Audit Diagnostics

CE

TOTAL PROTEIN

Total protein

Biuret. Colorimetric

Quantitative determination of total protein
IVD

Store at 2-8ºC

PRINCIPLE OF THE METHOD

Proteins give an intensive violet-blue complex with copper salts in an alkaline medium. Iodide is included as an antioxidant.
The intensity of the color formed is proportional to the total protein concentration in the sample[1,4].

CLINICAL SIGNIFICANCE

The proteins are macromolecular organic compounds, widely distributed in the organism. They act like structural and transport elements. The proteins of the serum are divide in two fractions, albumin and globulins
The determination of total proteins is useful in the detection of:
- High protein levels caused by hemoconcentration like in the dehydrations or increase in the concentration of specific proteins.
- Low protein level caused by hemodilution by an impared synthesis or loss (as by hemorrhage) or excessive protein catabolism[4,5].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R Biuret	Sodium potassium tartrate	15 mmol/L
	Sodium iodide	100 mmol/L
	Potassium iodide	5 mmol/L
	Copper (II) sulphate	5 mmol/L
	Sodium hydroxide	1000 mmol/L
T PROTEIN CAL	Bovine albumin primary standard	7 g/dL

PRECAUTIONS

Causes severe skin burns and eye damage. H412-Harmful to aquatic life with long lasting effects.
Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

The reagents are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 540 nm ≥ 0,22.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 540 nm.
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or heparinized plasma[1]:
Stability of the sample: 1 month at refrigerator (2-8ºC).

PROCEDURE

1. Assay conditions:
   Wavelength: . . . . . 540 (530-550) nm
   Cuvette: . . . . . 1 cm. light path
   Temperature: . . . . . 37ºC / 15-25ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
R (mL)	1,0	1,0	1,0
Standard(Note 1,2,3) (µL)	--	25	--
Sample (µL)	--	--	25

4. Mix and incubate 5 min at 37ºC or 10 min at room temperature.
5. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

$$\frac{(A)\text{Sample} - (A)\text{Blank}}{(A)\text{Standard} - (A)\text{Blank}} \times 7(\text{Standard conc.}) = \text{g/dL of total protein in the sample}$$

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: CONTROL Normal and Pathologic
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Adults: 6,6 – 8,3 g/dL
Newborn: 5,2 – 9,1 g/dL
These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0,007 g/dL to linearity limit of 14 g/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (g/dL)	6,53	4,89	6,77	5,08
SD	0,01	0,01	0,07	0,05
CV (%)	0,21	0,24	1,05	0,94

Sensitivity: 1 g/dL = 0,0825 A.
Accuracy: Results obtained using Audit Diagnostics reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0,97002
Regression equation y= 0,954x +0,511.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemoglobin and lipemia[1,4].
A list of drugs and other interfering substances with total protein determination has been reported by Young et. al[2,3].

NOTES

1. T PROTEIN CAL: Proceed carefully with this product because due its nature it can get contamined easily.
2. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.
4. **Audit Diagnostics has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

BIBLIOGRAPHY

1. Koller A. Total serum protein. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1316-1324 and 418.
2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
3. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
4. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
5. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

Manufactured By: AUDIT DIAGNOSTICS
Business & Technology Park, Carrigtwohill, Co. Cork (Ireland)
Tel: 00353 - (0) 21 – 4533 652 *Fax:* 00353 - (0) 21 – 4533 653
E-mail: info@auditdiagnostics.ie *Website:* www.auditdiagnostics.ie