MurphyLab@Emory

BIORAD PROTEIN ASSAY

Standard Curve Setup

1. In a borosilicate 13x100 mm tube, add 100 ul of γ-globulin standard (1.41 mg/ml) to 5.5 ml of QH2O for a final volume of 5.6 ml. This gives 25 ug/ml of protein.

2. Make duplicate dilutions of this standard as below in borosilicate 12X75 mm tubes.:

tube#	ug/ml standard	ul γ-globublin	ul QH2O
1	0 ug/ml	0 ul	800 ul
2	2.5	80	720
3	5.0	160	640
4	10	320	480
5	15	480	320
6	20	640	160
7	25	800	0

3. Make dilutions of unknown proteins into a final volume of 800 ul with QH2O, if protein sample is not limiting, try 1/8, 1/80 and 1/800 dilutions. For this example then, its 100 ul, 10 ul and 1 ul of unknown brought up to a final volume of 800 ul with QH2O.

4. Add 200 ul of Biorad dye reagent. Vortex each sample, and read OD595 with a single plastic cuvette. To do this, press 'Mode' on the spectrophotometer, then press '5', for Quantitative. If necessary, change parameters to 1) λ=595, 2) repeats = 2, 3) print data = yes, 4) standard curve = yes.

5. Calculate your unknown concentration based upon where its OD hits on the standard curve, and take into account your dilution factor. The spectrometer program will give you a value for your unknowns in ug/ml. Mulitply this value by your dilution factor (e.g.: 8X, 80X or 800X) to determine the protein concentration in you sample.