TOTAL PROTEIN

Diagnostic reagent for determination of Total Protein concentration.
Liquid. Monoreagent. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
A2300N	500 mL	DM2300	333 mL	L2300	600 mL	M4300	500 mL
A2301N	200 mL	D2300	540 mL	L2301	400 mL	M4301	300 mL
A2302N	100 mL	D2301	280 mL	L2302	240 mL	PL2300	240 mL
A2303N	200 mL	HN300	400 mL	MD300	450 mL	RD2300	400 mL
BB155	220 mL	HN301	230 mL	MD301	225 mL	S2301	200 mL
BY2300	700 mL	K2301	320 mL	M2300	500 mL	TB2300	400 mL
BY2301	500 mL	LB300	160 mL	M2301	300 mL	TB2301	200 mL
BZ2165	360 mL	LM068	240 mL	M3300	320 mL	T2300	900 mL
		PL2301	150 mL	M3301	70 mL	8A2300	700 mL
						8A2301	500 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of total protein in serum and plasma.

GENERAL INFORMATION

Serum proteins are a large group of transport proteins, enzymes, immunoglobulins, inhibitors and others.[1] Plasma proteins are synthesized mainly in the liver, plasma cells, lymph nodes, spleen and bone marrow.[2] Despite the functional differences between the various serum proteins, they share some common biophysical and biochemical properties: (1) a basic composition of carbon, hydrogen, nitrogen and oxygen; (2) a backbone of covalent peptide bonds linking amino acid units; and (3) maximum absorbance at ultraviolet and far ultraviolet wavelengths. Based on these properties, laboratory methods have been developed to determine the protein concentration in serum, assuming that each of the hundreds of individual proteins present in serum reacts similarly in chemical reactions.[1]

Total protein measurements are used in the diagnosis and treatment of various diseases involving the liver, kidney or bone marrow, as well as other metabolic or nutritional disorders.[2] The two main causes of changes in serum total protein concentration are volume changes in plasma fluid and changes in the concentration of one or more specific proteins in plasma. A decrease in plasma fluid volume (hemoconcentration) is reflected as relative hyperproteinemia as a result of the concentration of all protein increases to the same degree. This is seen in dehydration due to inadequate water intake or excessive water loss, such as severe vomiting, diarrhea, Addison's disease or diabetic acidosis.[1] In addition to severe dehydration, hyperproteinemia may be seen in diseases such as multiple myeloma. Changes in the relative percentage of plasma proteins may be due to a change in the percentage of a plasma protein fraction. Often in such cases the total amount of protein does not change.[2]

On the other hand, the increase in plasma fluid volume (hemodilution) is seen as relative hypoproteinemia, with the concentration of all proteins decreasing to the same degree. This occurs during syndromes of water intoxication or salt retention and excessive intravenous infusion. Severe protein depletion caused by dietary inadequacy, poor digestion or malabsorption can reduce the serum protein concentration, mainly albumin. Severe liver disease also reduces serum protein concentration. Renal diseases such as glomerulonephritis, nephrotic syndrome and severe proximal tubular disease can cause severe, chronic serum protein loss and decreased serum total protein levels. Edema is usually seen when serum protein concentration falls below 40 g/L.[1] Hypoproteinemia can also result from diseases and disorders such as blood loss, sprue (inadequate protein absorption), severe burns and Kwashiorkor (acute protein deficiency).[2]

TEST PRINCIPLE

Colorimetric measurement

The peptidic bonds of proteins react with divalent Cu^{+2} in alkaline solution to form a blue-violet biurea complex. The color intensity is measured photometrically at a wavelength of 520-560 nm and the absorbance value obtained is directly proportional to the protein concentration in the measured sample. Each Cu(II) can complex up to 6 peptide bonds. Sodium potassium tartrate in the reagent prevents precipitation of copper hydroxide, while potassium iodide prevents spontaneous reduction of copper.

REAGENT COMPONENTS

Cupric sulphate	≤ 8 mmol/L
Potassium sodium tartrate	≤ 24 mmol/L
Potassium iodide	≤ 8 mmol/L
NaOH	≤ 0.80 mmol/L

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[3]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. It can provide comparable results.[29] The plasma concentration of total protein is 2 to 4 g/L higher than the serum concentration due to the presence of fibrinogen.[1]

Multiple sample freezing and thawing should be avoided. The sample should be homogenized before testing.

