



Product Information

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}

$E^{1\%}(278\text{nm}) = 7.6-10.5^{1,2}$.

Isoelectric point: Alkaline phosphatase can exist as isozymes with a pI range of 4.4-5.8.^{3,4,5}

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 subunits¹. 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 diisopropyl 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 β-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.

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REFERENCES:

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