Alkaline Phosphatase (ALP)

Diagnostic reagent for determination of Alkaline Phosphatase (ALP) concentration.
Liquid. Dual Reagents (Ratio: R1/R2: 4/1). Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Package
MH-012	75 mL
MH-013	50 mL

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

INTENDED USE

This test is applied for the quantitative determination of ALP in human serum and plasma.

GENERAL INFORMATION

Alkaline phosphatase, (EC 3.1.3.1; orthophosphoric monoester phosphohydrolase [alkaline optimum]; ALP) catalyzes the alkaline hydrolysis of a wide variety of naturally occurring and synthetic substrates. Some divalent ions such as Mg^{+2}, Co^{+2} and Mn^{+2} are activators of the enzyme, while Zn^{+2} is a metal ion constitutively present in the enzyme. The correct ratio of Mg^{+2} to Zn^{+2} ions is necessary to avoid displacement of Mg^{+2} and ~~for~~ to have optimal activity of the enzyme. Phosphate, borate, oxalate and cyanide ions are inhibitors of ALP activity. Changes in Mg^{+2} and substrate concentrations alter the pH optimum required for enzyme activity. ALP activity is present in most organs of the body and is found fixed to the cell membrane via glycosylphosphatidylinositol ("ectoenzyme"), particularly in the mucosa of the small intestine and proximal convoluted tubules of the kidney, bone (osteoblasts), liver and placenta. Although the exact metabolic function of the enzyme is not yet understood, ~~ALP~~ it appears to be involved in lipid transport in the gut, the calcification process in bone and host defense through endotoxin dephosphorylation. ALP exists in multiple homodimeric forms (molecular weight ranges from 70 to 120 kDa), some of which are true isoenzymes encoded at separate genetic loci. Bone, liver and kidney forms of ~~ALP~~ it share a common primary structure encoded by the same genetic locus, but differ in their carbohydrate content. The ALP activity found in the serum of healthy adults is primarily of liver and bone origin in a ratio of approximately 1:1. Minimal amounts of intestinal ALP may also be present in the serum, particularly in individuals with blood type B or O. Increases in serum ALP activity usually originate from one or both of two sources: liver and bone.

Consequently, serum ALP measurements are particularly important in the investigation of two major conditions: Hepatobiliary disease and bone disease associated with increased osteoblastic activity. Serum ALP is one of the main enzymes used in the investigation of liver disease.[1] The liver induces ALP synthesis by hepatocytes in response to any obstruction in the biliary tract. The newly formed ectoenzyme is released from the cell membrane by the detergent effect of bile salts and enters the circulation to increase enzyme activity in serum.[2]

The increase in enzyme activity in extrahepatic obstruction (e.g. stones, pancreatic head cancer) can be more than four times the upper reference limit (URL). This increase is generally greater than those seen in intrahepatic cholestasis. The greater the obstruction, the higher the serum enzyme activity (which can reach 10 to 12 times the URL) and usually returns to normal within a week after surgical removal of the obstruction. A similar increase is also seen in patients with advanced primary liver cancer or extensive secondary liver metastasis. ALP elevation ~~is~~ can be predictive of outcomes such as liver transplantation or death in patients with primary biliary cholangitis (previously known as primary biliary cirrhosis) and can increase more than twice the URL.[3]

Similar prognostic information can also be obtained from ALP activity in primary sclerosing cholangitis. Liver diseases that primarily affect parenchymal cells, such as infectious hepatitis, typically show only moderately elevated (less than threefold) or even normal serum ALP activities. Increases may also be the result of a response to drug therapy and ALT-/ALP-based criteria remain the mainstay for detecting and characterizing the type of drug-induced liver injury.[1] In the third trimester of pregnancy, an increase of up to two to three times the URL is observed in women. This situaiton makes ALP an unreliable marker of hepatobiliary disease in pregnancy.
Studies have described a benign familial increase in serum ALP activity caused by increased intestinal ALP concentrations.[5] Transient, well-defined increases in serum ALP from both liver and bone forms can be seen in infants and children, often reaching up to 10 times the URL. These changes seem to reflect a reduction in the removal of ALP from blood caused by transient modifications of enzyme glycosylation.[6] Complexes between ALP and immunoglobulins or macro-ALP are occasionally seen in serum and cause abnormal ALP values, but have no specific diagnostic value in the present state of knowledge.[1]

Finally, loss-of-function mutations in the tissue-nonspecific ALP gene are associated with hypophosphatasia, a rare inherited disorder characterized by poor bone mineralization (due to the extracellular accumulation of inorganic pyrophosphate, a natural ALP substrate and a potent inhibitor of mineralization) and low serum ALP activity.[6]

TEST PRINCIPLE

Colorimetric Measurement [in accordance with International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method]

The IFCC reference measurement procedure uses 4-nitrophenyl phosphate (4-NPP) as substrate and 2-amino-2-methyl-1-propanol (AMP) as phosphate acceptor buffer. In accordance with the IFCC reference method, the test method contains 4-nitrophenyl phosphate (4-NPP) as substrate. Alkaline phosphatase in the sample catalyzes the hydrolysis of colorless 4-NPP to yield colorless 4-nitrophenol and inorganic phosphate in benzenoid form. In alkaline medium, 4-nitrophenol is converted to the yellow 4-nitrophenoxide ion form. The enzyme reaction is continuously monitored by observing the rate of formation of 4-nitrophenoxide ions at 405 nm. The rate of increase in absorbance at this wavelength is directly proportional to the ALP activity in the sample.

AMP was used as buffer in this assay. The type of buffer present affects the rate of enzyme activity. Accordingly, buffers can be classified as inert (carbonate and barbital), inhibitory (glycine and propylamine) or activating (AMP, tris[hydroxymethyl]aminomethane [TRIS] and diethanolamine). Activating buffers such as AMP buffer used in optimal concentration increase the enzyme activity two to six times over inert ones such as carbonate.[1]

Optimized concentrations of Mg^{+2} and Zn^{+2} ions are present as cofactors to activate ALP in the sample. The reagent component contains the activator Mg^{+2} and Zn^{+2} ions involved in the reaction as well as the chelating agent N-hydroxyethylenediaminetriacetic acid (HEDTA),[1] which acts as a metal ion buffer to maintain optimal concentrations of these ions.

REAGENT COMPONENTS

2-amino-2-methyl-1-propanol buffer pH 10.40 (30°C),	: ≤ 0.35 M
Magnesium acetate	: ≤ 2 mM
Zinc sulfate	: ≤ 1 mM
HEDTA	: ≤ 2 mM
4-NPP	: ≤ 16 mM

REAGENT PREPARATION

Reagents are 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 10 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.[7]

SAMPLE REQUIREMENTS

Serum or Li-heparin plasma collected by standard procedure can be used.Multiple sample freezing and thawing should be avoided.

ALP activity stability in serum and plasma:

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

Unit Conversion:

U/L x 0.0167 = µkat/L

Note

- Serum or heparinized plasma, free of hemolysis, should be used. Complexing anticoagulants -such as citrate, oxalate and EDTA must be avoided as they bind cations, such as Mg^{+2} and Zn^{+2}, which are essential cofactors for ALP activity. Blood transfusion (containing citrate) causes a transient decrease in serum ALP by a similar mechanism.
- It is recommended that freshly collected serum samples be kept at room temperature and tested as soon as possible, preferably within 4 hours after collection. In sera stored at a refrigerated temperature, ALP activity increases slowly (2%/d).
- Frozen samples should be thawed and kept at room temperature for 18 to 24 hours before measurement to achieve full enzyme reactivation.[1]

CALIBRATION AND QUALITY CONTROL

Calibration: Arcal Auto Calibrator should be used for this test.

Arcal Auto Calibrator
Ref.No: VT-003

Calibration stability is 10 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: VT-001

Arcon P Level 2 Control- Lyophilized
Ref.No: VT-002

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

Reference Range[18]

Age	Range (U/L)
0 to 14 days	90 – 273
15 days to < 1 year	134 – 518
1 year to < 3 years	156 – 369
3 to 5 years	144 – 327
6 to 10 years	153 – 367
11 to 15 years, Male	113 – 438
11 to 15 years, Female	64 – 359
16 to 21 years, Male	56 – 167
16 to 29 years, Female	44 – 107
22 to 79 years, Male	50 – 116
30 to 79 years, Female	46 – 122

- ALP activities in serum vary with age and, in minor amount, with sex.
- For women, a progressive increase in ALP activity has been described after menopause with a reference interval between 53 and 141 U/L.
- Infants and peripubertal children show higher ALP activity than healthy adults as a result of bone ALP leakage from osteoblasts during bone growth. Age-specific reference intervals are therefore crucial for the detection of hypophosphatasia. The decline in ALP activity to the typical adult intervals occurs on average 2 years earlier in females than in males.[1,8]

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 Clinical and Laboratory Standards Institute (CLSI) EP28-A3c protocol.[9]

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).[10]
The determined analytic measuring interval for ALP: 8-1000 U/L.

Detection Capability

Limit of Detection (LoD): 5 U/L

Limit of Quantitation (LoQ): 8 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.[11]

Linearity

This method shows measurement linearity in the activities up to 1000 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 1:10 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.[12]

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.[13]

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

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

Mean Concentration	SD*	CV%	n
83 U/L	0.59	0.71	80
205 U/L	0.90	0.44	80

***SD: Standard Deviation**

Note: This working system has been named “Within-Run Precision” in the previous CLSI - EP05-A2 manual.[14]

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

Mean Concentration	SD	CV%	n
83 U/L	2.35	2.83	80
205 U/L	6.79	3.31	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[14]

Method Comparison

Correlation with a comparative method is:
r= 0.999
According to Passing-Bablok Fit:
y= 0,95x +1,65

Interference

Endogenous interferant and analyte concentrations that have been used in the ALP scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[15,16]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from ALP interference scanning test is appropriate, is determined as ±10%.[17]

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

Interfering Substance and Concentration	ALP Target (U/L)	N*	Observed Recovery %
Hemoglobin 990 mg/dL	85	3	107
Bilirubin 58.5 mg/dL	165	3	98
Lipemia 825 mg/dL	89	3	94

** 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).*[16]

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.[16]

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 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
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practice
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limitation
	Consult Instructions for Use
	Caution
	Number of Tests