

Phospho-PKC α/β II (Thr638/641) Antibody

✓ 100 μ l
(10 Western mini-blot)

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This product is for *in vitro* research use only and is not intended for use in humans or animals. This product is not intended for use as a therapeutic or in diagnostic procedures.

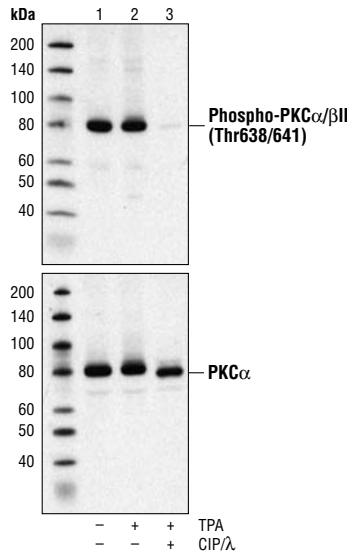
Applications W, IP	Species Cross-Reactivity* H, M, R, Mk	Molecular Wt. 80 kDa, 82 kDa	Source Rabbit**
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Background: Activation of PKC is one of the earliest events in a cascade leading to a variety of cellular responses such as secretion, gene expression, proliferation and muscle contraction (1,2). PKC isoforms have been classified into three groups: classical PKCs, which are calcium-dependent via their C2 domains and are activated by phosphatidylserine (PS), diacylglycerol (DAG) and phorbol esters (TPA or PMA) through their cysteine-rich C1 domains, novel PKCs, which are calcium-independent but are still regulated by PS, DAG and TPA and atypical PKCs, which are calcium-independent and do not require PS, DAG or TPA for their activation (3-7). Members of these three PKC groups contain a pseudo-substrate or autoinhibitory domain that binds to the substrate binding site in the catalytic domain, preventing its activation in the absence of cofactors or activators.

Other members have been recently added to the PKC superfamily based on homology within the catalytic domain. PKC, or PKD, is regulated by DAG and TPA through its C1 domain. However, PKD is distinguished by a PH domain, as well as by its unique substrate recognition and Golgi localization. The PKC-related kinases, or PRKs, lack a C1 domain and do not respond to DAG or phorbol esters. Instead, they can be activated by phosphatidylinositol lipids and their kinase activity is directly regulated by small GTPases of the Rho family through Rho binding to the homology region 1 (HR1).

The activity of PKC is under the control of three distinct phosphorylation events. Specifically, Thr500 in the activation loop, the Thr641 autophosphorylation site and the Ser660 hydrophobic site at the carboxy terminus of PKC β II are phosphorylated *in vivo* (2). For the atypical PKC isoforms, there is no phosphorylation in the hydrophobic region, which has a glutamic acid residue in place of the serine or threonine residue found in other PKC isoforms. The enzyme PDK1, or a close relative, is responsible for PKC activation.

Specificity/Sensitivity: Phospho-PKC α/β II (Thr638/641) Antibody detects PKC α only when phosphorylated at threonine 638 and PKC β II only when phosphorylated at Thr641. This antibody reacts weakly with phosphorylated PKC β I and γ .



Western blot analysis of extracts from 293 cells, untreated, TPA treated (200 nM), or treated with TPA and CIP and λ phosphatases using Phospho-PKC α/β II (Thr638/641) Antibody or PKC α Antibody #2056.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Thr638 of human PKC α . Antibodies are purified by protein A and peptide affinity chromatography.

Selected Application References:

Jost, M. et al. (2001) Epidermal growth factor receptor-dependent control of keratinocyte survival and Bcl-xL expression through aMEK-dependent pathway. *J. Biol. Chem.* 276, 6320-6326. Application: W.

Macpherson, P. et al. (2002) Mammalian Muscle Protein Kinase C and Calcium/Calmodulin-activated Protein Kinase II (CaMK II) Suppress Nicotinic Acetylcholine Receptor Gene Expression in Mammalian Muscle. *J. Biol. Chem.* 277 (18), 15638-15646. Application: W.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by Western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

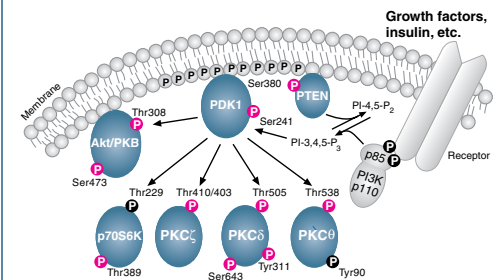
Western Blotting 1:1000
Immunoprecipitation 1:100

For application specific protocols please see the web page for this product at www.cellsignal.com.

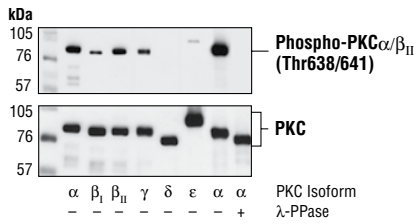
Companion Products:

- Phospho-(Ser) PKC Substrate Antibody #2261
- Phospho-PKC (pan) (BII Ser660) Antibody #9371
- Phospho-PKC δ (Thr505) Antibody #9374
- Phospho-PKC δ/θ (Ser643/676) Antibody #9376
- Phospho-PKC θ (Thr538) Antibody #9377
- Phospho-PKC Antibody Sampler Kit #9921
- Phospho-PKC ζ/λ (Thr410/403) Antibody #9378
- PKC ζ Antibody #9372
- Phospho-PKC (pan) (γ Thr514) Antibody #9379
- PKC α Antibody #2056
- PKC δ Antibody #2058
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003

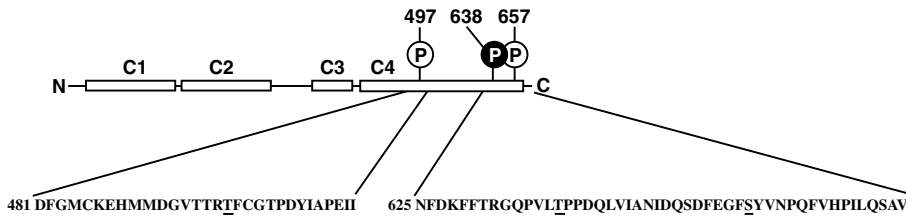
Please visit www.cellsignal.com for a complete listing of recommended companion products.



IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.



Western blot analysis of Baculovirus-expressed PKC isoforms α , β , γ , δ and ϵ , untreated or treated with λ protein phosphatase, using Phospho-PKC α/β II (Thr638/641) Antibody (upper) or PKC α , β , γ , δ , ϵ antibodies (lower).



Phosphorylation of PKC α

Background References:

- (1) Nishizuka, Y. (1984) *Nature* 308, 693–698.
- (2) Keranen, L.M. et al. (1995) *Curr. Biol.* 5, 1394–1403.
- (3) Newton, A.C. (1995) *J. Biol. Chem.* 270, 28495–28498.
- (4) Mellor, H. and Parker, P.J. (1998) *Biochem J.* 332 (Pt 2), 281–292.
- (5) Ron, D. and Kazanietz, M.G. (1999) *FASEB J.* 13, 1658–1676.
- (6) Way, K.J. et al. (2000) *Trends Pharmacol. Sci.* 21, 181–187.
- (7) Moscat, J. and Diaz-Meco, M.T. (2000) *EMBO Rep.* 1, 399–403.