Bradford Protein Assay (Bio-Rad)

Adapted from existing protocols by Vinh Pham
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MATERIALS

Spectrophotometer
Immunoglobulin G, 1mg/ml
Resuspend lyophilized gamma globulin in dH_2O, store at -20ºC
Disposable 1cm lightpath cuvettes
Polystyrene (340nm-750nm)
Methacrylate (275nm-750nm)
13 x 100 mm disposable borosilicate test tubes
Bio-Rad Protein Assay Dye (Bradford) Reagent Concentrate (5X)

PROCEDURE

1. Add the following volumes of the IgG protein standard to separate test tubes:

$[IgG]_{final}$ in μg/ml	Dilution Series	
	IgG (1mg/ml), μl	dH_2O, μl
0	0	100
2.5	2.5	97.5
5	5	95
7.5	7.5	92.5
10	10	90
12.5	12.5	87.5
15	15	85
17.5	17.5	82.5
20	20	80

Note: The linear range of the assay for IgG is 1.2 to 25 μg/ml.

2. Prepare working solution of Bradford reagent. For each sample (including the IgG standards), prepare 0.9ml of working solution. The working solution is a mixture of Bradford reagent concentrate and water in a 2:7 ratio (e.g., 200μl Bradford reagent concentrate to 700μl water).

   Note: Always prepare about twice as much working solution as is needed for each experimental sample in order to dilute samples with OD_{595} readings outside the range of the IgG standard curve.

3. Prepare the experimental samples. Samples can be used as is, or they can be diluted in dH_2O. The final volume should be 100μl.

   Note: Duplicate or triplicate samples should be prepared for more accurate [protein] measurements.

4. Transfer samples to separate test tubes.

5. Add 900μl Bradford reagent (working concentration) to each standard and experimental sample.

   Note: The Coomassie Brilliant Blue G-250 dye shifts from 465nm to 595nm when binding to basic and aromatic amino acid residues, especially arginine (source: Bio-Rad Protein Assay manual).

6. Incubate at RT for at least 5 minutes.

7. Measure OD_{595} absorption. If necessary, add excess Bradford reagent (plus 100μl dH_2O to make it a 1X working solution) prepared in step 2 to dilute samples to standard range.

8. Plot standard curve in a graphing utility (e.g., Microsoft Excel).

9. Plot a trendline (in Excel, go to Chart>Add Trendline…; click Options tab, check box for Display equation on chart). If not using a graphing utility, use y=m(x-a)+b to calculate the equation for the trendline.

10. For each sample, the [protein] equals $(OD_{595}-b)(1/m)$(dilution factor). The dilution factor is the final volume divided by the volume of protein sample (100μl or less).

    Note: Adjust accordingly if excess working solution was added to dilute the sample. For example, if 5μl of protein sample was diluted with working solution to a final volume of 2ml (=2000μl), then the dilution factor is 2000/5, or 400.