ARENA BioScien

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TOTAL PROTEIN– Biuret Reagent

Diagnostic reagent for the in-vitro quantitative determination of total protein in human serum and plasma on both manual and automated systems.

REF: BS.1/TP02.125.0250	250 test	REF: BS.1/TP01.500.0500	500 test
REF: BS.1/TP01.250.0250	250 test	REF: BS.1/TP04.250.1000	1000 test

CLINICAL SIGNIFICANCE [1-3]

Most of the plasma proteins are synthesized by the liver, with the exception of immunoglobulins which are produced by plasma cells found in the spleen, lymph nodes and bone marrow. Plasma proteins are involved in the maintenance of normal water distribution between tissues and blood, as well as acid-base balance. Total protein is useful for monitoring gross changes in protein levels caused by various disease states. It is usually performed in conjunction with other tests such as serum albumin, liver function tests or protein electrophoresis. An albumin/globulin ratio is often calculated to obtain additional information. Increased levels of serum protein are observed in dehydration (severe vomiting, diarrhea, Addison's disease, diabetic acidosis), multiple myeloma and chronic liver disease. Decreased levels are encountered by an impaired synthesis (severe malnutrition, chronic liver disease, intestinal mal-absorptive disease), or by an excessive protein loss due to a chronic kidney disease or severe burns.

METHOD PRINCIPLE [4]

Proteins give the characteristic pink to purple biuret complex with copper salts in an alkaline medium. The intensity of the color formed is proportional to the total protein concentration in the sample, and its absorbance is spectrophotometry measured at 545nm.

$$\text{Protein} + Cu_2 \xrightarrow{\text{Alkaline pH}} \text{Cu – protein complex}$$

Iodide is included as an antioxidant, and sodium potassium tartrate prevents copper hydroxide precipitation.

REAGENT COMPOSITION

R1: Standard	6 g/dl
R2: Reagent	
Sodium potassium tartrate	40.9 mmol/L
Sodium hydroxide	750 mmol/L
Potassium iodide	19.8 mmol/L
Copper (II) sulphate	12 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagents contain sodium hydroxide irritant (xi) chemical and copper (II) sulphate that is an environmentally dangerous reagent.

R36/38: Irritating to eyes and skin.

R52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment.

For further information, refer to the total protein reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien Total protein reagents are supplied ready-to-use. All reagents are stable until expiration date stated on label when properly stored refrigerated at 15-25°C. Only standard is needed to be kept refrigerated at 2-8°C.

Deterioration

The ***BioScien*** Total protein reagent is normally clear, do not use reagent if it is turbid or if the absorbance of blank is greater than 0.150 at 545 nm.

SPECIMEN COLLECTION AND PRESERVATION [5]

Serum or plasma

Specimen should be separated from the cells within 4 hours after blood collection. The only acceptable anticoagulants are heparin and EDTA, usually plasma results are higher due to fibrinogen. Stability is 1 day at 15-25°C; 4 weeks at 2-8°C and 6 months at -20°C.

SYSTEM PARAMETERS

Wavelength	545 nm (530 – 570 nm)
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample Reagent Ratio e.g.: Reagent volume Sample volume	1:50 1 ml 20 µl
Temperature	15-25°C
Incubation time	10 min at 15-25°C
Zero adjustment	Reagent blank
Sensitivity	1.0 g/dl
Linearity	12 g/dl

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	Blank	*Standard*	*Specimen*
Reagent	1.0 ml	1.0 ml	1.0 ml
Standard		20 µl	
Specimen			20 µl

Mix, incubate for 10 minutes at room temp. Measure absorbance of specimen and standard against reagent blank within 30 minutes.

CALCULATION

Total protein concentration (g/dl) = $\frac{\text{Abs. specimen}}{\text{Abs. standard}} \times 6$

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact ***BioScien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean g/dl	5.2	7.23	5.7	7.32
SD.	0.12	0.15	0.19	0.21
CV. %	2.47	2.2	2.53	2.24

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from ***BioScien*** Total Protein reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.978.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 1.0 g/dl Total Protein.

Linearity
The reaction is linear up to Total Protein concentration of 12 g/dl. Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result×2).

INTERFERING SUBSTANCES [6]

Haemolysis
No significant interference up to hemoglobin level of 7.5 g/L.

Icterus
Serum bilirubin levels higher than 30 mg/dl have a positive interference in Total Protein assay.

lipemia
Lipid may cause positive interference at absorbance higher than 0.150 measured at 600nmd.

Others
Other drugs and substances may interfere.

EXPECTED VALUES [5]

Serum and plasma	***g/dl***
Premature	3.6-6.0
Newborns	4.6-7.0
Children >1 year	4.8-7.5
Children 1-2 year	5.6-7.5
Children >3 year	6.0-8.0
Adult	6.6-8.3

DYNAMIC RANGE

1.0 – 12 g/dl.

REFERENCES

1. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
2. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
3. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001
4. Gornall AG, Bardawill CS, David MM. Determination of serum proteins by means of the Biuret reaction. J Biol Chem 1949; 177: 751-766.
5. Tietz N. W., Clinical Guide to Laboratory Tests, 4th Edition, (2000) p 916-917.
6. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

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CE