

## Product Information

### Anti-PC Specific PLD2, N-terminal

Developed in Rabbit, Affinity isolated antibody

Product Number **P 6618**

#### Product Description

Anti-PC Specific PLD2 (Anti-Phosphatidylcholine Specific Phospholipase D2), N-terminal was developed in rabbit using a synthetic peptide derived from the N-terminal region of mouse PC-specific PLD2 protein (amino acids 29-39) as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography.

The antibody specifically recognizes the N-terminal region of the PLD2 enzyme. It detects mouse recombinant and natural PLD2 (approx. 108 kDa). It does not crossreact with PLD1. It is used in immunoblotting and immunoprecipitation applications.<sup>1-3</sup>

Phosphatidylcholine specific phospholipase D (PLDs) are widely distributed enzymes found in bacteria, fungi, plants and animals. They are implicated in several important cellular functions and, in mammals, are under the control of many hormones, neurotransmitters, growth factors and cytokines. PLDs hydrolyze phosphatidylcholine to phosphatidic acid (PA) and choline. There are two mammalian PLD isoforms (PLD1 and PLD2), which occur as splice variants. These have four conserved sequences (I-IV) and pleckstrin homology (PH) and phox homology (PX) domains in tandem at their N-termini. These domains are implicated in phospholipid and protein binding. The mammalian PLDs have an absolute requirement for phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) for their activation.<sup>4-6</sup>

PLD2 is PIP<sub>2</sub>-dependent but differs from PLD1 in that it exhibits high basal constitutive activity *in vitro* and *in vivo* and shows little or no response to such PLD1 activating proteins as PKC $\alpha$ , ARF and Rho. The basal activity of PLD2 is approximately 1500 fold higher than that of PLD1, which is proof that this PLD form is expressed in the active state. The specific antibodies generated against PLD1 and PLD2 have been used in the studies of tissue expression, activation and function of PLDs.<sup>1,2,3</sup>

The selectivity of the isozyme-specific antibodies was first investigated by immunoprecipitating PLD from baculovirus-infected Sf9 cells expressing human PLD1 or mouse PLD2. The

PLD antibodies immunoprecipitated PLD activity only in the corresponding Sf9 cell lysate. Both antibodies were efficient in immunoprecipitating their respective proteins from whole-cell lysates: antibodies against PLD1 immunoprecipitated 95% of the total activity, whereas antibodies against PLD2 immunoprecipitated 88% of the total activity.<sup>3</sup> PLD2 is present in all mouse tissues, with highest levels detected in brain and lung. PLD2 localizes primarily to the plasma membrane. It is constitutively active and may be modulated *in vivo* by mechanisms involving protein inhibitors that have been identified using *in vitro* assays. These inhibitors include fodrin, synaptojanin, clathrin assembly protein-3 and synucleins. PLD2 is responsible for the PLD activity that generates diglyceride in caveolin-1-enriched detergent-resistant membrane microdomains (DRMs). PLD2 may be responsible for signal-induced cytoskeletal regulation and/or endocytosis. The two PLD isoforms may serve distinct but complementary functions in secretion.<sup>6,7</sup>

#### Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg<sup>2+</sup> and Ca<sup>2+</sup>), pH 7.3, with 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

#### Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

### Storage/Stability

Store at  $-70^{\circ}\text{C}$ . For continuous use, store at  $2-8^{\circ}\text{C}$  for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

### Product Profile

The supplied reagent is sufficient for 10 blots.

A recommended working dilution of 1:100 to 1:2000 is determined by immunoblotting using recombinant purified PLD2 protein. Due to the low expression levels of this protein, it is suggested that the protein be immunoprecipitated before immunoblotting.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

### References

1. Hammond, S. M., et al., Characterization of two alternately spliced forms of phospholipase D1. Activation of the purified enzymes by

- phosphatidylinositol 4,5-bisphosphate, ADP-ribosylation factor, and Rho family monomeric GTP-binding proteins and protein kinase C- $\alpha$ ., *J. Biol. Chem.*, **272**, 3860-3868 (1997).
2. Coley, W. C., et al., Phospholipase D2, a distinct phospholipase D isoform with novel regulatory properties that provokes cytoskeletal reorganization., *Curr. Biol.*, **7**,191-201 (1997).
3. Sciorra, V. A. and Morris, A. J., Sequential actions of phospholipase D and phosphatidic acid phosphohydrolase 2b generate diglyceride in mammalian cells., *Mol. Biol. Cell.*,**10**, 3863-3876 (1999).
4. Exton, J. H., Phospholipase D., *Ann. N. Y. Acad. Sci.*, **905**, 61-68 (2000).
5. Frohman, M.A., et al., Mammalian phospholipase D structure and regulation., *Biochim. Biophys. Acta*, **1439**, 175-186 (1999).
6. Powner, D. J., et al., The regulation of phospholipase D by inositol phospholipids and small GTPases., *FEBS Lett.*, **531**, 62-64 (2002).
7. Cockcroft, S., et al., Signaling role for ARF and phospholipase D in mast cell exocytosis stimulated by crosslinking of the high affinity Fc varepsilon R1 receptor., *Mol. Immunol.*, **38**, 1277-1282 (2002).

AH/PHC 09/04

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.