

Total Protein. Biuret. Colorimetric.
 Quantitative determination of Total Protein
 Only for in vitro use in clinical laboratory
 Store at 2-8°C

Ref.: PRO-013

2x125 mL

TOTAL PROTEIN



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 divided into 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 impaired 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	Sodium potassium tartrate	15 mmol/L
	Sodium iodide	100 mmol/L
	Potassium iodide	5 mmol/L
	Copper (II) sulphate	19 mmol/L
T PROTEIN CAL	Bovine albumin primary standard 7 g/dL	

PRECAUTIONS

Copper (II) sulphate: Environmentally dangerous (N); R52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
 S60: This material and its container must be disposed of as hazardous waste. S61: Avoid release to the environment. Refer to special instructions/safety data sheets.

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 2-8°C protected from light and contaminations prevented during their use.
 Do not use reagents over the expiration date.

T PROTEIN CAL Once open is stable up to 1 month when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 540 nm \geq 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 plasma¹:
 Stability of the sample: 1 month at refrigerator (2-8°C).

PROCEDURE

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

	Blank	Standard	Sample
R (mL)	1.0	1.0	1.0
Standard ^(Note 1-2) (µL)	--	25	--
Sample (µL)	--	--	25

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

CALCULATIONS

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

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.
 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 VALUES¹

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 0.20 g/dL to linearity limit of 15 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)	5.07	9.64	5.15	9.74
SD	0.04	0.08	0.06	0.14
CV (%)	0.88	0.90	1.23	1.43

Sensitivity: 1 g/dL = 0.07 A.

Accuracy: Results obtained using BSM 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): 0.9918.

Regression equation: $y = 1.0164x - 0.1264$.

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

- Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
- Use clean disposable pipette tips for its dispensation.
- BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

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