Rabbit (polyclonal) Anti-PKCι [pT^{555}]/PKCλ [pT^{563}] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44-968G (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 epitope-specific affinity 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 PKCι. The final product is generated by affinity chromatography using a PKCι-derived peptide that is phosphorylated at threonine 555.
Immunogen:	The antiserum was produced against a chemically synthesized phosphopeptide derived from a region of human PKCι that contains threonine 555. The sequence is conserved in mouse PKCι and human PKCλ.
Target Summary:	Protein Kinase Cι (PKCι) is a 76 kDa member of the atypical 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. Phosphorylation of PKCι plays a key role in protein trafficking. Phosphorylated PKCι is implicated in high glucose-induced T type II transforming growth factor-beta activity, TNF-α-stimulated NF-κB activation and Bcr/Abl-mediated anti-apoptotic effect.
Reactivity:	Human and mouse (100% homologous) PKCι, and human PKCλ. This antibody reacted with PKCλ immunoprecipitates, indicating cross-reactivity for PKCλ [pT^{563}]. PKCζ [pT^{560}] (83% homologous) has been shown to cross-react by peptide competition. Peptide competition also suggests that this antibody may partially cross-react with PKCβI [pS^{642}] (58% homologous) and PKCγ [pT^{655}] (42% homologous).
Applications:	This antibody has been used in 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:	Jurkat cells treated with PMA, a phorbol ester.

This product is for research use only. Not for use in diagnostic procedures.

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Related Products:

Antibodies:

PKCα [pT^{638}], Cat. # 44-962
PKCβII [pT^{641}], Cat. # 44-964G
PKCγ [pT^{514}], Cat. # 44-956
PKCγ [pT^{655}], Cat. # 44-965
PKCγ [pT^{674}], Cat. # 44-975
PKCδ [pY^{311}] (mouse), Cat. # 44-950
PKCδ [pS^{645}], Cat. # 44-966
PKCη [pT^{655}], Cat. # 44-969
PKCθ [pT^{676}], Cat. # 44-970
Akt/PKB [pT^{308}], Cat. # 44-602G
ERK1&2 [pTpY$^{185/187}$], Cat. # 44-680G
IKK-alpha [pSpS$^{176/180}$], Cat. # 44-714
IκB PhosphoELISA, Cat. # KHO0211

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

References :

Tisdale, E.J. et al. (2003) Atypical protein kinase C plays a critical role in protein transport from Pre-Golgi intermediates. J. Biol. Chem. [Jul 17 epub].

Chuang, L.Y. et al. (2003) Regulations of type II transforming growth factor-beta receptors by protein kinase C iota. Biochem. J. [Jul 4 epub].

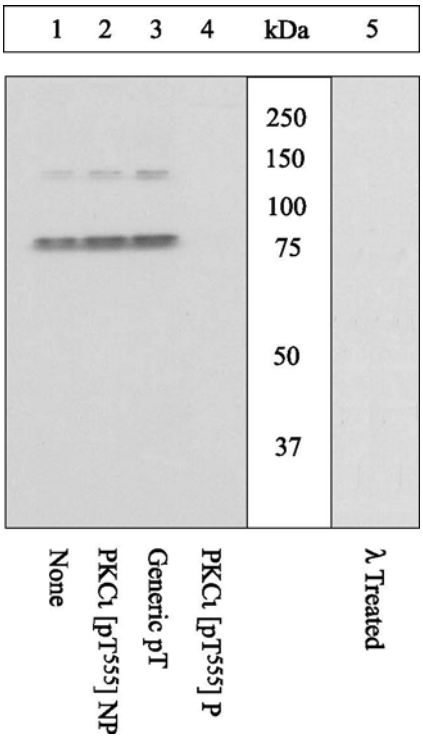
Standaert, M.L. et al. (2002) Skeletal muscle insulin resistance in obesity-associated type 2 diabetes in monkeys is linked to a defect in insulin activation of protein kinase C-zeta/lambda/iota. Diabetes 51(10):2936-2943.

Selzer, E. et al. (2002) Protein kinase C isoforms in normal and transformed cells of the melanocytic lineage. Melanoma Res. 12(3):201-209.

Acevedo-Duncan, M. et al. (2002) Human glioma PKC-iota and PKC-betaII phosphorylate cyclin-dependent kinase activating kinase during the cell cycle. Cell Prolif. 35(1):23-36.

Xie, J. et al. (2000) Protein kinase C iota protects neural cells against apoptosis induced by amyloid beta-peptide. Brain Res. Mol. Brain Res. 82(1-2):107-113.

Jamieson, L. et al. (1999) Protein kinase Ciota activity is necessary for Bcr-Abl-mediated resistance to drug-induced apoptosis. J. Biol. Chem. 274(7):3927-3930.



Peptide Competition and Phosphatase Treatment

Lysates prepared from Jurkat 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% low-fat milk-TBST buffer for one hour at room temperature, and incubated with PKCι [pT^{555}] antibody for two hours at room temperature in a 3% low-fat milk-TBST buffer, following prior incubation with: no peptide (1), 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 the phosphopeptide corresponding to PKCι [pT^{555}] blocks the antibody signal. The peptide corresponding to PKCζ [pT^{560}] blocks the antibody signal and the peptides corresponding to PKC isoforms βI [pT^{642}] and γ [pT^{655}] partially block the antibody signal (data not shown), suggesting cross-reactivity of the antibody with these sites. The antibody signal was not blocked by the corresponding peptides of any other PKC isoforms. 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% low-fat milk 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 low-fat dried milk
0.1 mL Tween 20

This product is for research use only. Not for use in diagnostic procedures.

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