Total Protein

(Biuret Method)

Code : 10021 (2x50 ml)

(For the Analyser/Colorimetric Estimation of Total Protein in Serum / Plasma)

In VITRO USE Only.

SUMMARY & EXPLANATION OF TEST:

Among the various methods available for the quantitative analysis of Proteins such as salt fractionation, electrophoresis, ultracentrifugation, etc Kjeldahl's digestion method is considered as a reference method. However, this method is time consuming and cumbersome.

Biuret reagent incorporate some modifications to ensure optimum performance & greater stability. The Protein standard use is standardized by kjeldahl's digestion method. Hence, TOTAL PROTEIN Kit becomes ideal for the quantitative analysis of Total Proteins.

PRINCIPLE:

Proteins Bind with copper ions in the alkaline medium of Biuret reagent and produce a purple colored complex, whose absorbance is proportional to the Protein concentration.

REAGENTS:

1. Biuret Reagent 2 x 50 ml
2. Standard 3 ml

The reagents are ready to use and usable upto the expiration date when stored at room temperature. Standard which is provided separately should be stored at 2-8°C.

SAMPLE :

Serum / E.D.T.A. Plasma. Serum should be separated as soon as possible after collection. Grossly hemolyzed / turbid sample should not be used.

EXPECTED RANGE:

Total Proteins	:	6.0 to 8.0 gm%
Urinary Proteins	:	40 to 150 mg%/24 hours collection
CSF	:	15 to 45 mg%

LINEARITY:

Total Proteins	:	10 gm%

INSTRUCTIONS:

1. If standard (2) shows any visible bacterial or fungal contamination, consider it unsuitable for use and discard it.
2. The reagent and sample volumes may be altered roportionately to accommodate different pectrophotometer requirements.

DIRECTIONS FOR USE ON ANALYSERS:

Total Protein Assay

Reaction Type	:	End point with std.
Wave Length	:	555 nm (yellow-green filter)
Incubation Temp	:	37°C
Incubation Time	:	10 min.
Standard	:	Value stamped on the vial
Linearity	:	10 gm%
Unit	:	gm%

PROCEDURE:

I. Total Protein Assay :

Pipette into clean dry test tubes labelled Blank (B), Standard (S), and Test (T).

	B	S	T
Biuret reagent (1)	1.0ml	1.0ml	1.0ml
Distilled water	2.0ml	2.0ml	2.0ml
Standard (3)	-	0.05ml	-
Serum/plasma	-	-	0.05ml

Mix well and incubate at 37°C, for 10 minutes. Measure the absorbance of Standard(S), and Test (T) against Blank (B) on a photocolorimeter with yellow-green filter or on a spectrophotometer at 555nm (Hg 546nm).

II. Urinary & CSF Protein Assay :

Working reagent preparation : Dilute Biuret reagent (1) 1+2 with distilled water.
Auxiliary Reagent : T.C.A. 10% (w/v)

Step I Precipitation of protein :

	(Tu)	(Tc)
	For Urine	For CSF
Specimen	5.0 ml	1.0 ml
T.C.A 10% (w/v)	2.0 ml	0.5ml

Mix well and allow it to stand at RT for 15 minutes. Centrifuge at 3000 rpm to get a clear supernatant. Drain out any residual supernatant by inverting the centrifuge tubes on filter paper.

Step II Assay of precipitated protein :

	(B)	(S)	(Tu)	(Tc)
			For Urine Precipitate From Step 1	For CSF Precipitate From Step 1
Diluted Biuret Reagent	5.0ml	5.0ml	5.0ml	1.0ml
Standard (3)	-	0.1ml	-	-

Mix well and incubate 37°C for 10 minutes. Measure the absorbance of standard (S), Test (Tu) for urine and Test (Tc) for CSF against blank at 555nm.

CALCULATIONS:

a) Total proteins in gm% = $\frac{\text{A of (T)}}{\text{A of (S)}}$ x Std. Conc.

b) Urinary proteins in mg% = $\frac{\text{A of (Tu)}}{\text{A of (S)}}$ x 20xStd. Conc.

c) CSF proteins in mg% = $\frac{\text{A of (Tc)}}{\text{A of (S)}}$ x 20xStd. Conc.

NOTES:

★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.

★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

BIBLIOGRAPHY:

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