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Polyclonal Antibody to Phospholipase D2 (PLD2) (internal) - Aff - Purified

Alternate names: PL-D2

Catalog No.: BP7150

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. The mechanisms that regulate PL-D2 are not known. This isoform is unaffected by the activators of PL-D1.

Host: Rabbit

Immunogen: Chemically synthesized peptide derived from the internal region of mouse PC-specific PL-D2 protein

Format: **State:** Liquid Ig fraction
Purification: Peptide affinity chromatography
Buffer System: Dulbecco's phosphate buffered saline (without Mg2+ and Ca2+), pH 7.3 (+/- 0.1), with 1.0 mg/mL BSA (IgG, protease free) as a carrier, containing 0.05 % sodium azide as preservative

Applications: 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 Western blotting).
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody detects PL-D2, Internal.
Species: Mouse.
Other species not tested.

Storage: Store at -80 şC. Upon initial thawing, aliquot and store at -80 şC. Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings: Colley, W.C. et al. (1997) Phospholipase D2, a distinct phospholipase D isoform with novel regulatory properties that provokes cytoskeletal reorganization. Curr. Biol. 7:191-201.
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.

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 ultracentrifugedat 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. 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°C.
12. Incubate the blocked blot with primary antibody at a dilution of 1:100 to 1:2000 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 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:

The protein was resolved using SDS-PAGE. The protein was transferred to PVDF membrane and incubated with a 1:2000 dilution of the rabbit (polyclonal) anti-PC Specific PL-D2 Internal antibody. The signal was detected using a Goat F(ab')2 anti-Rabbit IgG Alkaline Phosphatase antibody at a 1:5000 dilution and the membrane was incubated with CDP-substrate using the WesternStarTM method (Tropix). The membrane was then exposed to Kodak BioMax film.