Lowry Protein Assay (Bio-Rad)

Adapted from Bio-Rad DC *Protein Assay manual 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) or Methacrylate (275nm-750nm)
13 x 100 mm disposable borosilicate test tubes
Bio-Rad *DC* Protein Assay Reagents (A, B, & S)
0.1M Iodoacetamide in 0.1mM Tris, pH 8.0 (optional)

PROCEDURE

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

$[IgG]_{final}$ in μg/ml	Dilution Series	
	IgG (9.2mg/ml), μl	dH_2O+buffer*, μl
0	0	100
5	2.5	97.5
10	5	95
15	7.5	92.5
20	10	90
25	12.5	87.5
30	15	85
35	17.5	82.5
40	20	80

Note: The linear range of the assay for IgG is 5.0μg/ml to 250μg/ml.

**Use the buffer employed in the protein extraction step (e.g., cracking buffer). Add the same volume of buffer to the standard samples as the volume of the experimental samples, e.g., if 10μl of experimental sample is to be analyzed, then add 10μl of cracking buffer to each standard sample.*

2. Prepare working solution of reagent A by adding 10μl of reagent S to each 500μl of reagent A required (500μl per sample). Vortex and warm solution to 37ºC if a precipitate forms. This is the alkaline copper tartrate solution.

Note: Always prepare about twice as much working solution as is needed for each experimental sample in order to dilute samples with OD_{750} 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.

   Note: Reducing agents like dithiothreitol (DTT) and 2-mercaptoethanol (2-ME/_-ME) are not compatible with the Bio-Rad DC *Protein Assay. If these reducing agents are present in the protein extracts, add 100µl 0.1M iodoacetamide (in 0.1mM Tris, pH 8.0) to each experimental sample containing 2-ME (200µl for DTT), as well as to each standard. Vortex, incubate at 37ºC for 15', and let cool to RT for 5'. The iodoacetamide methylates (and, thus, inactivates) the reducing agents. Store the iodoacetamide solution in the dark and at 4ºC to prevent light-catalyzed formation of free radicals (leading to the formation of iodine, which turns the solution yellow).*

5. Add 500µl of reagent A (working concentration) to each standard and experimental sample. Vortex.

6. Add 4ml of reagent B to each test tube and vortex *immediately*. This is the Folin reagent, which, when reduced by the copper-treated protein (mostly by tyrosine and tryptophan residues, but also, to a lesser extent, by cystine, cysteine, and histidine) produces a blue color.

7. Incubate at RT between 15 minutes and 1 hour.

   Note: Ninety percent of the maximal color development is achieved in 15 minutes, and the color changes not more than 5% in 1 hour or 10% in 2 hours after the additon of reagents (source: Bio-Rad DC *Protein Assay manual).*

8. Measure OD_{750} absorption (absorbance measurements in the 650nm-750nm is also acceptable). If necessary, add excess reagent A (plus 100µl dH_2O to make it a 1X working solution) prepared in step 2, as well as the corresponding volume of reagent B, to dilute samples to standard range.

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

10. 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.

11. For each sample, the [protein] equals $(OD_{750}-b)(1/m)$(dilution factor). The dilution factor is 1000µl divided by the volume of protein sample (100µl or less).