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Polyclonal Antibody to PSAT1(M-49) - Aff - Purified

Catalog No.: 15-288-21256

Quantity: 0.1 mg

Background: Protein Kinase CβII (PKCβII) is an 80 kDa member of the conventional group (cPKCs: sensitive to diacylglycerol, phosphotidylserine and phorbol esters) of the PKC family of serine/threonine kinases that are involved in a wide range of physiological processes including mitogenesis, cell survival, transcriptional regulation and tumor promotion. PKCβII is phosphorylated on three sites, threonine 500 in the activation loop, threonine 641 in the turn loop and serine 660 in the hydrophobic loop. Threonine 641 mediates PKCβII binding to Hsp70 which regulates its stability and phosphorylation. Phosphorylation of threonine 641 is also crucial for PKCβII proper subcellular localization and catalytic function as well as for its autophosphorylation on serine 660.

Host / Isotype: Chicken

Immunogen: Chemically synthesized phosphopeptide derived from a region of human PKCβII that contains threonine 641.
Remarks: The sequence is conserved in mouse and rat.

Format:
State: Liquid Ig fraction
Purification: Sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated PKCβII. The final product is generated by affinity chromatography using a PKCβII-derived peptide that is phosphorylated at threonine 641.
Buffer System: Dulbecco's phosphate buffered saline (without Mg2+ and Ca2+), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier, containing 0.05 % sodium azide

Applications: Western blot (1:1000).
Positive Control Used: K562 cells treated with PMA, a phorbol ester.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody is specific for PKCβoI [pT642].
Species: Human, Mouse, Rat.
Others not tested.

Storage:

Store the antibody at -20 °C. Can be shipped at 2 - 8 °C.
Avoid repeated freezing and thawing.
Centrifuge vial before opening.
Shelf life: One year from despatch.

General References:

Gao, T. and A.C. Newton (2002) The turn motif is a phosphorylation switch that regulates the binding of Hsp70 to protein kinase C. J. Biol. Chem. 277(35):31585-31592.
Edwards, A.S., et al. (1999) Carboxyl-terminal phosphorylation regulates the function and subcellular localization of protein kinase C βII. J. Biol. Chem. 274(10):6461-6468.
Keranen, L.M., et al. (1995) Protein kinase C is regulated in vivo by three functionally distinct phosphorylations. Curr. Biol. 5(12):1394-1403.
Newton, A.C. (1995) Protein kinase C: structure, function, and regulation. J. Biol. Chem. 270(48):28495-28498.
Zhang, J., et al. (1994) Phosphorylation of Thr642 is an early event in the processing of newly synthesized protein kinase C β1 and is essential for its activation. J. Biol. Chem. 269(30):19578-19584.

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 1:1000 dilution in Tris buffered saline supplemented with 3% BSA and 0.1% Tween-20 overnight at 4°C or for one hour 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.

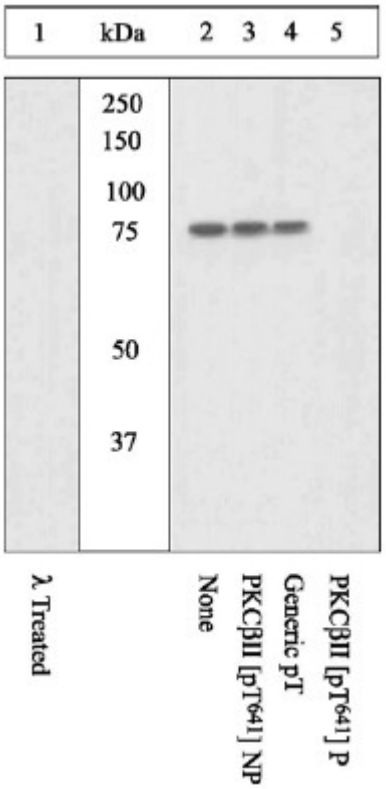
Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM Na4P2O7
2 mM Na3VO4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO)
or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin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



Peptide Competition and Phosphatase Treatment
Lysates prepared from K562 cells stimulated with PMA were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF. Membranes were either treated with lambda (λ) phosphatase (1) or, left untreated (2-5), blocked with a 5% BSA-TBST buffer for two hours at room temperature, and incubated with PKCβII [pT641] antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 2), the non-phosphopeptide corresponding to the immunogen (3), a generic phosphothreonine-containing peptide (4), or, the phosphopeptide immunogen (5). After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG HRP conjugate and bands were detected using the Pierce SuperSignalTM method. The data show that the peptide corresponding to PKCβII [pS641] blocks the antibody signal. The data also show that phosphatase stripping eliminates the signal, verifying that the antibody is phospho-specific.