

#9375 Store at -20°C

# Phospho-PKC $\alpha/\beta$ II (Thr638/641) Antibody

100  $\mu$ l  
 (10 western blots)



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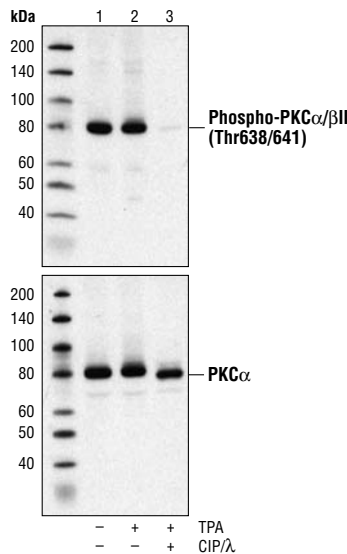
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP Endogenous	H, M, R, Mk	80 kDa, 82 kDa	Rabbit**

**Background:** Activation of protein kinase C (PKC) is one of the earliest events in a cascade that controls a variety of cellular responses, including secretion, gene expression, proliferation and muscle contraction (1,2). PKC isoforms belong to three groups based on calcium dependency and activators. Classical PKCs are calcium-dependent via their C2 domains and are activated by phosphatidylserine (PS), diacylglycerol (DAG) and phorbol esters (TPA, PMA) through their cysteine-rich C1 domains. Both novel and atypical PKCs are calcium-independent, but only novel PKCs are activated by PS, DAG and phorbol esters (3-5). Members of these three PKC groups contain a pseudo-substrate or autoinhibitory domain that binds to substrate-binding sites in the catalytic domain to prevent activation in the absence of cofactors or activators. Control of PKC activity is regulated through three distinct phosphorylation events. Phosphorylation of Thr500 in the activation loop, the autophosphorylation site at Thr641 and at carboxy-terminal hydrophobic site Ser660 occurs *in vivo* (2). Atypical PKC isoforms lack hydrophobic region phosphorylation, which correlates with the presence of glutamic acid rather than the serine or threonine residues found in more typical PKC isoforms. Either the enzyme PDK1 or a close relative is responsible for PKC activation. A recent addition to the PKC superfamily is PKC $\mu$  (PKD), which is regulated by DAG and TPA through its C1 domain. PKD is distinguished by the presence of a PH domain and by its unique substrate recognition and Golgi localization (6). PKC-related kinases (PRK) lack the C1 domain and do not respond to DAG or phorbol esters. Phosphatidylinositol lipids activate PRKs and small Rho-family GTPases bind to the homology region 1 (HR1) to regulate PRK kinase activity (7).

**Specificity/Sensitivity:** Phospho-PKC $\alpha/\beta$  II (Thr638/641) Antibody detects PKC $\alpha$  only when phosphorylated at threonine 638 and PKC $\beta$  II only when phosphorylated at Thr641. This antibody reacts weakly with phosphorylated PKC $\beta$  I and  $\gamma$ .

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr638 of human PKC $\alpha$ . Antibodies are purified by protein A and peptide affinity chromatography.



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

**Entrez-Gene ID** #5578, 5579  
**Swiss-Prot Acc.** #P17252, P05771

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA and 50% glycerol. Store at  $-20^{\circ}\text{C}$ . 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:**

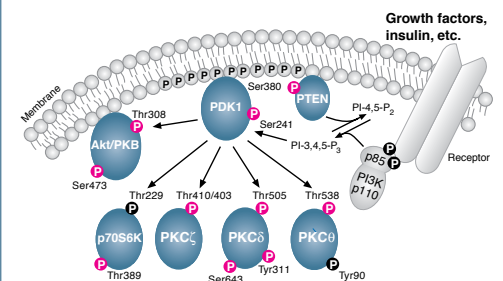
Western Blotting 1:1000  
 Immunoprecipitation 1:100

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

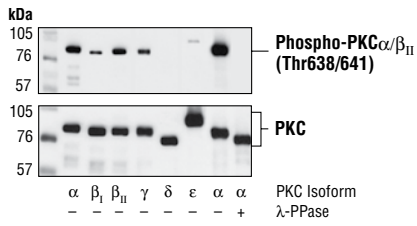
**Background References:**

- (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.

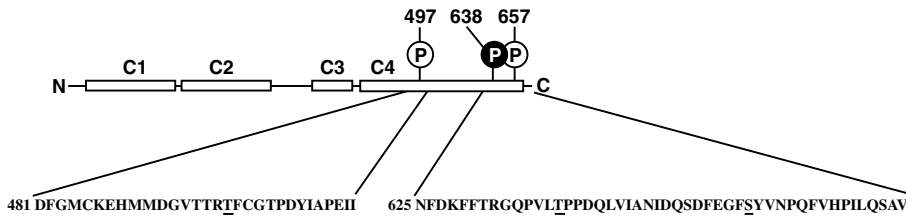


**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine  
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Western blot analysis of Baculovirus-expressed PKC isoforms  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$ , untreated or treated with  $\lambda$  protein phosphatase, using Phospho-PKC $\alpha/\beta_{II}$  (Thr638/641) Antibody (upper) or PKC $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$  antibodies (lower).



Phosphorylation of PKC $\alpha$