

## Total Protein Fluid monoreagent



IN VITRO TEST FOR THE QUANTITATIVE DETERMINATION OF TOTAL PROTEIN IN HUMAN SERUM AND PLASMA BY BIURET-METHOD.

REF	88 71 71	1x1000ml
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### CALIBRATION

Protein total standard (4 g/dl)	5ml	88 08 36
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### QUALITY CONTROL

Control Serum N	6x5ml	88 41 48B
Control Serum P	6x5ml	88 46 85B

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

### SUMMARY

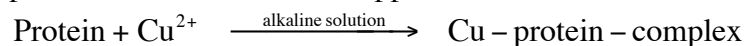
Plasma proteins are synthesized predominantly in the liver, plasma cells, lymph nodes, the spleen and in bone marrow. In the course of disease the total protein concentration and also the percentage represented by individual fractions can significantly deviate from normal values. Hypoproteinemia can be caused by diseases and disorders such as loss of blood, sprue, nephrotic syndrome, severe burns, salt retention syndrome and Kwashiorkor (acute protein deficiency). Hyperproteinemia can be observed in cases of severe dehydration and illnesses such as multiple myeloma. Changes in the relative percentage of plasma proteins can be due to a change in the percentage of one plasma protein fraction. Often in such cases the amount of total protein does not change. The A/G-ratio is commonly used as an index of the distribution of albumin and globulin fractions. Marked changes in this ratio can be observed in cirrhosis of the liver, glomerulonephritis, nephrotic syndrome, acute hepatitis, lupus erythematosus as well as in certain acute and chronic inflammations. Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow, as well as other metabolic or nutritional disorders.

### TEST PRINCIPLE

Colorimetric assay

Sample and addition of R1 (blank reagent)

Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored Biuret complex. Sodium potassium tartrate prevents the precipitation of copper hydroxide and potassium iodide prevents autoreduction of copper.



The color intensity is directly proportional to the protein concentration which can be determined photometrically.

### NOTES

For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents.

Warning. Bottle 1 contains sodium hydroxide. Sodium hydroxide can cause caustic burns. In the event of contact, flush affected area with copious amounts of water. Get immediate medical attention after contact with eyes, or if ingested.

### LIMITATIONS - INTERFERENCE

Criterion: Recovery within  $\pm 10\%$  of initial value.

Icterus: No significant interference up to an index I of 22 corresponding to approximately 22 mg/dl of bilirubin.

Hemolysis: No significant interference up to an index H of 1100 (approximate hemoglobin concentration: 1100 mg/dl).

This interference results from the fact that hemoglobin is reacting as a protein in the Total Protein assay.

Lipemia (Intralipid): No significant interference up to an index L of 1075 (approximate triglycerides concentration: 2150 mg/dl). There is poor correlation between turbidity and triglycerides concentration.



**MEASURING/REPORTABLE RANGE**

Measuring range: 0.2-13 g/dl (2.0-130 g/l)

Determine samples having higher concentrations via the rerun function.

On instruments without rerun function, manually dilute samples with 0.9% NaCl or distilled/deionized water (e. g. 1 + 2). Multiply the result by the appropriate dilution factor (e.g. 3).

**EXPECTED VALUES**

*Expected values according to Josephson*

Adults: 6.6-8.7 g/dl or 66-87 g/l

*Expected values according to Tietz:*

Unbilical Cord 4.8-8.0 g/dl

Pemature 3.6-6.0 g/dl

Newborn 4.6-7.0 g/dl

1 week 4.4-7.6 g/dl

7 months – 1 year 5.1-7.3 g/dl

1-2 years 5.6-7.5 g/dl

> 3 years 6.0-8.0 g/dl

Adult (ambulatory) 6.4-8.3 g/dl

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, total protein results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

**ANALYTICAL SENSITIVITY (LOWER DETECTION LIMIT)**

Detection limit: 0.2 g/dl or 2.0 g/l

The detection limit represents the lowest measurable protein concentration that can be distinguished from zero.

