Product Information

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Phospholipase C, Phosphatidylinositol-specific from *Bacillus cereus*

Catalog Number **P5542**
Storage Temperature –20 °C

CAS RN 37288-19-0
EC 4.6.1.13
Synonyms: PI-PLC; 1-Phosphatidyl-D-*myo*-inositol inositolphosphohydrolase, cyclic-phosphate forming

Product Description
The enzymatic action of phospholipase C, phosphatidylinositol specific (PI-PLC) results in the release of GPI (glycosylphosphatidylinositol) anchored proteins from cell membranes. This product is tested for the release of acetylcholinesterase from erythrocyte membranes. The enzyme from several microbial sources has been charaterized.[1-4]

Molecular mass:[1] 29 kDa (SDS-PAGE and gel filtration)

pI:[2] 5.4

Optimal pH:[2] 7.2–7.5 (purified yeast phosphatidyl-inositol as substrate)

Enzyme activity is increased by sodium deoxycholate with maximum effect at 0.16% with 2 mM substrate.[2]

There appears to be no requirement for divalent metal ions as the enzyme activity is not affected by 0.2 mM EDTA nor by 0.08 mM *o*-phenanthroline. Divalent metal ions, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+}, are inhibitory at 2–10 mM and high concentrations of NaCl show significant inhibition.[2]

This product is lyophilized from a solution containing phosphate buffer, EDTA, and a stabilizer. The EDTA serves as a chelator of any zinc ions, which may be present. Zn^{2+} is a cofactor of phospholipase C (PLC, lecithinase), which is present in the preparation. Phospholipase C is a similar enzyme with broader substrate specificity, not specific for just the phosphatidylinositol moiety. The EDTA chelates Zn^{2+} ions and effectively inactivates PLC, resulting in activity due only to phosphatidylinositol-specific phospholipase C. Removal of EDTA from solutions made with this product may result in lecithinase activity. However, if this activity is not a concern and EDTA interferes with the intended application, three methods suggested for removing EDTA are dialysis against 10 mM Tris buffer, pH 7-8; ultrafiltration (cut-off of 10,000 Da); or gel filtration (desalting column).

Specific activity: ≥1,000 units/mg protein

The specific activity measures acetylcholinesterase release from erythrocyte membranes by phosphatidylinositol-specific phospholipase C. The activity of the released acetylcholinesterase is determined by a stopped spectrophotometric rate determination assay measuring the conversion of 5,5′-dithiobis(2-nitrobenzoic acid) and acetylthiocholine iodide. One unit of phosphatidylinositol-specific phospholipase C will liberate one unit of acetylcholinesterase per minute from a membrane bound crude preparation (Catalog Number C5021) at pH 7.4 at 30 °C.

Other activities:
phospholipase C (lecithinase) ≤1 unit/mg protein
sphingomyelinase ≤40 units/mg protein

Protein content: ≤1% (Lowry)

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
The enzyme can be solubilized in 10 mM Tris-HCl, pH 7.4, containing 144 mM NaCl and 0.05% BSA.

Storage/Stability
Store the product at –20 °C. The lyophilized product, when stored properly, retains activity for at least 2 years.

A preparation of this enzyme (Catalog Number P8804) in 60% (v/v) glycerol containing 10 mM Tris-HCl, pH 8.0, and 10 mM EDTA retains activity at 2–8 °C for at least 3 years.

The enzyme is fairly unstable in acidic medium and can be inactivated by heating.[2]

References

1. Sundler, R., et al., Enzymatic Properties of Phosphatidylinositol Inositolphosphohydrolase from *Bacillus cereus*. Substrate Dilution in Detergent-phospholipid Micelles and Bilayer Vesicles. J. Biol. Chem., **253(12)**, 4175-4179 (1978).
2. Ikezawa, H., and Taguchi, R., Phosphatidyl-inositol-Specific Phospholipase C from *Bacillus cereus* and *Bacillus thuringiensis*. Meth. Enzymol., **71-C**, 731-741 (1981).
3. Heinz, D.W., et al., Crystal Structure of Phosphatidylinositol-specific Phospholipase C from *Bacillus cereus* in Complex with Glucosaminyl (α 1$\rightarrow$6)-D-*myo*-inositol, an Essential Fragment of GPI Anchors. Biochemistry, **35(29)**, 9496-9504 (1996).
4. Taguchi, R., et al, Purification and Properties of Phosphatidylinositol-specific Phospholipase C of *Bacillus thuringiensis*. Biochim. Biophys. Acta, **619(1)**, 48-57 (1980).

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