Total Protein

Colorimetric Test – Biuret Method

IVD CE

PRODUCT CODE
CS015

INTENDED USE
For the quantitative determination of Total Protein in serum or plasma

CLINICAL SIGNIFICANCE
Proteins form the major portion of dissolved substances in the plasma. They form the basic structural components of the body. They constitute the enzymes present in our body & also act as secondary source of energy. The other functions include distribution of water, buffering, transport of various components, defense & coagulation of blood in our body.
-Increased levels are found in dehydration & myeloma.
-Decreased levels are found in liver disorders, Nephrotic syndrome, malnutrition & protein due to haemorrhage.

PRINCIPLE
Protein in serum or plasma forms a blue/violet complex when mixed with copper ions in alkaline solution (Biuret reaction) each copper ion binding with 5 or 6 peptide bonds. Tartrate is added as a stabilizer and iodide is used to prevent auto reduction of the alkaline copper complex. The absorbance of this complex at 546 nm is proportional to the protein concentration.

REAGENT COMPOSITION
TOTAL PROTEIN (SL)

Sodium hydroxide	200 mmol/L
Potassium sodium tartrate	32 mmol/L
Copper Sulphate	18 mmol/L
Potassium Iodide	30 mmol/L
Protein Standard	
Protein standard concentration	8 g/dL

REAGENT PREPARATION
Reagent and standard are ready for use.

REAGENT STORAGE AND STABILITY
The color reagent and standard are stable up to the expiry date when stored at 2-8° C. Contamination after opening must be avoided.

SPECIMEN
Serum, heparinized or EDTA plasma, Serum is stable for 1 month at 2-8° C or 1 week at 15 - 25° C.

PRECAUTION
To avoid contamination, use clean laboratory wares.
Avoid direct exposure of reagent to light.

ASSAY

Wavelength	546nm
Cuvette	1 cm light path
Temperature	20-25°C
Measurement	Against reagent blank

PROCEDURE

Pipette into cuvettes	Blank	Standard	Sample
T. Protein reagent	1000 µL	1000 µL	1000 µL
Standard	--	20 µL	--
Sample	--	--	20 µL
Mix and incubate for 5 minutes at 20-25°C (RT). Measure the absorbance of the sample (As) and the standard (Astd) against the reagent blank within 30 minutes.			

CALCULATION

$$\text{Total Protein Conc. (g/dL)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 8 \text{ (Std.conc.)}$$

LINEARITY
The test is linear up to a protein concentration of 12 g/dl; Sample with a higher concentration should be diluted 1:1 with physiological saline (0.9%). Repeat the estimation and multiply the result by 2.

NORMAL RANGE

New born	(4.6 - 7.0) g/dL
Children/Adults	(6.6 - 8.7) g/dL

QUALITY CONTROL
All control sera with Protein values estimated by this method may be used.

NOTES
1- The color reagent contains sodium hydroxide which is an irritant. In case of contact with skin or mucous membrane wash immediately with water.
2- The standard contains sodium azide as a preservative. Do not swallow. Avoid contact with skin and mucous membranes.
3- Slight sediment may develop as the reagent ages. This may be removed by filtration or centrifugation.
4- Sample blank is needed for Haemolytic and lipemic sera. using the same procedure, pipette 0.02 ml serum into 1 mL of 0.9% saline, read absorbance against saline and subtract from As.

SYMBOL ON LABELS

Symbols	Signify	Symbols	Signify
REF	Catalogue Number	SIZE	Pack Size
	Expiry Date	VOL	Volume
	Storage Condition	LOT	Lot Number
	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests	2	For Single Use Only
EC REP	EC Representative	CE	European conformity

BIBILOGRAPHY

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