TOTAL PROTEIN CE

REF 1153005	REF 1153010	REF 1153020
2 x 50 mL	4 x 100 mL	4 x 250 mL
CONTENTS	**CONTENTS**	**CONTENTS**
R1.Reagent 2 x 50 mL	R1.Reagent 4 x 100 mL	R1.Reagent 4 x 250 mL
CAL.Standard 1 x 3 mL	CAL.Standard 1 x 3 mL	CAL.Standard 1 x 3 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]

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

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). Concentration value is traceable to Standard Reference Material 927.

STORAGE AND STABILITY

Store at 2-8ºC.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.
Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 540 nm > 0.150 in 1cm cuvette.

REAGENT PREPARATION

The Reagent and Standard are ready-to-use.

SAMPLES

Serum or heparinized plasma.
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

- Lipemia (intralipid) may affect the results.
- Bilirubin (20 mg/dL) does not interfere.
- Hemoglobin may affect the results.
- Other drugs and substances may interfere[3].
- Dextrans used as plasma volume 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	CAL. Standard
R1.Biuret	1.0 mL	1.0 mL	1.0 mL
Sample	–	20 µL	–
CAL. Standard	–	–	20 µL

2. Mix and incubate the tubes 5 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

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

Samples with concentrations higher than 12 g/dL should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

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 higuer 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.

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

LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN
Telf. (+34) 934 694 990; E-mail: info@linear.es ; website: www.linear.es NIF-VAT:B60485687

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.

If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

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.

Hyperproteinemia o hyperalbuminemia, usually occurs during multiple myeloma 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 or 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

- ***Detection Limit :*** 0.31 g/dL
- ***Linearity :*** Up to 12 g/dL
- ***Precision:***

g/dL	Within-run		Between-run	
Mean	4.33	8.99	4.33	8.99
SD	0.05	0.14	0.07	0.24
CV%	1.20	1.59	1.58	2.65
N	10	10	10	10

- ***Sensitivity :*** 0.05 A / g/dL proteins.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:

  N = 64 r = 0.95 y = 0.99x + 0,20

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

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 DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Tietz. N.W. Fundamentals of Clinical Chemistry, p. 940. W.B. Saunders Co. Philadelphia, PA. (1987).

B1153--2/0901
R1.ing

LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN
Telf. (+34) 934 694 990; E-mail: info@linear.es ; website: www.linear.es NIF-VAT:B60485687