TOTAL PROTEIN CE

CONTENTS			
REF	1153005	Total Protein	2 x 50 mL
	1153010	Total Protein	4 x 100 mL
	1153020	Total Protein	4 x 250 mL
For *in vitro* diagnostic use only			

PROTEIN

TOTAL

Colorimetric method

ENDPOINT

PRINCIPLE

In the biuret reaction, a chelate is formed between the Cu^{2+} ion and the peptide bonds of the proteins in alkaline solutions to form a violet colored complex whose absorbance is measured photometrically. The intensity of the color produced is proportional to the concentration of protein in the sample.[1-2]

$$Cu^{2+} + \text{Serum protein} \xrightarrow[25\text{-}37^{\circ}C]{pH > 12} \text{Copper-protein complex}$$

REAGENT COMPOSITION

R1 **Biuret reagent**. Cupric sulfate 6 mmol/L, sodium-potassium-tartrate 21 mmol/L, potassium iodide 6 mmol/L, sodium hydroxide 0.75 mol/L. **C R:34**

CAL **Protein standard**. Bovine serum albumin 7 g/dL (70 g/L). Secondary standard traceable to SRM 927.

STORAGE AND STABILITY

Store at 2-8ºC.
The Reagent and Standard are stable until the expiry date stated on the label.

REAGENT PREPARATION

The reagents are ready-to-use.

SAMPLES

Serum, EDTA or heparinized plasma, and exudates.
Total protein is stable in serum and plasma for 1 week at room temperature, for at least 1 month refrigerated at 2-8ºC, and for up to 2 months at –20ºC.

INTERFERENCES[3]

- Grossly hemolytic or lipemic samples result in a positive interference.
- Dextrans used as plasma volumen expanders for the treatment of low blood pressure, complex with copper and tartrate forming a precipitate.

MATERIALS REQUIRED

- Photometer or colorimeter capable of measuring absorbance at 540 ± 20 nm.
- Constant temperature incubator set at 37ºC.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Pipette into labelled tubes:

TUBES	Blank	Sample	Standard
Biuret	1.0 mL	1.0 mL	1.0 mL
Sample	–	20 µL	–
Standard	–	–	20 µL

2. Mix and incubate the tubes 10 minutes at 37ºC.
3. Read the absorbance (A) of the samples and the standard at 540 nm against the reagent blank.

The color is stable for at least 1 hour.

CALCULATIONS

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = \text{g/dL total protein}$$

If results are to be expressed as SI units apply: g/dL x 10 = g/L.

REFERENCE VALUES[4]

Serum, plasma

Adults	6.6-8.7 g/dL (66-87 g/L)
Prematures	3.6-6.0 g/dL (36-60 g/L)
Newborns	5.3-8.9 g/dL (53-89 g/L)
Pregnancy	Concentration lowers from 69 to 61 g/L

Total serum protein is lower by 4 to 8 g/L with the subject supine than with the subject ambulatory.

Plasma

Plasma protein is 2 to 4 g/L higher due to the presence of fibrinogen in the sample.

Joaquim Costa, 18, 2ª planta. 08390 Montgat – Barcelona (Spain). Tel. (+34) 93 4694990 Fax (+34) 93 4693435
Almacén: Sant Antoni Mª Claret, 8 bis. 08390 Montgat – Barcelona (Spain) www.linear.es e-mail: info@linear.es

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.
To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of total protein. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of total protein. Assayed.

CLINICAL SIGNIFICANCE

The serum content of the soluble proteins, those circulating in extracellular and intracellular fluids, has been used as a marker to aid in clinical diagnosis. The main diagnostic tests are those measuring serum total protein and serum albumin.
Collectively, serum total protein including albumin is mainly involved in the maintenance of normal water distribution between tissues and the blood and responsible for maintaining the oncotic pressure of plasma and is used to transport many substances including macromolecules.

Hiperproteinemia o hiperalbuminemia, usually occurs during multiple mieloma caused by high levels of the monoclonal immunoglobulins, dehydration, excessive water loss, as in severe vomiting, diarrhea, Addisons´s disease or diabetic acidosis. The *hemoconcentration*, decrease in the volume of plasma water, is reflected as a relative hyperproteinemia since concentration of all the individual plasma proteins are increased to the same degree.

Hypoproteinemia o hypoalbuminemia usually occurs in edema, malnutrition, nephrotic syndrome, malabsortion and severe liver cirrhosis. Since albumin is present in such high concentration low levels of this protein alone may also cause hypoproteinemia.

ANALYTICAL PERFORMANCE

- ***Linearity.*** Up to 12 g/dL
- ***Precision***

mg/dL	Within-runl			Between-runl		
Mean	4.91	5.76	7.22	4.91	5.76	7.3
SD	0.03	0.03	0.06	0.03	0.029	0.02
CV%	0.61	0.52	0.83	0.67	0.5	0.27
N	10	10	10	10	10	10

Replicates: 10 for each level. Replicates: 10 for each level
Instrument: CECIL CE 2021 for 8 days.

- ***Sensitivity.*** Using a 1:50 sample/reagent at 540 nm, 1 g of protein will produce a net absorbance of approximately 0.076.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 25 r = 0.985 y = 0.962x + 0,2727

REFERENCES

1. Gornall, A.G., Bardawill, C.S., and David, M.M. J. Biol. Chem. 177 : 751 (1949).
2. Falkner, W.R., and Meites, S. Selected Methods of Clinical Chemistry, 9, 319, AACC., Washington, D.C. (1982).
3. Young, D.S., Pestaner, L.G. and Gibberman, P. Clin. Chem. 21, Vol. 5, 10-432D (1975).
4. Tietz. N.W. Fundamentals of Clinical Chemistry, p. 940. W.B. Saunders Co. Philadelphia, PA. (1987).

B1153-1/0312
R1.ing

Joaquim Costa, 18, 2ª planta. 08390 Montgat – Barcelona (Spain). Tel. (+34) 93 4694990 Fax (+34) 93 4693435
Almacén: Sant Antoni Mª Claret, 8 bis. 08390 Montgat – Barcelona (Spain) www.linear.es e-mail: info@linear.es