BIO-RAD Protein Assay:

A dye binding assay based on the differential color change of a dye in response to various concentrations of protein.

Based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs.

Procedure: For Microplate Readers

1. Prepare dye reagent by diluting one (1) part Dye Reagent concentrate with 4 parts distilled, deionized (DDI) water (a 1:5 dilution). Filter through Whatman #1 filter to remove particulates. This diluted reagent may by used for approximately 2 weeks when kept at room temperature.

2. Prepare three to five dilutions of a protein standard. The protein standard that we will use is Bovine Serum Albumin (BSA). The stock solution is 1 mg/ ml (1000µg/ml). {a 10µl aliquot of this stock solution will contain 10 µg, a 20 µl aliquot will contain 20 µg, ...

3. Assay the protein standards and protein solutions in triplicate.

4. To prepare standards for assay:
    a. Set up 6 test tubes with the following volume of water
        1. 100 µl water
        2. 97.5 µl water + 2.5 µl BSA from stock
        3. 95 µl water + 5 µl BSA from stock
        4. 90 µl water + 10 µl BSA from stock
        5. 80 µl water + 20 µl BSA from stock
        6. 60 µl water + 40 µl BSA from stock

5. To prepare unknown protein sample from tissues:
    a. Dilute your sample 1:50 and 1:100 with water (for 1:50 dilution, add 20µl of your sample from the tissue to 980 µl water: for the 1:100 dilution, **<u>either</u>** take 10 µl of your tissue sample and add to 990 µl of water, **OR** take 500 µl of your 1:50 dilution and add to 500 µl of water.

6. Add 10 µl of each standard to triplicate wells of the microtiter plate, and add 10 µl of each sample dilution to triplicate wells of the microtiter plate.

7. Add 200 µl of the diluted dye reagent to each well. Mix the solution in each well by repeatedly depressing the plunger of the pipette (you can use a multiwell pipette if available). Change the tip of the pipette after each mix.

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

9. Measure absorbance at 595 nm.

10. Plot the OD at 595 against the standard concentrations. This should give a relatively straight line. You can then interpolate from your standard curve the µg/ml that is present in your tissue samples by finding the OD of the tissue sample and dropping a line straight down to determine the concentration.

OR

11. You can plot the standard curve using Sigma Plot, obtain a regression line for the plot and determine the slope and "y" intercept. Using the equation: **Y= Mx + B**, where Y= OD, M=slope (b(1)), and B=y intercept (b(0)), you should be able to figure out the concentration of your sample.