PROTEIN HS

Diagnostic reagent for determination of Protein HS (Protein High Sensitive) 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
BB150	160 mL	HN360	400 mL	MDP21	150 mL	RD2297	200 mL
BY2305	250 mL	HN361	230 mL	MSP20	400 mL	TA490	100 mL
BZ2160	240 mL	LB295	160 mL	MSP21	200 mL	TBSP20	400 mL
DM2315	333 mL	LM070	120 mL	M3P20	240 mL	TBSP21	150 mL
D2400	540 mL	LSP20	600 mL	M4P20	400 mL	TSP20	900 mL
D2402	280 mL	LSP21	160 mL	M4P21	200 mL	TSP21	250 mL
D2403	175 mL	KSP21	240 mL			8A2305	250 mL

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

INTENDED USE

The test is applied for the quantitative determination of protein in urine and cerebrospinal fluid (CSF).

GENERAL INFORMATION

The concentration of total protein in cerebrospinal fluid (CSF) is an indicator of blood-COS permeability. CSF usually has a total protein concentration about 100 times lower than plasma and has a different protein composition as most proteins have limited transmission across the blood-brain barrier. Methods with higher sensitivity or increased sample volume are required to measure total serum or plasma protein. More than 80% of the protein content of CSF originates from plasma via ultrafiltration and pinocytosis; the rest is derived from intrathecal synthesis. Since CSF is essentially an ultrafiltrate of plasma, the protein content of CSF is dominated by low to medium molecular weight plasma proteins such as prealbumin, albumin and transferrin. No protein with a molecular weight greater than IgG is present in sufficient concentration to be visible on electrophoresis.[1] The normal plasma/COS gradient is approximately 14 for prealbumin, 240 for albumin, 140 for transferrin, 800 for IgG and more than 1000 for larger proteins such as IgA, AMG, fibrinogen, IgM and β-lipoprotein.[2]

Analyses of total protein and specific proteins in CSF are primarily used to detect increased permeability of the blood-CSF barrier, increased intrathecal synthesis or increased protein release from neural and glial tissue. Conditions such as viral meningitis, encephalitis, increased intracranial pressure, spinal cord tumors, trauma and bleeding can all disrupt the blood-brain barrier and lead to increased CSF protein. Increased CSF protein is observed in demyelinating diseases of the CNS, such as multiple sclerosis (MS), in which increased intrathecal synthesis of immunoglobulins (Ig), especially IgG, is observed. Similar to MS, CSF protein concentration may increase in neurosyphilis due to increased local Ig. In tuberculous meningitis and brain abscess, CSF protein levels may increase due to both increased capillary permeability and increased local Ig production. CSF protein levels may also increase after myelography due to inflammatory reaction.[1]

Low CSF protein concentrations may be seen in samples taken from ventricles or cisternae.[3] Other causes include recurrent LP, chronic CSF leakage, idiopathic intracranial hypertension, acute water intoxication and some children between 6 months and 2 years of age.[4]

A more specific measurement of the permeability of the blood-CSF barrier involves determining the ratio of albumin concentration in CSF to plasma. This ratio, the CSF/serum albumin index, is usually calculated in milligrams per deciliter for CSF albumin and grams per liter for serum protein, with values multiplied by 1000. A CSF-serum albumin index below 9 indicates a stable blood-CSF barrier. Values between 9 and 14 represent mild impairment, values between 14 and 30 represent moderate impairment and values above 30 represent severe impairment.[1]

High molecular weight proteins are retained in circulation by the glomerular filtrate, while low molecular weight proteins are freely filtered, reabsorbed and catabolized within the tubular cells. As a result, the appearance of significant amounts of protein in the urine is suggestive of kidney disease. Although the term "proteinuria" is generally used for the total amount of all types of protein in the urine, it can also be classified as glomerular or tubular, depending on the form of proteinuria observed. A third category, overflow proteinuria, is seen in conditions such as Bence Jones proteinuria and myoglobinuria and occurs when filtration of excessive amounts of low molecular weight protein exceeds the tubular capacity for reabsorption. Furthermore, proteinuria is a strong risk marker for progressive kidney disease and reducing protein excretion is a therapeutic goal.[5]

TEST PRINCIPLE

Colorimetric measurement

Proteins form a colored complex with pyrogallol red; the absorbance of this complex is measured at 600 nm and is directly proportional to the protein concentration in the sample.

REAGENT COMPONENTS

Succinate buffer	≤ 0.08 M
Pyrogallol red	≤ 0.06 mM,
Sodium molibdate	≤ 0.15 mM,
Sodium oxalate	≤ 1.2 mM,
Sodium benzoate	≤ 0.37 mM,
SDS	≤ 0.12 mM.

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.[6]

SAMPLE REQUIREMENTS

Urine and CSF can be used and are collected according to the standard procedures. Multiple sample freezing and thawing should be avoided. The sample should be homogenized before testing.

Urine: Random or 24-hour urine samples should be used. Do not use preservatives. The specimen should be stored in a refrigerator after collection.

Protein activity stability in urine[24]:

1 day at +20/+25ºC
7 days at +2/+8ºC
1 month at -20ºC

Annotation:

- It is generally accepted that the definitive way to demonstrate and quantify the presence of proteinuria is 24-hour specimen collection. However, it is widely recognized that this is a difficult procedure to control effectively and that inaccuracies in urine collection can lead to errors in the estimation of protein loss.[5]
- For most purposes, spot urine and albumin/creatinine ratio (or protein/creatinine ratio) are used instead of 24-hour urine collection.[7-9]
- Overnight urine collection, first morning urine, second morning urine or a random urine sample can be used.[5] However, first morning urine is preferred because it correlates well with 24-hour protein loss and is not affected by orthostatic (postural) proteinuria.[10]

Cerebrospinal Fluid (CSF): No special additives required. The presence of blood in the CSF sample invalidates the protein value. Samples for urine/CSF protein should be collected before or at least 24 hours after fluorescein administration.

Follow the tube manufacturer's instructions when processing samples in primary tubes (sample collection systems). Centrifuge samples containing sediment before performing the test. Uncentrifuged samples may give high results.

Annotation:

- CSF protein may be increased in samples obtained from traumatic lumbar puncture (LP).[4]
- Protein concentration increases from the ventricle to the lumbar region (rostrocaudal gradient), reflecting the change in the permeability of the blood-brain barrier and increased time for equilibration in the lumbar region. Low CSF protein concentrations may be seen in samples from the ventricle or cisternae.[3]
- CSF protein concentration may increase due to repeated LP.[4]

Protein activity stability in CSF[24]:

1 day at +20/+25ºC
6 days at +2/+8ºC
1 year at -20ºC

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of a MicroProtein Calibrator.

MicroProtein Calibrator
Ref.No: MPCL4

Calibration stability is 15 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:

Microprotein Control-I
Ref.No: MPCN1

Microprotein Control-II
Ref.No: MPCN2

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

Expected values:

Urine[11]:

24 hour	: < 140 mg/24s
Random	: < 150 mg/L

Centrifuged CSF Samples[23]:
Reference interval: 150-450 mg/L

Annotation:

- In premature and term neonates, CSF protein concentrations normally range between 200 and 1700 g/L.[12]

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.[13]

Unit Conversion:
mg/dL x 10 = mg/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).[14]

The determined analytic measuring interval for Protein HS is 7-250 mg/L.

Detection Capability

Limit of Detection (LoD): 1 mg/L

Limit of Quantitation (LoQ): 7 mg/L

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.[15]

Linearity

This method shows measurement linearity in the activities up to 250 mg/L. 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.[16]

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.[17]

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

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

Mean Concentration	SD	CV%	n
37.1 mg/L	0.74	2.00	80
103.7 mg/L	1.27	1.22	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[18]

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

Mean Concentration	SD	CV%	n
37.1 mg/L	0.79	2.12	80
103.7 mg/L	2.46	2.37	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[18]

Method Comparison

As a result of the statistical evaluation of the method comparison data:
Passing-Bablok equation:[19]
y= 0.97x – 0.54 mg/L
r=0.978

Interference

Endogenous interferant and analyte concentrations that have been used in the Protein HS scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[20,21]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Protein HS interference scanning test is appropriate, is determined as ±10%.[22]

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

Ascorbic Acid : ≤ 200 mg/dL

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.[21]

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.

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

Archem Sağlık Sanayi ve Tic. A.Ş.
Mahmutbey Mah. Halkalı Cad. No:124 Kat:4
Bağcılar/İstanbul/Türkiye
Tel: + 90 212 444 08 92
Fax: +90 212 629 98 89
info@archem.com.tr **www.archem.com.tr**

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