

PROTEIN DETERMINATION
Lowry/TCA Method

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

METHOD: Colorimetric

REAGENTS:

- A. 0.85% Sodium Chloride Solution (NaCl)
(Use Stock No. 430AG-4 **or** prepare 100 ml in deionized water using Sodium Chloride, Prod. No. S-9625.)
- B. 0.01% Lowry Protein Standard (LoPS)
(Prepare 10 ml in Reagent A using WPS prepared as per Working Protein Standard Procedure.)
- C. 100 mM Sodium Hydroxide Solution (NaOH)
(Prepare 1 liter in deionized water using Sodium Hydroxide, Prod. No. S-5881.)
- D. 2% (w/v) Sodium Carbonate Solution (NaCO₃)
(Prepare 1 liter in Reagent C using Sodium Carbonate, Prod. No. S-2127.)
- E. 2% (w/v) Sodium Potassium Tartrate Solution (SPT)
(Prepare 10 ml in deionized water using Sodium Potassium Tartrate, Tetrahydrate, Prod. No. S-2377.)
- F. 1% (w/v) Cupric Sulfate Solution (CuSO₄)
(Prepare 10 ml in deionized water using Cupric Sulfate, Pentahydrate, Prod. No. C-7631.)
- G. 50% Folin-Ciocalteu's Reagent (FC)
(Prepare 100 ml in deionized water using Folin-Ciocalteu Reagent, Prod. No. F-9252. **Store in an amber bottle-Sensitive to light. PREPARE FRESH.**)
- H. Trichloroacetic Acid (TCA)
(Use Stock No. 490-10.)

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REAGENTS: (continued)

- I. Protein Sample Solution (Pro)
(Prepare a solution containing 0.03 - 0.080 mg protein/ml of Sample in Reagent A.)

PROCEDURE:

Prepare the Lowry Reagent by pipetting (in milliliters) the following reagents into a suitable container:

Reagent D (NaCO ₃)	100.0
Reagent E (SPT)	1.0
Reagent F (CuSO ₄)	1.0

Mix.

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	0.80	0.60	0.40	0.20	1.00
Reagent B (LoPS)	----	0.10	0.20	0.40	0.60	0.80	----
Reagent I (Pro)	1.00	----	----	----	----	----	----

Mix. Then add:

Reagent H (TCA)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
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Mix thoroughly and place in an ice bath for 10 minutes. Centrifuge at 6000 rpm for 20 minutes. Discard the supernatant. Then add:

Reagent A (NaCl)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
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Mix thoroughly. Then add:

Lowry Reagent	5.00	5.00	5.00	5.00	5.00	5.00	5.00
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Mix thoroughly by vortexing and incubate for 10 minutes at 25°C. Then add:

Reagent F (FC)	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. Transfer to suitable cuvettes and record the

absorbance at 750 nm for Test, Standards, and Blank.

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CALCULATIONS:

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

Plotting the $r A_{750\text{nm}}$ Standards vs Protein concentration.

Sample Determination:

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

Determine the mg protein from the Standard curve.

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

For Products that are liquid:

$$\text{mg Protein/ml} = \frac{(\text{mg Protein}) (\text{Dilution})}{(\text{ml Reagent D})}$$

100 = Conversion to percentage

REFERENCES:

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

Thorne, C.J.R. (1978) *Techniques in Protein and Enzyme Biochemistry* 2-18.

NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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