TOTAL PROTEIN

(BIURET METHOD)

CHEMPAK

Last update 09-2020

Ref. CC3-TOP.045, 4x60 ml

INTENDED USE

Reagent kit for quantitative estimation of Total Protein in serum or plasma.

INTRODUCTION

The plasma proteins form a complex mixture in serum with varieties of functions. Many plasma proteins, including albumin, fibrinogen and almost all globulins are formed in the liver. Some of the globulins are produced by the reticuloendothelial system and plasma cells. All proteins fulfill some physiological and biological functions. Often the known biological property of a protein is the basis of a method for its detection and quantification.

Methods which have been devised for the determination of total protein include measurement of specific gravity, refractive index and absorbance of light in the ultra-violet region, and reactions of proteins with Folin Ciocalteau's, & Biuret reagent.

Historically, total protein was first determined by the Kjeldahl method which still remains as a reference method. The use of the biuret reaction to estimate protein in plasma was first introduced by Reigler. This was further improved by Gornell et al. by addition of sodium potassium tartrate to act as a complexing agent. Thus forming a stable copper-protein complex.

Total Protein uses biuret reaction where in protein reacts with the reagent in an alkaline medium to develop a blue-violet colored complex.

DIAGNOSTIC SIGNIFICANCE

Total protein estimation is usually performed in conjunction with other tests such as serum albumin, liver function tests or protein electrophoresis. An albumin/globulin ratio is often calculated to obtain additional information.
Increased levels are found in dehydration, multiple myeloma, chronic liver diseases and chronic infection while decreased levels are found in renal disease, malnutrition, albuminuria and terminal liver failure.

PRINCIPLE

In an alkaline medium, protein reacts with the copper in the biuret reagent causing an increase in absorbance. The increase in absorbance, at 540 nm (530-570 nm or with GREEN/YELLOW filter) due to formation of the coloured complex, is directly proportional to the concentration of protein.

Protein + Cu^{++} ⟶ Blue - Violet complex

PRESENTATION

	No. of Bottles 4 x 60 ml
• Total Protein Reagent (Biuret Reagent) Store at room temperature	4
• Total Protein Standard, 5 gm/dL Store at 2-8^{0}C. (Provided separately)	1

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• Copper Sulphate	15 mmol/L
• Sodium Potassium tartarate	50 mmol/L
• Potassium Iodide	4 mmol/L

Total Protein Standard (5 gm/dl)
Also contains non-reactive filters and Stabilizers.

PRECAUTION

The reagent offered in the kit is for *IN-VITRO* diagnostic use. Protect eyes from splashes of the reagent. Use of automated pipettes or dispensing devices is recommended.
Reagent contains Sodium Azide. DO NOT INGEST.

PREPARATION OF WORKING REAGENT

Total Protein reagent (Liquid) is ready to use.

REAGENT STORAGE AND STABILITY

The biuret reagent is stable at room temperature until the expiry date stated on the label.

SPECIMEN COLLECTION

Fresh, clear serum under fasting condition with no hemolysis is the specimen of choice, however, plasma may also be used. Anticoagulants such as heparin, oxalate, citrate, fluoride, EDTA can be used. Report as total plasma protein or Total serum protein as per the sample used.

REACTION PARAMETERS

- Type of Reaction : End Point
- Wavelength : 540 nm
- Flowcell Temp. : 30^{0}C
- Incubation Time : 10 min, at R. T.
- Std. Concentration: 5 gm / dL
- Sample volume : 0.05ml / 0.02 ml.
- Reagent Volume : 2.5 ml / 1.0 ml.
- Zero setting with : Reagent Blank
- Light Path : 1.0 cm.

TEST PROCEDURE

Pipette Into Test Tube	Procedure for 2.5 ml.			Procedure for 1 ml.		
	BLK	STD.	TEST	BLK	STD.	TEST
Reagent (ml)	2.5	2.5	2.5	1.0	1.0	1.0
Sample (ml)	-	-	0.05	-	-	0.02
STD. (ml)	-	0.05	-	-	0.02	-
Dist. Water (ml)	0.05	-	-	0.02	-	-

Mix well and allow to stand at room temperature for ten minutes. Read absorbance of test and standard at 540 nm (530-570 nm or with GREEN/YELLOW filter) against reagent blank.
The color of the final reaction mixture is stable for one hour.

TEST RESULTS

$$\text{Total protein concentration (gm/dL)} = \frac{\text{Abs. of test}}{\text{Abs. of Standard}} \times 5$$

$$\frac{\text{Albumin}}{\text{Globumin}} \text{ ratio} = \frac{\text{Albumin (gm/dl)}}{\text{(Total protein (gm/dl)-Albumin (gm/dl)}}$$

NORMAL VALUES

6 to 8.5 gm/dl

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LINEARITY

The biuret method for protein estimation is linear upto 10 gm/dl. For total protein values above 10 gm/dl dilute the sample suitably with 0.9% saline and repeat the assay. Apply proper dilution factor to obtain the test result.

NOTE

Total Protein is free from interferences from moderate levels of hemoglobin, bilirubin, salicylates and lipids, Severely lipemic or icteric samples require the use of a sample blank which may be prepared using 50 microliters of sample and 2.5 ml of distilled water.

REFERENCES

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