Product Information

Phosphatase, Alkaline from bovine intestinal mucosa

Ammonium sulfate suspension, ≥2,000 DEA units/mg protein

P5521

Product Description

CAS Registry Number: 9001-78-9

Enzyme Commission (EC) Number: 3.1.3.1

Synonym: alkaline phosphomonoesterase, phosphomonoesterase, glycerophosphatase, alkaline phosphohydrolase, alkaline phenyl phosphatase, orthophosphoric-monoester phosphohydrolase (alkaline optimum)

K_M:

- 1.5×10^{-3} M (*p*-nitrophenyl phosphate)
- 19×10^{-3} M (phosphoenolpyruvate)

Molecular mass:[1,2] 140–160 kDa

$E_{278}^{1\%}$ = 7.6–10.5

Isoelectric point:[3-5] several isozymes with a pI range of 4.4-5.8

Bovine intestinal alkaline phosphatase is a dimeric, membrane-derived glycoprotein.[1,2,6] At least three isoforms exist, which typically possess two N-linked and one or more O-linked glycans per monomer.[1] The enzyme requires zinc, and magnesium or calcium divalent ions for activity.[3] The enzyme contains 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] Maximal activity with alkaline phosphatase is achieved in the presence of magnesium.[7]

Alkaline phosphatase has a broad specificity for phosphate esters of alcohols, amines, pyrophosphate, and phenols. It is routinely used to dephosphorylate proteins and nucleic acids.[8-10] Other applications of alkaline phosphatase include conjugation to antibodies and other proteins for ELISA, Western blotting, and histochemical detection.[11,12]

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 and other proteins.[14,15]

Several publications,[16,17] theses,[18,19] and dissertations[20-24] have cited use of product P5521 in their research protocols.

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.[4]
- The enzyme activity for this product is determined at pH 9.8, using a diethanolamine (DEA) buffer enzyme assay.

Substrates[9,10,25]

Alkaline phosphatase catalyzes the hydrolysis of phosphate monoesters. 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
- β-nicotinamide adenine dinucleotide phosphate

Inhibitors[5,10]

- Chelating agents
- Arsenate
- Cysteine
- Iodine
- Inorganic phosphate
- Pyrophosphate
- Diisopropyl phosphate
- Triphenylphosphate
- Diisopropyl fluorophosphate
- L-phenylalanine

Levamisole (such as Cat. No. L9756) is typically used to inhibit endogenous alkaline phosphatase activity, while only slightly inhibiting the intestinal enzyme.[26,27]

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Product

This product is supplied as an ammonium sulfate suspension, in 3.2 M ammonium sulfate containing 1 mM magnesium chloride ($MgCl_2$) and 0.1 mM zinc chloride ($ZnCl_2$), pH 7.0.

Specific Activity: ≥2,000 DEA units/mg protein

Unit Definition: One DEA unit will hydrolyze 1 µmole of 4-nitrophenyl phosphate per minute at pH 9.8 at 37 °C. Diethanolamine (DEA) units are measured in a 1.0 M DEA buffer (pH 9.8) containing 0.5 mM $MgCl_2$, with a substrate concentration of 15 mM.

Storage/Stability

Store the product, as supplied, at 2-8 °C. **Do not freeze ammonium sulfate suspensions**.

Preparation Instructions

Dilute solutions of alkaline phosphatase should be prepared in 10 mM Tris HCl (pH 8.0), 1-5 mM $MgCl_2$, and 0.1-0.2 mM $ZnCl_2$. 50% glycerol can be included for long term storage at 2-8 °C.[12]

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