

## Polyclonal Antibody to Phospholipase D1 (PLD1) (internal) - Aff - Purified

<b>Alternate names:</b>	PL-D1
<b>Catalog No.:</b>	BP7148
<b>Quantity:</b>	0.1 mg
<b>Concentration:</b>	0.5 mg/ml
<b>Background:</b>	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.
<b>Host:</b>	Rabbit
<b>Immunogen:</b>	Synthetic peptide from the internal region of the human PC-specific PL-D1 protein
<b>Format:</b>	<b>State:</b> Liquid Ig fraction <b>Purification:</b> Peptide affinity chromatography <b>Buffer System:</b> Dulbecco's phosphate buffered saline, pH 7.2, with 1 mg/mL BSA, containing 0.05 % sodium azide.
<b>Applications:</b>	Western blot (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 use. Recommended positive control: Recombinant Protein, U937 cells. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
<b>Specificity:</b>	This antibody detects Phospholipase D1, internal. <b>Species:</b> Human. Other species not tested.
<b>Storage:</b>	Store (in aliquots) at -80 °C. Avoid repeated freezing and thawing. Shelf life: one year from despatch.
<b>General Readings:</b>	1. 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. 2. 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 PLD1 and PD2 antibodies).

Protocols:

Western Blotting Procedure

1. Lyse approximately  $10 \times 10^7$  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  $14,000 \times g$  for 10 minutes. Alternatively, lysates may be ultracentrifuged at  $100,000 \times g$  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  $100^\circ\text{C}$ .
5. Load 10-30  $\mu\text{g}$  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 ddH<sub>2</sub>O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the 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) for one hour at room temperature or overnight at  $4^\circ\text{C}$ .
12. Incubate the blocked blot with primary antibody at a concentration of  $\sim 0.1$ - $1.0 \mu\text{g}/\text{mL}$  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')<sub>2</sub> anti-rabbit IgG alkaline phosphatase conjugate or goat F(ab')<sub>2</sub> 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 Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>
- 2 mM Na<sub>3</sub>VO<sub>4</sub>
- 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 Cat. # 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

Pictures:

Using SDS-PAGE, proteins were resolved from (a) recombinant PLD2 protein, (b) recombinant PLD1 protein, and (c) the detergent-insoluble fraction of U937 cells. The proteins were transferred to PVDF membrane and incubated with a 1:2000 dilution of rabbit (polyclonal) anti-PC-specific PLD1 Internal antibody. The signal was detected using a Goat F(ab')<sub>2</sub> anti-Rabbit IgG Alkaline Phosphatase antibody at a 1:5000 dilution and the membrane was incubated with CDP-substrate using the WesternStar™ method (Tropix). The membrane was then exposed to Kodak BioMax film.

