Rabbit (polyclonal) Anti-PKCβI [pT^{642}] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number: 44-963G (10 mini-blot size)

Lot Number: See product label

Volume: 100 µL

Form of Antibody: Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier.

Preservative: 0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)

Purification: Purified from rabbit serum by 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 PKCβI. The final product is generated by affinity chromatography using a PKCβI-derived peptide that is phosphorylated at threonine 642.

Immunogen: The antiserum was produced against a chemically synthesized phosphopeptide derived from a region of human PKCβI that contains threonine 642. The sequence is conserved in rat.

Target Summary: Protein Kinase CβI (PKCβI) 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. PKCβI is phosphorylated on three sites, threonine 500 in the activation loop, threonine 642 in the turn loop and serine 661 in the hydrophobic loop. Autophosphorylation of PKCβ1 on threonine 642 is crucial for its activation and catalytic activity. Threonine 642 mediates PKCβ1 ability to activate c-Fos in response to phorbol esters.

Reactivity: Human PKCβI. Rat (100% homologous) PKCβI has not been tested, but is expected to react. This antibody does not react with PKCβII [pT^{641}], α [pT^{638}], γ [pT^{655}], ε [pT^{710}], ι [pT^{555}], η [pT^{655}], or ζ [pT^{560}] as determined by peptide competition experiments.

Applications: The antibody has been used for Western blotting applications.

Suggested Working Dilutions: For Western blotting applications, we recommend using the antibody at a 1:1000 dilution. The optimal antibody concentration should be determined empirically for each specific application.

Storage: Store at −20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at −20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.

Expiration Date: Expires one year from date of receipt when stored as instructed.

Positive Control Used: K562 cells treated with PMA, a phorbol ester.

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This antibody is manufactured under a licensed process covered by Patent # 5, 599, 681.

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Related Products:

Antibodies:

PKCα [pT638], Cat. # 44-962

PKCβII [pT642], Cat. # 44-964G

PKCγ [pT514], Cat. # 44-956

PKCγ [pT655], Cat. # 44-965

PKCγ [pT674], Cat. # 44-975

PKCδ [pY311] (mouse), Cat. # 44-950

PKCδ [pS645], Cat. # 44-966

PKCη [pT655], Cat. # 44-969

PKCθ [pT676], Cat. # 44-970

PKCι [pT555], Cat. # 44-968G

anti-PKCβI pan, Cat. # AHO0712

Akt/PKB [pT308], Cat. # 44-602G

Akt/PKB [pS473], Cat. # 44-621G

Other: See also antibodies, substrates and inhibitors to PKCs in the Invitrogen catalog.

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.

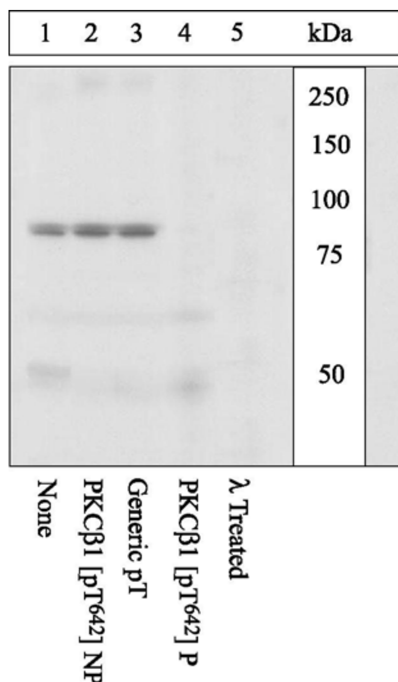
Paula, D., et al. (2000) Oxidative stress induces increase in intracellular amyloid beta-protein production and selective activation of betaI and betaII PKCs in NT2 cells. Biochem. Biophys. Res. Commun. 268(2):642-646.

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.



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 left untreated (1-4) or treated with lambda (λ) phosphatase (5), blocked with a 5% BSA-TBST buffer for one hour at room temperature, and incubated with PKCβI [pT642] antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 5), the non-phosphopeptide corresponding to the immunogen (2), a generic phosphothreonine-containing peptide (3), or, the phosphopeptide immunogen (4). After washing, membranes were incubated with goat $F(ab')_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and bands were detected using the Pierce SuperSignal™ method.

The data show that only the peptide corresponding to PKCβI [pT642] blocks the antibody signal. The data also show that phosphatase stripping eliminates the signal, verifying that the antibody is phospho-specific.

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Western Blotting Procedure

1. Lyse approximately 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. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). 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 ddH_2O for 5 minutes. Alternatively, nitrocellulose may be used.
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) 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 (Cat. # ALI4405) or goat F(ab')$_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) 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 $Na_4P_2O_7$
2 mM Na_3VO_4
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

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