OriGene Technologies Inc.

9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

OriGene EU

Acris Antibodies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

Polyclonal Antibody to Phospholipase D1 (PLD1) (N-term) - Aff - Purified

Alternate names: PL-D1

Catalog No.: BP7149

Quantity: 0.1 mg

Background: Activation of PL-D results in the generation of second messengers, phosphatidic acid and diglycerides, and appears to be involved in secretion, vesicle trafficking, mitosis and meiosis. In leukocytes, PL-D regulates cytoskeletal-dependent antimicrobial responses such as phagocytosis and oxidant generation. PL-D1 is regulated by GTP-binding proteins, (ARF and Rho families) and by protein kinase C.

Host: Rabbit

Immunogen: A chemically synthesized peptide derived from the N-terminal region of human PC-specific PL-D1 protein

Format:
State: Liquid
Purification: Peptide affinity chromatography
Buffer System: PBS, pH 7.2, with 0.1% BSA and 0.09% sodium azide

Applications: Western blotting: use at a dilution of 1:100 to 1:2000 against purified recombinant protein. Due to the low expression levels of this protein, it is suggested that the protein be immunoprecipitated before Western blotting.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody specifically recognizes the N-terminal region of the PL-D1 enzyme.
Species: Human.
Other species not tested.

Storage: Store (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings:
Hammond S.M., et al. (1997) 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 (6):3860-3868.
Sciorra, V.A. and A.J. Morris (1999) Sequential actions of phospholipase D and phosphatidic acid phosphohydrolase 2b generate diglyceride in mammalian cells. Mol. Biol. Cell 10:3863-3876 (cites the use of the PL-D1 and PL-D2 antibodies).
Zhong, M., et al. (2002) Elevated phospholipase D activity induced apoptosis in normal rat fibroblasts. Biochem. Phys. Res. Comm. 298:474-477 (cites the use of the PL-D1 and PL-D2 antibodies).

Protocols:

Western Blotting Procedure
1. Lyse approximately 10e7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH2O for 5 minutes.
7. Soak the PVDF membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4°C.
12. Incubate the blocked blot with primary antibody at appropriate concentration in Tris buffered saline supplemented with 3% BSA and 0.1% Tween 20 for two hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat F(ab)2 anti-rabbit IgG alkaline phosphatase conjugate or goat F(ab)2 anti-rabbit IgG horseradish peroxidase conjugate in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM Na4P2O7
2 mM Na3VO4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO)
or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin

1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma catalog number P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add 1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
5 gm BSA
0.1 mL Tween 20