

Clinical Enzymology



Isoenzymes or isozymes

Are multiple forms of the same enzyme that catalyze the same chemical reaction

Different chemical and physical properties

Amino acid sequence

Kinetic properties



Property	E.g.
Electrophoretic mobility	Isoenzymes of lactate dehydrogenase have different electrophoretic mobility
Heat stability	alkaline phosphatase Isoenzymes are either heat labile or heat stable.
Inhibitor	An inhibitor can inhibit only one Isoenzymes of an enzyme e.g. Acid phosphatase
Co- factors	Mitochondrial isocitrate dehydrogenase require NAD^+ , cytosolic form require $NADP^+$
Tissue localization	LDH γ is present in heart LDH δ present in liver
Antibodies	For creatine kinase, each Isoenzymes bound only by a specific antibody

Alkaline Phosphatase (ALP)

EC 3, 1, 1

Alternative names

Alkaline
phosphomonoesterase.

Glycerophosphatase.

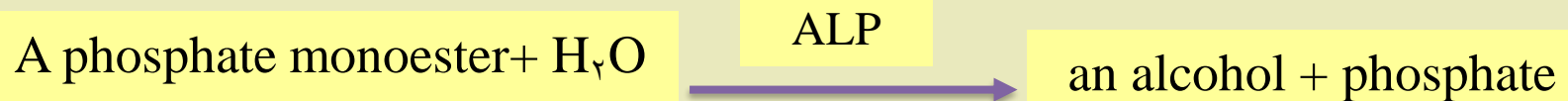
Phosphomonoesterase



Reaction catalyzed:

is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids.

The process of removing the phosphate group is called dephosphorylation



Co – factors: Mg; Zn



Physiology:

In humans, alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone, and the placenta.

Diagnostic use:

Normal ALP levels in adults are •
approximately 20 to 140 IU/L.
levels are significantly higher in •
children and pregnant women.



alkaline phosphatase isozymes:

Alpha \backslash ALP

epithelial cells of biliary canaliculi

Alpha γ ALP

hepatic cells

Labile heat

Placenta

Stable heat

Pre beta ALP

bone

Gamma ALP

intestinal cells

Leukocyte alkaline phosphatase

WBCs



alkaline phosphatase isozymes:

ALP I

intestinal

ALP L

tissue-nonspecific (liver / bone / kidney)

ALP P

placental (Regan isozyme)



Clinical significance

Abnormally high blood levels of ALP may indicate two groups of conditions:

Hepatobiliary disease

bile duct obstruction

Bone disease

associated with increased osteoblastic activity: Paget's disease (*a chronic disorder that typically results in enlarged and deformed bones*), rickets.

**Clinical
significance**

**Abnormally low blood levels of ALP
may indicate**

Hypophosphatasia

Postmenopausal women

magnesium deficiency

severe anemia

Hypothyroidism





Assay for ALP activity

Kinetic UV method using the reagent kits purchased from BioSystems, betalab, Barcelona (Spain)

Principle:

Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from ξ -nitrophenylphosphate to γ -amino- γ -methyl- γ -propanol (AMP), liberating ξ -nitrophenol. The catalytic concentration is determined from the rate of ξ -nitrophenol formation, measured at $\xi \cdot 0$ nm.

ξ -nitrophenylphosphate + AMP

ALP

AMP – phosphate + ξ -nitrophenol



Reagent:

Reagent A

γ -Amino- γ -methyl- β -propanol

Zinc sulfate

N-hydroxyethylethylenediaminetriacetic acid

Magnesium acetate, pH 10.5

Reagent B

ξ -Nitrophenylphosphate

Reagent preparation:

Transfer the contents of one Reagent B vial into a Reagent A bottle. Mix gently.



Procedure:

- Wavelength: 400 nm .1
Cuvette: light path: 1 cm .2
Temperature: $30/37^{\circ}\text{C}$.3
Adjust the instrument to zero against air or d. water. .4

Sample	$20\ \mu\text{l}$
Working Solution	$1\ \text{ml}$

- Mix and insert the cuvette into the photometer.
- Record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
 - Calculate the difference between consecutive absorbances, and the average absorbance difference per minute ($\Delta A/\text{min}$).



Calculation:

$$ALP \text{ (U/l)} = \Delta A \times 2764$$

Normal range:

	30°C	37°C
Male	87 U/l	110 U/l
Female	80 U/l	100 U/l



Serum Alkaline Phosphatase

What the Blood Test Means

Tom Wade MD

