Lowry Protein Assay

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The Lowry procedure is one of the most venerable and widely-used protein assays, being first described in 1951 [Lowry et al., *J. Biol. Chem.* 193: 265-275 (1951)]. Under alkaline conditions, copper complexes with protein. When folin phenol reagent (phospho-molybdic-phosphotungstic reagent) is added, the Folin-phenol reagent binds to the protein. Bound reagent is slowly reduced and changes color from yellow to blue.

While widely used, the Lowry procedure is less preferable an assay than some other protein assays since it is more subject to interference by a wide variety of chemicals. Among the chemicals reported to interfere with the Lowry procedure are barbital, CAPS, cesium chloride, citrate, cysteine, diethanolamine, dithiothreitol, EDTA, EGTA, HEPES, mercaptoethanol, Nonidet P-40, phenol, polyvinyl pyrrolidone, sodium deoxycholate, sodium salicylate, thimerosol, Tricine, TRIS and Triton X-100.

There is also much protein-to-protein variation in the intensity of color development. Ideally, the standard should be similar to the unknown. For example, if one is measuring IgG concentrations, an immunoglobulin standard would be ideal. For serum, use bovine serum albumin as a standard since albumin is a major component of serum.

An easy and accurate alternative, based on the binding of protein to Coomassie Blue G-250 dye, is the Bradford procedure. In addition, a modification of the Lowry procedure exists based on use of bicinchoninic acid (BCA) in place of the Folin-phenol reagent [Smith et al., *Anal Biochem.*150, 76-85 (1985)]. The BCA is less prone to interference than the Lowry procedure and is more sensitive.

Stock Solutions

Lowry A: 2% Na_2CO_3 in 0.1 M NaOH
Lowry B: 1% $CuSO_4$ in diH_2O
Lowry C: 2% sodium potassium tartrate ($NaKC_4H_4O_6 \cdot 4H_2O$)

Reagents

Lowry stock reagent
- 49 ml Lowry A
- 0.5 ml Lowry B
- 0.5 ml Lowry C

Folin's Reagent: Phenol reagent - 2N (Folin - Ciocalteau reagent). Dilute 1:1 in diH_2O before use.

Standard: Bovine serum albumin (BSA), lysozyme or other protein. If the standard is weighed out before use, make sure that the protein preparation used to prepare the standard is salt-free to avoid inaccurate results. The standard should be dissolved at a concentration of 1 mg/ml in a buffer similar to the solution the unknown is in (use PBS for biological samples)and diluted as follows:

micrograms protein standard	microliters standard	microliters buffer
0	0	100
10	10	90
20	20	80
30	30	70
50	50	50
75	75	25
100	100	0

Procedure

1) Add 100 ul of sample (sample + buffer = 100 ul) per tube.

2) Add 1.0 ml of Lowry stock reagent to each tube.

3) Incubate 30 min at room temperature.

4) Add 100 ml of Folin's reagent to each tube.

5) Incubate 30 min at room temperature.

6) Read in a spectrophotometer at 595 nm.

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