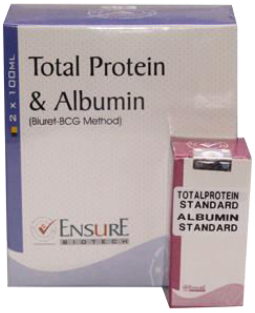
TOTAL PROTEIN & ALBUMIN



PRINCIPLE

Biuret method. The peptide bond of protein reacts with Copper ll ions in Alkaline solution to form a coloured Complex. The color formation is directly proportional to the Protein concentration in the sample and is measured at 540nm (520-560nm)
The most commonly used method for determination of Albumin is dye binding of which Bromocresol Green(BCG) is the most popular. Albumin in buffered medium binds with BCG causing proportional to the concentration in the sample

CLINICAL SIGNIFICANCE

Total protein is useful for measuring gross changes in protein levels caused by various disease states. Albumin is quantitatively the major single contributor to the Total protein. Measurement of the Total protein levels alone may be mis-leading and may be normal in view of the quite marked changes in the constituent protein. An Albumin Globulin ratio is often calculated to obtain additional information. Increased protein levels observed in dehydration and Diarrhea, where as in Multiple Myeloma increased protein levels with normal or low Albumin level is observed.
Low Albumin level ios usually found in severe liver diseases. Levels of protein &Albumin are low in malnutrition as well as severe renal disease. Increased globulin level is usually found in various infectious diseases. Decreased globulin levels is found in Hypogammaglobulinemia

SAMPLE COLLECTION

Fresh, Clean, Unhemolysed serum is preifered .

Serum is stable for 4 hours at RT and 2 days at 2-8°C.

Plasma collected with EDTA or oxalate may also be used

KIT CONTENTS & STORAGE

1 Total protein (Biuret) Reagent	1x100 ml	25x1ml
2 Albumin (BCG) Reagent	1x100 ml	25x1ml
3 Protein standard (5.5 gm/dl)	2 ml	1 Vial
4 Albumin standard	2 ml	1 Vial

The reagents are stable at room temperature till the expiry date mentioned on the label

SYSTEM PARAMETERS

	Total Protein	**Albumin**
Reaction Type	End point	End point
Slope of Reaction	Increasing	Increasing
Wave Length	546nm	630nm
Flow Cell Temp	37^0c	37^0c
Sample volume	10ul	10ul
Reagent volume	1.0 ml	1.0 ml
Standard	5.5	3.5
Incubationmin	5 min	1 min
Zero setting	Reagent Blank	Reagent Blank
Units	gm/dl	gm/dl

PROCEDURE

Pipette in a clean dry Test tubes labeled Blank (B) Standard(S),Test(T)

	B	**S**	**T**
Protein Reagent	1.0 ml	1.0 ml	1.0 ml
Standard		10 µl	
Sample			10 µl

Mix well incubate at RT for 5mins and then read the absorbance at 540nm

CALCULATIONS

Serum Protein Conc = (Abs of Test/Abs of Standard)x Conc of standard (5.5) gm/dl

Albumin

Pipette in a clean dry test tubes labeled Blank (B) Standard (S), Test(T)

	B	**S**	**T**
AlbuminReagent	1.0 ml	1.0 ml	1.0 ml
Standard		10 µl	
Sample			10 µl

Mix well incubate at RT for 3mins and then read the absorbance at 630nm

CALCULATIONS

Serum Albumin Conc = (Abs of Test/Abs of Standard)x Conc of standard (3.5) gm/dl

LINEARITY

This method is linear for Albumin and Protein up to 10 gm/dl. Sample exceeding 20 mg% should be diluted and re-assayed. The result has to be multiplied by the dilution factor.

EXPECTED VALUES

Total Proteins	:	6.00	-	8.5 gm/dl
Albumin	:	3.20	-	5.55 gm/dl

Due to variation in inter-laboratory assay conditions, instruments and demography it is recommended that each laboratory should establish its own normal range.

BIBILOGRAPHY:

1. Webster D clin chem. 23.663 (1977)
2. KalpanASzaboo LL, clin chemistry interpretation for Techniques p 403
3. Henry RJ Canon DC and Winkelman