

# Product Information

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## Phospholipase C, Phosphatidylinositol-specific from *Bacillus cereus*

Catalog Number **P8804**

Storage Temperature 2–8 °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 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 characterized.<sup>1-4</sup>

Molecular mass:<sup>1</sup> 29 kDa (SDS-PAGE and gel filtration)

pI:<sup>2</sup> 5.4

Optimal pH:<sup>2</sup> 7.2–7.5 (purified yeast phosphatidylinositol as substrate)

Enzyme activity is increased by sodium deoxycholate with maximum effect at 0.16% with 2 mM substrate.<sup>2</sup>

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<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, and Zn<sup>2+</sup> are inhibitory at 2–10 mM and high concentrations of NaCl show significant inhibition.<sup>2</sup>

This product is supplied in a solution containing 10 mM Tris-HCl, pH 8.0, with 10 mM EDTA, and 60% (v/v) glycerol. The EDTA serves as a chelator of any zinc ions, which may be present. Zn<sup>2+</sup> 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<sup>2+</sup> ions and effectively inactivates PLC, resulting in activity due only to phosphatidylinositol-specific phospholipase C. Removal of EDTA from this product may result in lecithinase activity.

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

### 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.

### Storage/Stability

Store the product at 2–8 °C. When stored properly, the product retains activity for at least 3 years.

The enzyme is fairly unstable in acidic medium and can be inactivated by heating.<sup>2</sup>

## 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 ( $\alpha$  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).

ES-S,RBG,RLG,RXR,MAM 02/08-1

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