Store at -20°C

#9379

Phospho-PKC (pan) (γ Thr514) Antibody

Cell Signaling TECHNOLOGY®

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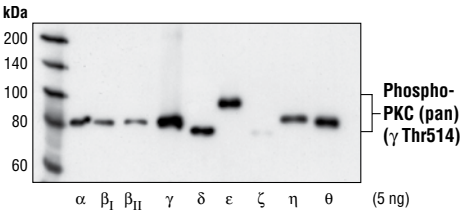
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP	H, M, R, Mk	78, 80, 82, 85 kDa	Rabbit**

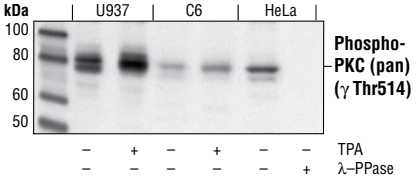
Background: Activation of protein kinase C (PKC) is one of the earliest events in a cascade that controls a variety of cellular responses, including secretion, gene expression, proliferation and muscle contraction (1,2). PKC isoforms belong to three groups based on calcium dependency and activators. 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 PKCμ (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 (pan) (γ Thr514) Antibody detects endogenous levels of PKC α, βI, βII, γ, δ, ε, η and θ isoforms only when phosphorylated at a residue homologous to Thr514 of human PKCγ. This antibody does not detect PKC phosphorylated at other sites.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr514 of human PKCγ. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of Baculovirus-expressed PKC isoforms using Phospho-PKC (pan) (γ Thr514) Antibody.



Western blot analysis of extracts from U937, C6 and HeLa cells, untreated, TPA-treated, or λ phosphatase-treated as indicated using Phospho-PKC (pan) (γ Thr514) Antibody.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/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.

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.