

PROTEIN DETERMINATION
Biuret Method using TCA Precipitation

CONDITIONS: T = 25°C, A_{540nm}, 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. 1% Working Protein Standard (WPS)
 (Use WPS prepared as per Working Protein Standard Procedure.)
- C. Biuret Reagent (Biu)
 (Use Stock No. 540-2.)
- D. Trichloroacetic Acid (TCA)
 (Use Stock No. 490-10)
- E. Protein Sample Solution (Pro)
 (Prepare a solution containing 1 - 3 mg protein/ml of Sample in Reagent A.)

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)	----	0.98	0.95	0.90	0.70	0.50	1.00
Reagent B (WPS)	----	0.02	0.05	0.10	0.30	0.50	----
Reagent E (Pro)	1.00	----	----	----	----	----	----

Mix. Then add:

Reagent D (TCA)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
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Mix thoroughly and place the containers in an ice bath for 10 minutes. Centrifuge for 20 minutes in a clinical centrifuge. Remove and discard the supernatant. Then add:

	<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)	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Mix thoroughly until pellet dissolves. Then add:

Reagent C (Biu)	4.00	4.00	4.00	4.00	4.00	4.00	4.00
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Mix thoroughly and seal the containers. Incubate for 30 minutes at 25°C. Transfer to suitable cuvettes and record the absorbance at 540 nm for Test, Standards, and Blank.

CALCULATIONS:

$$A_{540\text{nm}} \text{ Standard} = A_{540\text{nm}} \text{ Std} - A_{540\text{nm}} \text{ Blank}$$

Find the slope (M) by plotting the $A_{540\text{nm}}$ Standards vs Protein concentration.

Sample Determination:

Determine the concentration of protein using the following equation:

$$\text{mg Protein} = \frac{A_{540\text{nm}} \text{ Test} - A_{540\text{nm}} \text{ Blank}}{(M)}$$

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

For Products that are liquid:

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

100 = Conversion to percentage

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REFERENCES:

Gornal, A.C., Bardawill, C.J. and David, M.M. (1949) *J. Biol. Chem.* **177**, 751-766.

Ryan, M.T. and Chopra, R.K. (1976) *Biochim. Biophys. Acta* **427**, 337-349.

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.