3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Phosphatase, Alkaline
from bovine intestinal mucosa

Catalog Number **P0114**
Storage Temperature 2–8 °C

CAS RN 9001-78-9
EC 3.1.3.1
Synonyms: alkaline phosphomonoesterase; phosphomonoesterase; glycerophosphatase; alkaline phosphohydrolase; alkaline phenyl phosphatase; orthophosphoric-monoester phosphohydrolase (alkaline optimum)

Product Description

Bovine intestinal alkaline phosphatase is a dimeric, membrane-derived glycoprotein.[1-3] At least three isoforms exist, which typically possess two N-linked and one or more O-linked glycans per monomer.[2] The enzyme requires zinc, and magnesium or calcium divalent ions for activity.[4]

The enzyme has a broad specificity for phosphate esters of alcohols, amines, pyrophosphate, and phenols. It is routinely used to dephosphorylate proteins and nucleic acids.[5-7]

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

Molecular mass:[2,3] 140–160 kDa
$E_{278}^{1\%}$ = 7.6–10.5

Isoelectric point:[4,8,9] isozymes with a pI range of 4.4–5.8

pH Optimum: The enzyme is most stable in the pH range 7.5–9.5.[3] The pH optimum for enzymatic activity is pH 8–10. The pH optimum will change depending upon substrate, substrate concentration, and ionic concentration.[8] The enzyme activity for this product is determined at pH 9.8 (diethanolamine buffer enzyme assay).

Applications:
Alkaline phosphatase conjugation to antibodies and other proteins for ELISA, Western blotting, and histochemical detection.[10,11]

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.[12] It has also been used to dephosphorylate casein and other proteins.[13,14]

This product is supplied as a solution in 50% glycerol containing 5 mM Tris, 5 mM $MgCl_2$, and 0.1 mM $ZnCl_2$, pH 7.0

Specific Activity: ≥7,500 units/mg protein (DEA units)

Unit Definition: One DEA unit will hydrolyze 1 μmole of 4-nitrophenyl phosphate per minute at pH 9.8 at 37 °C. One glycine unit is equal to ~3 DEA units.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Dilute solutions of alkaline phosphatase should be prepared in 10 mM Tris HCl, pH 8.0, 1–5 mM $MgCl_2$, 0.1–0.2 mM $ZnCl_2$. 50% Glycerol can be included for long term storage at 2–8 °C.

Storage/Stability

Store the solution, as supplied, at 2–8 °C. The product remains active for at least 1 year.

Related Products

Substrates: Sigma-Aldrich offers a broad range of powdered, liquid, and tableted alkaline phosphatase substrate systems for ELISA, Western blotting, histochemistry, activity quantitation, and more. Visit the Enzyme Explorer on line for more details: sigma-aldrich.com/enzymeexplorer.

Inhibitors:
Chelating agents, arsenate, cysteine, iodine, inorganic phosphate, pyrophosphate, diisopropyl phosphate, triphenylphosphate, diisopropyl fluorophosphate, and L-phenylalanine.[9,10]

Levamisole (Catalog Number L9756) is typically used to inhibit endogenous alkaline phosphatase activity, while only slightly inhibiting the intestinal enzyme.[15,16]

References

1. Hsu, H.H.T., *et al*., J. Biol. Chem., **260**, 1826-1831 (1985).
2. Neumann, H., and Lustig, A., European Journal of Biochemistry, **109**, 475-480 (1980).
3. Fosset, M., *et al*., Biochemistry, **13**, 1783-1788 (1974).
4. Besman, M., and Coleman, J.E., J. Biol. Chem., **260**, 11190-11193 (1985).
5. Fernley, H.N., in: The Enzymes (Boyer, P.D., ed.), Vol. IV, 3rd ed., 417-447 (1971).
6. Morton, R. K., Biochemical Journal, **61**, 232-240 (1955).
7. Morton, R.K., Biochemical Journal, **61**, 240-244 (1955).
8. Latner, A.L., *et al*., Enzymologia, **40**, 1-6 (1970).
9. Lazdunski, M., *et al*., Canadian Journal of Chemistry, **43**, 2222-2235 (1965).
10. Harlow, E., and Lane, D., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY: 1988) Chapter 9, p. 349.
11. O'Sullivan, M.J., and Marks, V., Meth. Enzymol., **73**, 147-166 (1981).
12. Maunders, M.J., in Enzymes of Molecular Biology (Burrell, M.M., ed.), 331-341 (1993).
13. Kalan, E.B., and Telka, M., Archives of Biochemistry and Biophysics, **85**, 273-275 (1959).
14. Green, M.R., *et al*., Analytical Biochemistry, **56**, 43-51 (1973).
15. Stagni, N., *et al*., Biochim. Biophys. Acta, **761**, 246-251 (1983).
16. Van Belle, H., Biochim. Biophys. Acta, **289**, 158, (1972)

RBG,MAM 07/08-1