**IMPRECISION**

Reproducibility was determined using controls in an internal protocol. The following results were obtained

Sample	Within run		
	Mean mg/gl	SD mg/dl	CV %
Sample 1	5.20	0.039	0.73
Sample 2	5.37	0.039	0.75
Sample 3	5.70	0.037	0.65

Sample	Between day		
	Mean mg/gl	SD mg/dl	CV %
Sample 1	5.15	0.070	1.36
Sample 2	5.48	0.086	1.57
Sample 3	5.95	0.085	1.43

**METHOD COMPARISON**

A comparison of AXIOM Total Protein Fluid monoreagent (y) with a commercial obtainable assay (x) gave following results:

$$y = 0.951 x + 2.75; r=0.999$$

**REAGENT CONCENTRATION****R1:**

Potassium iodide 30 mmol/l

Potassium sodium tartrate 32 mmol/l

Copper sulphate 18 mmol/l

Sodium hydroxide 200 mmol/l

**PREPARATION AND STABILITY:**

Reagent and Standard are ready to use.

Unopened kit components up to the expiration date at +2°C to +8°C.

**SPECIMEN**

Collect serum using standard sampling tubes.

Heparin or EDTA plasma

Stability:                      3 days                      at +2°C to +8°C  
    6 months                      at -20°C

Separate the serum or plasma within 4 hours from the clot or cells. The total protein concentration is by 0.4 to 0.8 mg/dl lower when the sample is collected from a patient situated in the recumbent position rather than upright. Centrifuge samples containing precipitate before performing the assay.

### TESTING PROCEDURE

#### Materials provided

- Working solutions as described above

#### Additional materials required

- Controls as indicated below

#### **Manual procedure:**

Wavelength                      Hg 546 nm, (530 – 570 nm)  
 Temperature                      +25°C to +37°C  
 Cuvette                              1cm light path  
 Zero adjustment                      against reagent blank

	<i>blank</i>	<i>Standard / Calibrator</i>	<i>Sample</i>
<i>RI</i>	1000 $\mu$ l	1000 $\mu$ l	1000 $\mu$ l
<i>Standard</i>	---	20 $\mu$ l	---
<i>Sample</i>	---	---	20 $\mu$ l

Mix and incubate 10 minutes. Measure absorbance of sample and standard against reagent blank.

#### **Calculation:**

$$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{standard conc.} = \text{Total Protein conc. (g/dl)}$$

or calculate by factor:  $19 \times \Delta A_{\text{sample}} = \text{Total proteine g/dl}$

A sample blank must be determined for haemolytic and lipemic sera by pipetting 20  $\mu$ l serum to 1000  $\mu$ l NaCl (0.9%) and measurement against distilled water. The absorbance of the sample blank has to be subtracted from the absorbance of the sample

### DISPOSAL

Please note the legal regulations.

### LITERATURE

1. Bablok W. et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988; 26:783-790.
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4. Josephson B, Gyllenswärd C. The Development of the Protein Fractions and of Cholesterol Concentration in the Serum of Normal Infants and Children. Scand J Clin Lab Investigation 1957; 9:29.
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8. Weichselbaum T.E. Amer J Clin Path 1946;16:40.



### AXIOM Product range Clinical Chemistry

<b>Enzymes</b>	<b>Ions</b>	<b>Other Metabolites</b>
Acid Phosphatase	Ammonium fluid	Bilirubin T/D
Alkaline Phosphatase	Copper fluid	Creatinine fluid
$\alpha$ -Amylase direct	Calcium fluid	Glucose GOD-PAP fluid
CK-NAC actived	Chloride fluid	Glucose Hexokinase fluid
CK-MB (NAC- actived)	Inorganic Phosphorus UV fluid	Urea Enzymatic fluid
$\gamma$ -GT fluid	Iron fluid	Urea UV fluid
LDH fluid	TIBC	Uric Acid PAP fluid
Cholinesterase	Magnesium fluid	
GOT/ASAT fluid	Potassium fluid	
GPT/ALAT fluid	Sodium fluid	<b>Controls</b>
Lipase UV fluid		Control Serum N
Lactate PAP		Control Serum P
$\alpha$ -HBDH	<b>Proteins</b>	
<b>Lipids</b>	Albumin	
	CSF-Protein fluid	
Cholesterol fluid	Microprotein fluid	
HDL Cholesterol	Hemoglobin	
LDL Cholesterol	Protein Total fluid	
Triglycerides fluid		