Total protein activity stability in serum and plasma:

6 days at +20/+25ºC
30 days at +2/+8ºC
1 year at -20°C

Annotation:

- A fasting sample is not required but may be requested to reduce lipemia.[1]
- Samples collected in gel and gel-free separator tubes give comparable results.[4]
- No significant difference has been observed in samples collected in glass or plastic tubes.[5]
- Changes in body fluid between the vascular bed and interstitial spaces can cause significant changes in serum protein concentration. For example, total serum protein is 0.4 to 0.8 g/dL lower when the patient is in the supine position than when standing or upright.[6]

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Arcal Auto Calibrator or Total Protein Calibrator.

Arcal Auto Calibrator-Lyophilized
Ref.No: A39052
Ref.No: A39054
Ref.No: A39055 (For Olympus AU series.)

Total Protein Calibrator-Liquid
Ref.No: A230S
Ref.No: A230D

Calibration stability is 30 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N Level 1 Control- Lyophilized
Ref.No: A3910
Ref.No: A3912 (For Olympus AU series.)
Ref.No: A3913 (For BS series.)
Ref.No: A3914 (For Erba.)

Arcon P Level 2 Control- Lyophilized
Ref.No: A3920
Ref.No: A3922 (For Olympus AU series.)
Ref.No: A3923 (For BS series.)
Ref.No: A3924 (For Erba.)

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Adults, ambulatory	: 6.3 - 8.3 g/dL
Adults, recumbent	: 6.0 - 7.8 g/dL

After age 60, levels are about 0.2 g/dL lower.

Annotation:

- In adults, there is a slight and probably insignificant decrease in serum protein concentration with age.[7]
- The mean serum total protein concentration in neonates is 5.7 g/dL, increasing to 6.0 g/dL (±4 g/L) at 6 months[8] and reaches full adult concentrations by approximately 3 years of age.[1]
- Compared to full-term infants, premature infants may have a much lower total protein concentration, ranging from 3.6 to 6.0 g/dL.[6,9]
- During pregnancy, a mean decrease in serum protein concentration after delivery from 6.9 g/dL to 6.1 g/dL has been noted.[10]
- In addition, an increase in serum protein concentration of 0.4 to 0.8 g/dL can be recorded for several hours after intense exercise.[11]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[12]

Unit Conversion:
g/dL x 10 = g/L

PERFORMANCE CHARACTERISTICS

Measuring Interval
According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's

lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[13]

The determined analytic measuring interval for Total Protein is 0.80 – 12 g/dL.

Detection Capability

Limit of Detection (LoD): 0.10 g/dL

Limit of Quantitation (LoQ): 0.80 g/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[14]

Linearity

This method shows measurement linearity in the activities up to 12 g/dL. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[15]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[16]

Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of Total Protein have been given in the table 1 and 2 respectively.

Table 1. Total Protein Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
3.62 g/dL	0.07	1.93	80
7.17 g/dL	0.14	1.95	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[17]

Table 2. Total Protein Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
3.62 g/dL	0.09	2.59	80
7.17 g/dL	0.27	3.70	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[17]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablok equation:[18]
y= 1.02x – 0.11 g/dL
r=0.97

Interference

Endogenous interferant and analyte concentrations that have been used in the Total Protein scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[19,20]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Total Protein interference scanning test is appropriate, is determined as ±10%.[21]

In Total Protein test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	Total Protein Target (g/dL)	N*	Observed Recovery %
Bilirubin 48 mg/dL	6.48	3	92
Lipemia 433 mg/dL	7.16	3	108

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[20]

Annotation:

- Non-hemolyzed samples should be used.
- Interferences due to drug treatments or endogenous substances may affect the results.
- Interference with the Biurea method is rare and most is easily eliminated. Visibly hemolyzed, icteric or lipemic samples may cause increased endogenous absorbance at 540 nm and result in positive interference.[1]
- Hemolysis may cause a 3% increase in the apparent total protein concentration for every 1 g/L of hemoglobin present in the sample.[22]

- Low molecular mass dextrans interfere with the biurea method.[23,24] Dextranes used as plasma volume expanders form complexes with copper and tartrate in the reaction mixture, resulting in a gelatinous, light blue precipitate. The degree of interaction that occurs varies greatly with the concentration of dextran and the composition of the biurea reagent.[25,26] Interference can range from 3% to 50% at typically encountered dextran concentrations. The use of glycerol or low concentrations of NaOH[27,28] has been reported to prevent interference from dextran.[1]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[20]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests