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ProductInformation

Anti-PC Specific PLD1, N-Terminal
Developed in Rabbit, Affinity isolated antibody

Product Number **P 5743**

Product Description

Anti-Phosphatidylcholine (PC) Specific PLD1, N-terminal antibody was developed in rabbit using as immunogen a synthetic peptide derived from the N-terminal region of human PC-specific PLD1 protein (aa 1-15). The antiserum is affinity purified using epitope-specific affinity chromatography. Anti-PC-specific PLD1 antibody specifically recognizes the N-terminal region of the PLD1 enzyme. The antibody detects human recombinant and natural (~120 kDa) PLD1. It does not crossreact with PLD2. It is used in immunoblotting and immunoprecipitation applications.[1-3]

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_2) for their activation.[4-6]

PLD1, a 124 kDa protein, is a member of a novel but highly conserved PLD gene family. PLD1 likely encodes the gene product responsible for the most widely studied endogenous PLD activity. PLD1 has an extremely low level of basal activity and can be activated independently by the members of each of the known categories of PLD activators (PKCα, ARF and Rho). In the presence of these activators, PLD1 activity is increased approximately 250-fold over basal levels. PLD2 is also PIP_2-dependent but differs from PLD1 in that it exhibits a high basal constitutive activity *in vitro* and *in vivo* (about 1500-fold greater than that of PLD1) and shows little or no response to activating proteins such as PKCα, ARF and Rho. The specific antibodies generated against PLD1 and PLD2 are used to study tissue expression, activation and function of PLDs.[1,2,3]

The selectivity of 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.[3] PLD1 localizes solely to peri-nuclear regions (the endoplasmic reticulum, Golgi apparatus and late endosomes), where it promotes ARF-mediated intravesicular membrane trafficking. It is suggested that ARF, by activating PLD1 and PIP5-kinase, regulates PA and $PI(4,5)P_2$ levels, both of which are critical components of the exocytosis machinery in mast cells. The two isoforms may serve distinct but complementary functions in secretion.[6,7]

Reagent

Anti-PC-specific PLD1 antibody is supplied at approximately 0.5 mg/ml as a solution in phosphate buffered saline, pH 7.2, containing 0.1% BSA and 0.05% sodium azide. The amount of the reagent is sufficient for 10 blots.

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 °C. For extended storage upon initial thawing, freeze in working aliquots. Do not store in frost-free freezers. Avoid repeated freezing and thawing to prevent denaturing the antibody. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months when stored appropriately.

Product Profile
A recommended working dilution of 1:100 to 1:2000 is determined by immunoblotting using recombinant purified PLD1 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

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