PROTEIN DETERMINATION
Biuret Method

PRINCIPLE:

Copper + Protein $\xrightarrow{\text{Alkaline pH}}$ Copper-protein complexes

CONDITIONS: T = 25°C, A_{540nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 0.85% (w/v) 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. 0.5% (w/v) Working Protein Standard (WPS)
(Use WPS prepared per Sigma "Preparation of Working Protein Standard for use in Protein Determination Procedure." (See attached procedure)

C. Biuret Reagent (Biuret)
(Use Total Protein Reagent, Sigma Stock No. 541-2.)

D. Protein Sample Solution (Protein)
(Prepare a solution containing 0.5 - 4 mg protein/ml of Sample in Reagent A.)

PROCEDURE:

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

	~~Test~~	~~Std 1~~	~~Std 2~~	~~Std 3~~	~~Std 4~~	~~Std 5~~	~~Blank~~
Reagent A (NaCl)	----	0.96	0.90	0.80	0.40	----	1.00
Reagent B (WPS)	----	0.04	0.10	0.20	0.60	1.00	----
Reagent D (Protein)	1.00	----	----	----	----	----	----

Mix by swirling. Then add:

Reagent C (Biuret)	4.00	4.00	4.00	4.00	4.00	4.00	4.00

Mix thoroughly by vortexing and incubate for 30 minutes at 25°C. Transfer to suitable cuvettes and record the absorbance at 540 nm for Test, Standards, and Blank.

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CALCULATIONS:

r A_{540nm} Standard = A_{540nm} Std - A_{540nm} Std Blank

Prepare a standard curve by plotting the A_{540nm} of the Standards vs mg of protein.

Sample Determination:

r A_{540nm} Sample = r A_{540nm} Test - A_{540nm} Test Blank

Determine the mg of protein using the Standard curve.

mg Protein = (mg of protein from the Standard curve)(df)

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

For Products that are liquid:

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

100 = Conversion to percentage

REFERENCES:

Gornall, A.G., 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. This assay is based on the cited references.

2. 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.