

#9376 Store at -20°C

Phospho-PKC δ/θ (Ser643/676) Antibody

100 μ l
 (10 western blots)



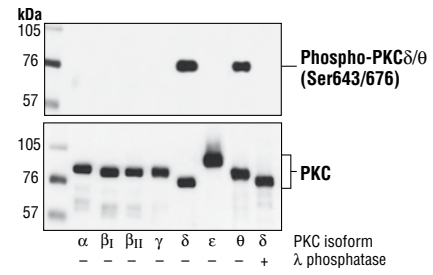
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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IHC-P Endogenous	H, M, R, Mk, X	78 kDa	Rabbit**

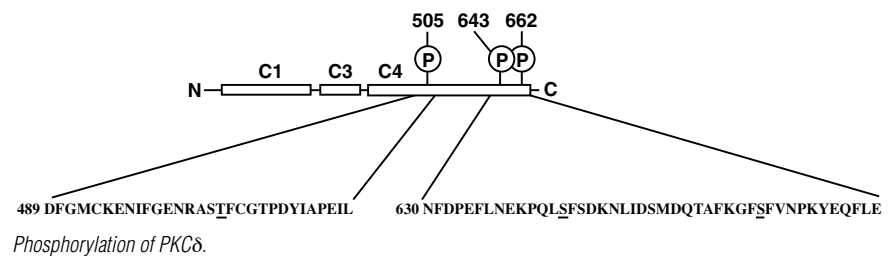
Background: Activation of protein kinase C (PKC) is one of the earliest events in a cascade that controls a variety of cellular responses, including secretion, gene expression, proliferation and muscle contraction (1,2). PKC isoforms belong to three groups based on calcium dependency and activators. 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 μ (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).



Western blot analysis of Baculovirus expressed PKC isoforms α , β , γ , δ and ϵ , untreated or λ protein phosphatase-treated using Phospho-PKC δ/θ (Ser643/676) Antibody (upper) or PKC α , $-\beta$, $-\gamma$, $-\delta$ and $-\epsilon$ antibodies (lower).

Specificity/Sensitivity: Phospho-PKC δ/θ (Ser643/676) Antibody detects endogenous levels of PKC δ only when phosphorylated at Ser643, and PKC θ only when phosphorylated at Ser676. This antibody does not cross-react with the phosphorylated PKC isoforms α , β , γ or ϵ .

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser643 of rat PKC δ . Antibodies are purified by protein A and peptide affinity chromatography.



Entrez-Gene ID #5580, 5588
Swiss-Prot Acc. #Q05655, Q04759

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:

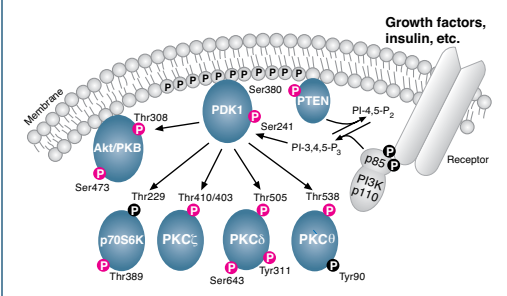
Western Blotting	1:1000
Immunohistochemistry (Paraffin)	1:50
Unmasking buffer:	Citrate
Antibody diluent:	TBST-5%NGS

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

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

Background References:

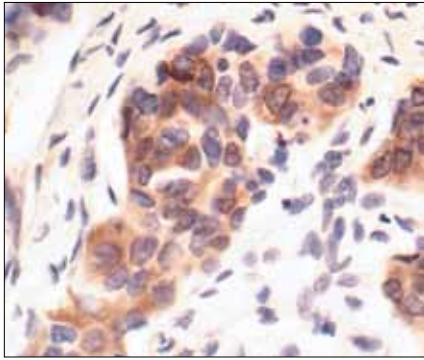
- (1) Nishizuka, Y. (1984) *Nature* 308, 693-698.
- (2) Keranen, L.M. et al. (1995) *Curr. Biol.* 5, 1394-1403.
- (3) Newton, A.C. (1995) *J. Biol. Chem.* 270, 28495-28498.
- (4) Mellor, H. and Parker, P.J. (1998) *Biochem J.* 332 (Pt 2), 281-292.
- (5) Ron, D. and Kazanietz, M.G. (1999) *FASEB J.* 13, 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.



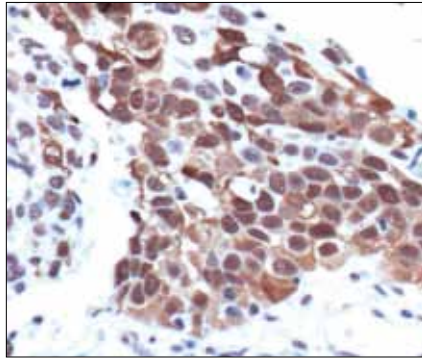
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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.

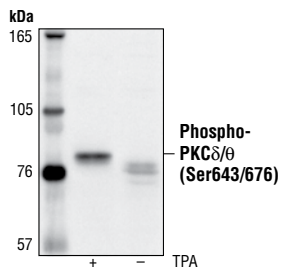
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Immunohistochemical analysis of paraffin-embedded human breast carcinoma using Phospho-PKC δ / θ (Ser643/676) Antibody.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma using Phospho-PKC δ / θ (Ser643/676) Antibody.



Western blot analysis of extracts from U-937 cells, untreated or TPA-treated (0.2 μ M) using Phospho-PKC δ / θ (Ser643/676) Antibody.