (12 μ g/ml)	60	140	800
BSA standard (16 μ g/ml)	80	120	800
BSA standard (20 μ g/ml)	100	100	800
Protein sample (unknown)	100	100	800