PHOSPHATASE, ALKALINE
from Bovine Intestinal Mucosa
Sigma Prod. No. P5521

CAS: 9001-78-9
ENZYME COMMISSION NUMBER: 3.1.3.1
SYNONYMS: Phosphomonoesterase, Alkaline Phosphomonoesterase

PHYSICAL DESCRIPTION:
Appearance: White suspension.
Molecular weight: 140,000-160,000\(^1\,^2\)
\(E\% (278\text{nm}) = 7.6-10.5\)^\(^1\,^2\).
Isoelectric point: Alkaline phosphatase can exist as isozymes with a pI range of 4.4-5.8\(^3\,^4\,^5\).
P pH Optimum: The enzyme is most stable in the pH range 7.5-9.5\(^2\). The pH optimum for enzymatic activity is pH 8-10. The pH optimum will change depending upon substrate, substrate concentration, and ionic concentration\(^6\). The enzyme activity for this product is determined by Sigma at pH 9.8 (diethanolamine buffer enzyme assay).
Salts present: This product is a suspension in 3.2 M ammonium sulfate containing 1 mM magnesium chloride and 0.1 mM zinc chloride, pH 7.0.

COMPOSITION:
Alkaline phosphatase is a dimer consisting of two equal subunits\(^1\). The enzyme is a glycoprotein containing approximately 12% carbohydrate (6% hexoses and 6% other neutral sugars)\(^2\). Each molecule of alkaline phosphatase contains four zinc atoms and four disulfide bridges\(^2\).

ACTIVATORS:
Maximal activity with alkaline phosphatase is achieved in the presence of magnesium\(^7\).

INHIBITORS:
Strong inhibitors of alkaline phosphatase include arsenate, cysteine, iodine, inorganic phosphate, pyrophosphate, diisopropyl phosphate, triphenylphosphate, and disopropyl fluorophosphate, and L-phenylalanine\(^8\,^9\,^10\).
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SUBSTRATES:

Alkaline phosphatase catalyzes the hydrolysis of phosphate monesters. Substrates that can be hydrolyzed by alkaline phosphatase include p-nitrophenyl phosphate, phenyl phosphate, phenolphthalein phosphate, α-glycerol phosphate, β-glycerol phosphate, 2-phosphorylglycerate, triosephosphate, glucose 6-phosphate, glucose 1-phosphate, fructose 1-phosphate, fructose 6-phosphate, adenosine 5-phosphate, adenosine 3-phosphate, phosphoenolpyruvate, and B-nicotinamide adenine dinucleotide phosphate.8,11,12

For p-Nitrophenyl phosphate, \(K_m = 1.5 \times 10^{-3}\) M
For Phosphoenolpyruvate, \(K_m = 19 \times 10^{-3}\) M

APPLICATIONS:

One of the most common uses of alkaline phosphatase is the use of it as a "reporter" in detection systems in which the alkaline phosphatase is conjugated to a protein (antibody, streptavidin, etc.) which specifically recognizes a target molecule. In addition, alkaline phosphatase may be used to dephosphorylate the 5’ termini of DNA or RNA to prevent self-ligation. DNA or RNA can also be tagged with radiolabeled phosphate (via T4 polynucleotide kinase) after dephosphorylation with alkaline phosphatase.13 Alkaline phosphatase has also been used to dephosphorylate casein.14,15

METHOD OF PREPARATION:

This product is prepared from bovine intestinal mucosa and a method of preparation is described in Preparative Biochemistry, 12, 29, 1982.

STABILITY / STORAGE AS SUPPLIED:

This product is stable for at least two years when stored at 2-8°C.

SOLUBILITY / SOLUTION STABILITY:

A clear and colorless solution is observed when this product is solubilized at a concentration of 1 mg protein/ml in deionized water. Dilute solutions of alkaline phosphatase should be made in 10 mM Tris HCl, pH 8.0, 1-5 mM magnesium chloride, 0.1-0.2 mM zinc chloride, and 50% glycerol and stored at 2-8°C.13

UNIT DEFINITION:

One unit will hydrolyze 1.0 umole of p-nitrophenol phosphate per minute at 37°C. Diethanolamine (DEA) units are measured in a 1.0 M diethanolamine buffer, pH 9.8, containing 0.5 mM magnesium chloride, substrate concentration 15 mM.
REFERENCES: