

Reagent kit for quantitative estimation of Total Protein in Serum or Plasma.

DIAGNOSTICS SIGNIFICATION:

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.



KIT PRESENTATION:

Pack Size	2 X 50 ml	2 X 100 ml	5 X 100 ml
Total Protein Reagent	2 X 50 ml	2 X 100 ml	5 X 100 ml
Total Protein Standard	1 X 1 ml	1 X 1 ml	1 X 1 ml

SPECIMEN COLLECTION:

Fasting, clear serum is preferred. Plasma also used. Report as Total plasma protein or Total serum protein as per the sample used.

REAGENT STORAGE AND STABILITY:

Total Protein reagent is stable at room temperature until expiry date printed on the label. **The standard is stable at 2-8°C until the expiry date indicated on the label.**

ASSAY PARAMETER:

Reaction : End point	Sample Volume : 20 µl
Wavelength : 540 nm	Reagent Volume : 1.0 ml
Zero Setting : Reagent Blank	Standard Conc. : 5 gm/dl
Incub.Temp. : RT	Linearity : 15 gm/dl
Incub Time : 5 min.	Unit : gm/dl

PROCEDURE:

Pipette into TT	Blank	Std	Test
Total Protein Reagent	1.0 ml	1.0 ml	1.0 ml
Total Protein Std (5 gm/dl)	--	20 µl	--
Sample (Test)	--	--	20 µl

Mix and incubate at RT for 5 minutes. Read absorbance of Standard (S) and Test (T) after 5 minutes against reagent blank at 540 nm (530-570 nm or with Green/Yellow filter).

STABILITY OF FINAL REACTION MIXTURE:

The color of the final reaction mixture is stable for 1 hour.

CALCULATION:

Total Protein concentration (gm/dl) = Abs T ÷ Abs S X 5

Many times, a ratio of Albumin to Globulin is considered. For getting the ratio, calculate globulin by using

Globulin = Serum Total Protein - Serum Albumin.

NORMAL VALUES:

Serum Total Protein: 6 to 8 gm/dl.
Each laboratory should establish its own reference range.

LINEARITY:

The procedure is linear up to 15 gm/dl. If values exceed this limit, dilute the sample with Distilled Water and repeat the assay. Multiply the result with proper dilution factor.

REFERENCE:

1. RODKEY F. L., Direct Spectrophometric Determination of Albumin in Human Serum, Clinical Chemistry 11, 478-487 (1965).
2. KAPLAN A., SZABO L.L., Chemistry; Clinical Interpretation and Techniques, 2nd Edition (1983) Lea & Febiger, Philadelphia, P-403

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