

Protein Assay
Dye-binding method (Bradford)

Procedure:

- 1- Add 1.4 ml of 1X Bradford reagent in each microfuge tube.
- 2- Into the standard tubes place the following amounts of BSA protein standard (1 mg/ml).

<u>Tube</u>	<u>μl BSA</u>	<u>μl H₂O</u>	<u>μg protein added</u>
1	0.0	30.0	0.0
2	0.0	30.0	0.0
3	5.0	25.0	5.0
4	5.0	25.0	5.0
5	10.0	20.0	10.0
6	10.0	20.0	10.0
7	15.0	15.0	15.0
8	15.0	15.0	15.0
9	20.0	10.0	20.0
10	20.0	10.0	20.0
11	25.0	5.0	25.0
12	25.0	5.0	25.0
13	30.0	0.0	30.0
14	30.0	0.0	30.0

3- Add up to 30 μl of unknown (record volume) to each test tube, add the appropriate volume of H₂O to QS to 30 μl . Briefly vortex and compare to the standards. The unknown should be darker than the color in tubes 3 and 4 but less intense than tubes 13 and 14. If your sample does not fit into this range dilute at least in half and check again.

4- Determine the absorbance at 595 (vis lamp)

5- Plot the graph and find the linear regression line for the standard curve.

6- Divide the total protein in determined in the above step by the volume of the sample to get the mg/ml. Be certain to account for any dilutions of the unknown sample.