



**Rabbit (polyclonal)
Anti-PKC β I&II [pT⁵⁰⁰]
Phosphospecific Antibody, Unconjugated**

PRODUCT ANALYSIS SHEET

Catalog Number:	44955G (10 mini-blot size)
Lot Number:	See product label
Volume:	100 μ L
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 PKC β . The final product is generated by affinity chromatography using a PKC β -derived peptide that is phosphorylated at threonine 500.
Immunogen:	The antiserum was produced against a chemically synthesized phosphopeptide derived from a region of human PKC β I&II that contains threonine 500.
Target Summary:	Protein Kinase C β (PKC β) is an 80 kDa member of the conventional group (cPKCs: sensitive to diacylglycerol, phosphatidylserine 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. PKC β has been implicated in diabetes and carcinogenesis. PKC β isoforms I&II are phosphorylated on three sites, threonine 500 in the activation loop, β I threonine 642 (β II 641) in the turn loop and β I serine 661 (β II 660) in the hydrophobic loop. Phosphorylation of PKC β I&II on threonine 500 by PDK1 is a prerequisite for its autophosphorylation and catalytic competence.
Reactivity:	Human PKC β I&II. Mouse and rat PKC β I&II have not been tested, but are expected to react. This antibody cross-reacts with PKC α [pT ⁴⁹⁷] (88% homologous) and partially cross-reacts with PKC γ [pT ⁵¹⁴] (63% homologous) and ϵ [pT ⁵⁶⁶] (75% homologous), as determined by antibody-peptide competition experiments.
Applications:	The antibody has been used in Western blotting. Other applications may work but have not been tested.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The exact concentration is not determined for each lot; however, the typical range is 0.1-1.0 mg/mL. 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.

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

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Related Products:**Antibodies:**PKC γ [pT⁵¹⁴], Cat. # 44956PKC β II [pT⁶⁴¹], Cat. # 44964GPKC θ [pT⁵³⁸], Cat. # 44960GPKC β I, Cat. # AHO0712PKC β I [pT⁶⁴²], Cat. # 44963GPKC β II, Cat. #s AHO0722, AHO0632**Other:** See also antibodies, substrates and inhibitors to PKCs in the Invitrogen catalog.**References:**

Newton, A.C. (2003) Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm. *Biochem. J.* 370(Pt 2):361-371.

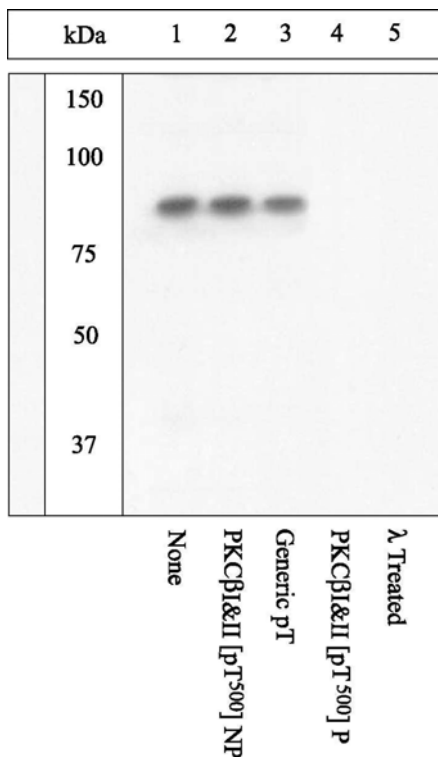
Yoshida, M., et al. (2003) Involvement of PKC betaII in anti-proliferating action of a new antitumor compound gnidimacrin. *Int. J. Cancer.* 105(5):601-606.

Helliwell, P.A., et al. (2003) Intestinal sugar absorption is regulated by phosphorylation and turnover of protein kinase C betaII mediated by phosphatidylinositol 3-kinase- and mammalian target of rapamycin-dependent pathways. *J. Biol. Chem.* 278(31):28644-28650.

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.

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.

**Peptide Competition and Phosphatase Treatment**

Extracts of K562 cells stimulated with 100 μ M PMA for 30 minutes were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was left untreated (1-4) or treated with lambda (λ) phosphatase (5), blocked with a 5% BSA-TBST buffer for one hour at room temperature, then incubated with the PKC β I&II [pT⁵⁰⁰] 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 phosphopeptide immunogen (2), a generic phosphothreonine-containing peptide (3), or the phosphopeptide immunogen (4). After washing, the membrane was incubated with goat F(ab')₂ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignalTM method.

The data show that only the phosphopeptide corresponding to PKC β I&II [pT⁵⁰⁰] blocks the antibody signal, demonstrating the specificity of the antibody. The data also show that phosphatase stripping eliminates the signal, further 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 (Invitrogen 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 ddH₂O 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) overnight at 4°C or for one hour at room temperature.
12. Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween-20 overnight at 4°C or 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 (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 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 Ig-free BSA
0.1 mL Tween 20

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