COD 11800 1 x 50 mL	COD 11500 2 x 250 mL	COD 11572 1 x 250 mL	COD 11553 1 x 1 L
STORE AT 2-30ºC			
Reagents for measurement of protein concentration Only for *in vitro* use in the clinical laboratory			

PROTEIN (TOTAL)

BioSystems

CE

PROTEIN (TOTAL)
BIURET

PRINCIPLE OF THE METHOD

Protein in the sample reacts with copper (II) ion in alkaline medium forming a coloured complex that can be measured by spectrophotometry[1].

CONTENTS

	COD 11800	COD 11500	COD 11572	COD 11553
A. Reagent	1 x 50 mL	2 x 250 mL	1 x 250 mL	1 x 1 L
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent. Copper (II) acetate 6 mmol/L, potassium iodide 12 mmol/L, sodium hydroxide 1.15 mol/L, detergent.

DANGER: H314: Causes severe skin burns and eye damage. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

S. Protein Standard. Bovine albumin. Concentration is given on the label. Concentration value is traceable to the Standard Reference Material 927 (National Institute of Standards and Technology, USA).

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Reagent (A): Store at 2-30ºC.

Protein Standard (S): Store at 2-30ºC, once opened.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.150 at 545 nm.

– Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer able to read at 545 ± 20 nm

SAMPLES

Serum or heparinized plasma collected by standard procedures. Stable for 4 weeks at 4-8ºC[2].

Anticoagulants other than heparin should not be used.

PROCEDURE

1. Pipette into labelled test tubes: (Note 1)

	Blank	Standard	Sample
Distilled water	20 µL	—	—
Protein Standard (S)	—	20 µL	—
Sample	—	—	20 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

2. Mix thoroughly and let stand the tubes for 10 minutes at room temperature.
3. Read the absorbance (A) of the Standard and the Sample at 545 nm against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

The protein concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = C_{Sample}$$

REFERENCE VALUES

Serum, adults[3]:

Ambulatory	64-83 g/L
Recumbent	60-78 g/L

Concentrations are lower in child. Plasma total protein concentration is 2 to 4 g/L higher due to the presence of fibrinogen as well as some other trace proteins[3].

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 4.6 g/L

– Linearity limit: 150 g/L For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean Concentration	CV	n
44 g/L	1,1 %	20
57 g/L	0,9 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
44 g/L	1,8 %	25
57 g/L	1,9 %	25

– Sensitivity: 5 mA·L/g

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.

– Interferences: Hemoglobin (2.5 g/L) and lipemia interfere. Bilirubin (20 mg/dL) does not affect the results. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Most of the plasma proteins are synthesized by the liver. The major exception to this is the immunoglobulins which are produced by plasma cells found in the spleen, lymph nodes and bone marrow.

The two general causes of alterations of serum total protein are a change in the volume of plasma water and a change in the concentration of one or more of the serum proteins.

Hyperproteinemia can be caused by dehydration (inadequate water intake, severe vomiting, diarrhea, Addison's disease, diabetic acidosis) or as a result of an increase in the concentration of specific proteins (immunoglobulins in chronic infections, multiple myeloma)[3,5].

Hypoproteinemia may be caused by hemodilution (salt retention syndromes, massive intravenous infusions), by an impaired synthesis (severe malnutrition, chronic liver disease, intestinal malabsorptive disease), or by an excessive protein loss due to a chronic kidney disease or severe burns[3,5].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. This reagent may be used in several automated analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

1. Gornall AG, Bardawill CS, David MM. Determination of serum proteins by means of the Biuret reaction. *J Biol Chem* 1949; 177: 751-766.
2. Word Health Organization (WHO). Use of anticoagulants in diagnostic laboratory investigations. Document WHO/DIL/LAB/99.1, Rev.2; 2002. http://www.who.int/iris/handle/10665/65957.
3. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
5. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.