CHOLINESTERASE (CHE)

Diagnostic reagent for determination of Cholinesterase concentration.
Liquid. Dual reagents. 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
AB2130N	360 mL	DM3010	342 mL	MBC30	350 mL	RD2130	150 mL
AB2131N	120 mL	HN330	300 mL	M3C30	200 mL	TBC30	240 mL
BB065	192 mL	LBC30	240 mL	M4C30	350 mL	8AC2130	450 mL
BYC2130	450 mL	LB093	192 mL				
BZ2065	170 mL						

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

INTENDED USE

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

GENERAL INFORMATION

Polymorphic serine esterases that catalyze the hydrolysis of acyl esters to related alcohols and carboxylates are called cholinesterases.[1] Two related enzymes have the ability to hydrolyze acetylcholine. One is acetylcholinesterase, called true cholinesterase or choline esterase I (EC 3.1.1.7; acetylcholine acetylhydrolase). True cholinesterase is found in erythrocytes, lungs and spleen, nerve endings and the gray matter of the brain. It is responsible for the rapid hydrolysis of acetylcholine released from nerve endings to mediate the transmission of nerve impulses across the synapse. For the nerve to depolarize, acetylcholine must be broken down so that the nerve is re-polarized at the next conduction event. The other cholinesterase is acylcholine acylhydrolase (EC 3.1.1.8; acylcholine acylhydrolase; CHE), also called pseudocholinesterase, serum cholinesterase, butyrylcholinesterase or choline esterase II, found in the liver, pancreas, heart, brain white matter and serum. Although the activity of CHE in the human body is about three times higher than that of acetylcholinesterase, its biological role is not fully understood. It has been suggested that CHE has a physiological role in the deactivation of octanoyl ghrelin, a hormone that stimulates feeding and influences weight gain through its metabolic effects.[2]

Atypical (genetic) variants of the enzyme, found in the serum of a small proportion of apparently healthy people and characterized by decreased activity towards acetylcholine and other substrates, are clinically important. The gene on chromosome 3 that controls CHE synthesis can be found in many allelic forms. The four most common forms are E^u, E^a, E^f and E^s. These four allelic genes can be combined to produce one normal and nine abnormal genotypes. At least 40 other forms exist and are at another gene locus (E2). The normal, most common phenotype is known as E^uE^u or UU (usual form). The E^a gene is called an atypical gene; sera from people homozygous for this gene (E^aE^a = AA) are weakly active against most substrates for CHE and show an increased resistance to inhibition of enzyme activity by dibucaine. The E^f gene (fluoride-resistant form) has a weak level of enzyme activity but strong resistance to fluoride inhibition. The es gene (silent form) is associated with the absence of the enzyme or the presence of a protein with minimal or no catalytic activity. Variant enzymes (allelozymes) are less efficient catalysts than the usual form and their affinity for competitive inhibitors such as dibucaine or fluoride is similarly less. This gives rise to the dibucaine- or fluoride-resistant traits used in the characterization of genetic variants.[2]

Cholinesterases are a group of enzymes primarily cleaved from choline and thiocholine esters. The names Serum Cholinesterase and Pseudocholinesterase are commonly used. ChE measured in serum and plasma is synthesized by the liver and is detected with protein loss (exudative enteropathy) in nephritic syndromes of liver diseases and in the diagnosis of intestinal diseases. Rapidly decreasing values may be indicative of poisoning by rodents. ChE measurement is also part of preoperative diagnostics, necessary for inactivation of muscle relaxants used in surgery. Homozygous forms of AA and FF are found in 0.3% to 0.5% of the white population, with an even lower incidence in blacks.[2]

Measurements of CHE activity in serum are used (1) to test liver function, (2) as an indicator of possible pesticide poisoning, and (3) to identify patients with atypical forms of the enzyme who are at risk of prolonged response to certain muscle relaxants used in surgical procedures.[3]

In the absence of genetic causes or known inhibitors, any decrease in CHE activity reflects impaired synthesis of the enzyme by the liver. Serial measurement of CHE is recommended as an indicator of prognosis in patients with liver failure and for monitoring liver function after liver transplantation.[2]

Organic phosphorus compounds that inhibit cholinesterase activity include many insecticides such as parathion, sarin and tetraethyl pyrophosphate. If sufficient quantities are ingested to inactivate all acetylcholinesterase in the nervous tissue, it will result in death. In case of poisoning, both cholinesterases are inhibited, but the activity of the serum enzyme decreases faster than that of the erythrocyte enzyme. A 40% drop in CHE activity occurs before the first symptoms appear and

an 80% drop is required for neuromuscular effects to become apparent. Concentrations of enzyme activity close to zero require immediate treatment with enzyme reactivators such as pyridine-2-aldoxime.[2]

Succinyldicholine (sucsamethonium) and mivacurium, muscle relaxants used to assist endotracheal intubation in surgical procedures, are hydrolyzed by CHE and their pharmacological effects normally only last long enough to meet the needs of the surgical procedure. In individuals with low enzyme activity or a weakly active variant, degradation of the drug will not occur fast enough and the person may enter a prolonged period of paralysis of the respiratory muscles (apnea) requiring mechanical ventilation until the effects of the drug gradually fade.[2]

TEST PRINCIPLE

Spectrophotometric method

In the reaction catalyzed by CHE, butyrylthiocholine undergoes hydrolysis and butyric acid and thiocholine are formed as products. In the subsequent reaction, thiocholine reduces yellow potassium hexacyanoferrate (III) to colorless potassium hexacyanoferrate (II). The rate of color loss, measured spectrophotometrically at a wavelength of 405 nm, is proportional to the cholinesterase activity in the sample.

$$\text{Butyrylthiocholine} + H_2O \xrightarrow{\text{Cholinesterase}} \text{Thiocholine} + \text{Butyrate}$$

$$2\ \text{Thiocholine} + 2\ [Fe(CN)6]^{3} + H_2O \longrightarrow \text{Choline} + 2\ [Fe(CN)_6]^{4} + H_2O$$

REAGENT COMPONENTS

Reagent 1:

Pyrophosphate	: pH 7.6
Potassium	: ≤ 77 mmol/L
Hexacyanoferrate(III)	: ≤ 2.4 mmol/L

Reagent 2:

Butyrylthiocholine	: ≤ 18 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 23 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.[4]

SAMPLE REQUIREMENTS

Serum and heparinised plasma 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.

