

# PKC $\theta$ Antibody

100  $\mu$ l  
 (10 western blots)

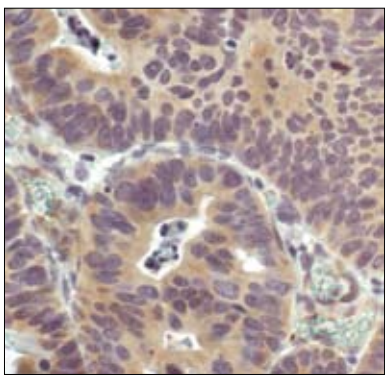
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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

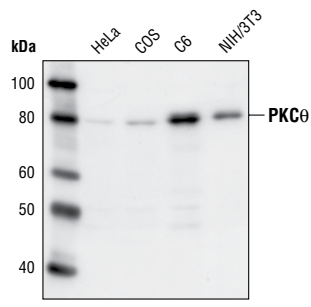
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IHC-P Endogenous	H, M, R, Mk	80 kDa	Rabbit**

**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.



Immunohistochemical analysis of paraffin-embedded human renal adenocarcinoma using PKC $\theta$  Antibody.

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).



Western blot analysis of extracts of HeLa, COS, C6 and NIH/3T3 cells using PKC $\theta$  Antibody.

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 $\beta$  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.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide derived from the sequence of human PKC $\theta$ . Antibodies are purified by protein A and peptide affinity chromatography.

**Specificity/Sensitivity:** PKC $\theta$  Antibody detects endogenous levels of total PKC $\theta$ . The antibody does not crossreact with endogenous levels of other PKC isoforms.

**Entrez-Gene ID** #5588  
**Swiss-Prot Acc.** #Q04759

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ 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)	1:25
IHC Protocol:	Citrate/TBST

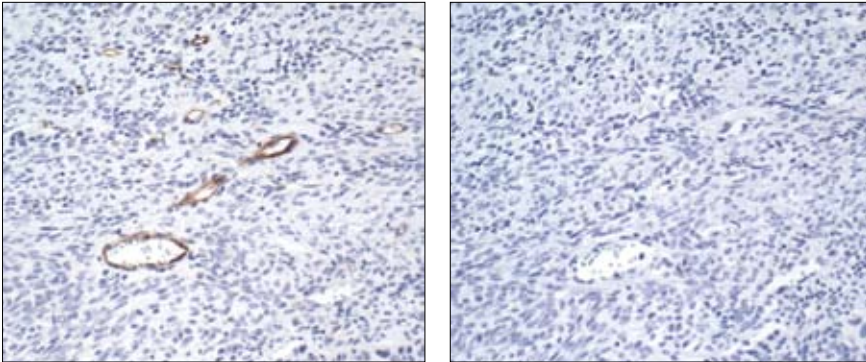
For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

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

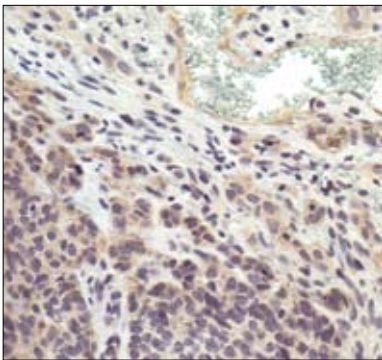
**Background References:**

- (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.**



Immunohistochemical analysis of paraffin-embedded human ovarian adenocarcinoma using PKC $\theta$  Antibody in the presence of control peptide (left), or antigen-specific peptide (right). Note specific staining of endothelial cells.



Immunohistochemical analysis of paraffin-embedded human basal cell carcinoma, showing cytoplasmic staining of tumor and endothelial cells using PKC $\theta$  Antibody.

