Total protein Biuret Colorimetric

IVD *For In-Vitro diagnostic and professional use only*

Store at 2° to 8° C

INTENDED USE

For the quantitative determination of total protein concentration in human serum or plasma.

INTRODUCTION

Proteins are macromolecular organic compounds, widely distributed in the body. They act as structural and transport elements. Serum proteins are divided into albumin and globulins.

The determination of total proteins is useful in the detection of:

- High protein levels caused by hemoconcentration like in dehydration or increase in the concentration of specific proteins.
- Low protein levels caused by hemodilution by impared synthesis or loss (as by hemorrhage) or excessive protein catabolism.

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

PRINCIPLE

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 sample.

REAGENTS

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

EQUIPMENTS NEEDED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 540 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

PRECAUTIONS

- Copper (II) sulphate: Environmentally dangerous (N).
- R: Harmful to aquatic organisms may cause long-term adverse effects in the aquatic environment.
- This material and its container must be disposed of as hazardous waste.
- Avoid release to the environment. Refer to special instructions/safety data sheets.

PREPARATION

Reagents are ready to use.

STORAGE AND STABILITY

- All components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C 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 $\geq$ 0.22.

SAMPLES

Serum or heparinized plasma.

Sample is stable for 1 month in the refrigerator (2-8°C).

PROCEDURE

1. Assay conditions:
   Wavelength:…… 540 (530-550) nm
   Cuvette light path: ……..1 cm.
   Temperature:……37°C / 15-25°C
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 (µL)	--	25	--
Sample (µL)	--	--	25

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

CALCULATIONS

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

REFERENCE VALUES

Adults: 6.6 – 8.3 g/dL

Newborn: 5.2 – 9.1 g/dL

NOTE

These values are for orientation purposes; each laboratory should establish its own reference range.

QUALITY CONTROL

- 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.

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 to half 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 (%)	0.15	0.20	1.03	0.98

Sensitivity:

1 g/dL = 0,0825 A.

Accuracy:

Results obtained using ATLAS reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were as follow:

Correlation coefficient $(R)^2$: 0.97002.

Regression equation: y= 0.954x + 0.511.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemoglobin and lipemia.
A list of drugs and other substances interfering with total protein determination has been reported.

NOTES

1. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
2. Use clean disposable pipette tips for its dispensation.
3. Total protein standard: Proceed carefully with this product as, due its nature, it can get contaminated easily.

REFERENCES

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.
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PPI1463A01
Rev A (02.09.2019)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry