

PROTEIN DETERMINATION
Modified Lowry Method

PRINCIPLE:

Copper + Protein $\xrightarrow[\text{phenol reagent}]{\text{Alkaline pH}}$ Copper-protein complex (Blue color)

CONDITIONS: T = 25°C, A_{750nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 0.85% Sodium Chloride Solution (NaCl)
(Use Sodium Chloride Solution, 0.85%, Sigma Stock No. 430AG-4, **or** prepare 100 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)
- B. 0.01% Lowry Protein Standard (WPS)
(Prepare 10 ml in Reagent A using WPS prepared per Sigma "Working Protein Standard Procedure." See attached procedure.)
- C. Modified Lowry Reagent (LOW)
(Prepare by reconstituting in deionized water per label instructions a vial of Lowry Reagent, Modified, Sigma Prod. No. L-1013.)¹
- D. Protein Sample Solution (Sample)
(Prepare a solution containing 10 - 100 µg protein/ml of sample in Reagent A.)
- E. 330 mM Folin and Ciocalteu's Phenol Reagent (F & C)
(Prepare by diluting an appropriate volume of Folin and Ciocalteu's Phenol Reagent, Sigma Prod. No. F-9252 in deionized water and mix thoroughly. Prepare fresh immediately prior to use.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Blank</u>
Reagent A (NaCl)	----	0.90	0.70	0.50	0.30	----	1.00
Reagent B (WPS)	----	0.10	0.30	0.50	0.70	1.00	----
Reagent D (Sample)	1.00	----	----	----	----	----	----
Reagent C (Low)	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Mix thoroughly by swirling and incubate for 10 minutes at 25°C.² Then add:

Reagent E (F & C)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
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Mix thoroughly by vortexing and incubate for 30 minutes at 25°C.³ Transfer the solutions to suitable cuvettes and record the A_{750nm} for Test, Standards, and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$r A_{750nm} \text{ Standard} = A_{750nm} \text{ Std} - A_{750nm} \text{ Blank}$$

Prepare a standard curve by plotting the r A_{750nm} of the Standards vs µg of protein.

Sample Determination:

$$r A_{750nm} \text{ Test} = A_{750nm} \text{ Test} - A_{750nm} \text{ Blank}$$

Determine the µg protein from the Standard curve.

$$\mu\text{g Protein} = (\mu\text{g of protein from the Standard curve})(df)$$

df = Dilution factor

$$\% \text{ Protein} = \frac{(\mu\text{g Protein}) (100)}{(\mu\text{g solid/ml Reagent D})}$$

100 = Conversion to percentage

For Products that are liquid:

$$(\mu\text{g Protein})$$

$$\mu\text{g Protein/ml} = \frac{\text{_____}}{(\text{ml sample/ml Reagent D})}$$

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REFERENCES:

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) *J. Biol. Chem.* **193**, 265-275.

Peterson, G.L. (1979) *Analytical Biochemistry*, 100, 201-220

NOTES:

1. This solution can be stored for approximately one month or until the solution becomes cloudy.
2. Incubation time must be at least 10 minutes to allow for the copper solution to react with peptide bonds. This time can be as long as 24 hours if needed.
3. Incubation time must be at least 30 minutes but no longer than 60 minutes, because color development will continue after 30 minutes. All standards, samples, and blank absorbances must be read on spectrophotometer within a short time of each other.
4. This assay is based on the cited references.
5. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.