ProductInformation

PHOSPHATASE, ALKALINE
from Bovine Intestinal Mucosa
Sigma Prod. No. P5778

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

PHYSICAL DESCRIPTION:

Appearance: White powder.
Molecular weight: 140,000-160,000[1,2]
$E^{1\%}$(278nm) = 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 lyophilized powder containg approximately 60% protein; with the balance being primarily citrate buffer salt, pH 7.2.

STRUCTURE:

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 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 B-nicotinamide adenine dinucleotide phosphate.[8,11,12]
For p-Nitrophenyl phosphate, Km = 1.5×10^{-3} M
For Phosphoenolpyruvate, Km = 19×10^{-3} M

APPLICATIONS:

One common use of alkaline phosphatase is 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 one year when stored at -OEC.

SOLUBILITY / SOLUTION STABILITY:

A clear and colorless solution is observed when this product is solubilized at a concentration of 1 mg/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 2-8EC.[13]

UNIT DEFINITION:

One unit will hydrolyze 1.0 umole of p-nitrophenol phosphate per minute at 37EC. 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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