Cholinesterase activity stability in serum and plasma[5]:

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

Annotation:

- Samples should be preserved from direct exposure to light.
- Samples should be stored and shipped cold to avoid temperature extremes.[1]
- Samples should be centrifuged and serum or plasma should be rapidly separated from clots or cells, avoiding hemolysis.[1]

CALIBRATION AND QUALITY CONTROL

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

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

Calibration stability is 7 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

Women	3,930 – 10,800 U/L
Men	4,620 – 11,500 U/L

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.[6]

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).[7]

The determined analytic measuring interval for Cholinesterase is 50 – 25000 U/L.

Detection Capability

Limit of Detection (LoD): 40 U/L

Limit of Quantitation (LoQ): 50 U/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.[8]

Linearity

This method shows measurement linearity in the activities up to 25000 U/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.[9]

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.[10]

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

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

Mean Concentration	SD	CV%	n
4188 U/L	39.8	0.95	80
8805 U/L	44.3	0.50	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[11]

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

Mean Concentration	SD	CV%	n
4188 U/L	50.7	1.21	80
8805 U/L	215.7	2.45	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[11]

Method Comparison

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

Passing-Bablok equation:[12]
y= 0.948x + 89 U/L
r= 0.994

Interference

Endogenous interferant and analyte concentrations that have been used in the Cholinesterase scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[13,14]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Cholinesterase interference scanning test is appropriate, is determined as ±10%.[15]

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

Ascorbic acid	: ≤ 30 mg/dL
Bilirubin	: ≤ 45 mg/dL
Hemoglobin	: ≤ 500 mg/dL
Lipemia	: ≤ 1400 mg/dL

Annotation:

- If the serum is well centrifuged to remove red blood cell ghosts, moderate hemolysis does not affect the measurement method.[16]
- The presence of oxalate or fluoride has been reported to significantly reduce measured cholinesterase activities.[17]

- Similarly, citrate anticoagulant, heavy metals, borate, some serum separators, insecticides, organophosphates, carbamates, neuromuscular relaxants, phenothiazines, both anabolic and glucocorticosteroids, oral contraceptives, estrogens, radiographic agents such as iopanoic acid, streptokinase, testosterone and many other such compounds have been observed to reduce cholinesterase activity.[1]
- Plasma cholinesterase activity has been found to decrease after plasmapheresis and to increase after transfusion in patients with abnormally low cholinesterase activity.[18]

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.[14]

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.

REFERENCES

1. Pesce, A. J., & Kaplan, L. D. (2009). Methods in Clinical Chemistry: Kaplan and Pesce's: Clinical Chemistry: Theory, Analysis, Correlation: Vol. I (5th ed.), Chapter: Cholinesterase, p.393-404. Elseviers.
2. Rifai, N., Chiu, R. W., & Young, I., et al., (2023) Tietz Textbook of Laboratory Medicine (7th ed.), Chapter 32: Serum Enzymes, p.350-350.e36, Elsevier, St. Louis, Missouri 63043
3. McQueen MJ. Clinical and analytical considerations in the utilization of cholinesterase measurements. Clin Chim Acta 1995;237:91–105.
4. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI Document EP25-A. Wayne, PA: CLSI; 2009.
5. Young DS. Effects of Preanalytical variables on Clinical LaboratoryTests. 3rd ed. Washington, DC: AACC Press; 2007:259
6. Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. CLSI Document EP28-A3c. Wayne, PA: CLSI; 2010.
7. Clinical and Laboratory Standards Institute (CLSI). Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking – 1st Edition. CLSI Document EP34. Wayne, PA: CLSI; 2018.
8. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.
9. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach - 1st Edition. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.
10. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.
11. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second

Edition. CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.
12. Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.
13. Clinical and Laboratory Standards Institute (CLSI). Supplemental Tables for Interference Testing in Clinical Chemistry - First Edition. CLSI Document EP37. Wayne, PA: CLSI; 2018.
14. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry - Third Edition. CLSI Document EP07. Wayne, PA: CLSI; 2018.
15. CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register July 11, 2022;87(131:41194-242.
16. Dietz AA, Rubenstein, HM, Lubrano T. Colorimetric determination of serum cholinesterase and its genetic variants by the propionylthiocholine-dithiobis (nitrobenzoic acid) procedure. In: Cooper GR, King JS, eds. Selected Methods for the Small Clinical Chemistry Laboratory. Vol. 8. Washington, DC: American Association for Clinical Chemistry; 1977:41-46.
17. Kramer S, Watson LL, Lodato DT, Widsor- Long K, Gilmore JM, Augustyn JM. Assay of pseudocholinesterase on the dimension clinical chemistry system. Clin Chem 1991;37:913.
18. Evans RT, MacDonald R, Robinson A. Suxamethonium apnoea associated with plasmapheresis. Anaesthesia 1980;35:198-201.

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**

CE

SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
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