#13643 Store at -20°C

PKCθ (E1I7Y) Rabbit mAb

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 06/22/15

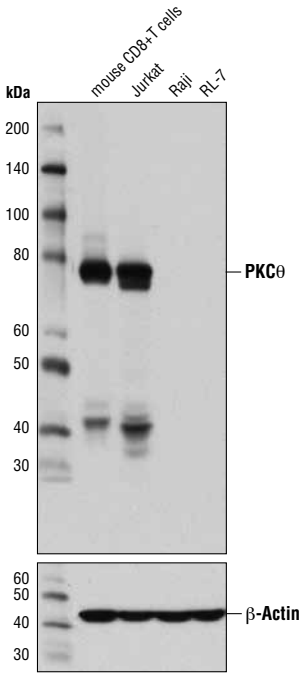
For Research Use Only. Not For Use In Diagnostic Procedures.

Entrez Gene ID #5588
UniProt ID #Q04759

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, F Endogenous	H, M, R, (B)	78 kDa	Rabbit IgG**

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 occurs *in vivo* at Thr500 in the activation loop, at Thr641 through autophosphorylation, and at the carboxy-terminal hydrophobic site Ser660 (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. 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).

PKCθ is a novel protein kinase C that is predominantly expressed in T cells (8). Recruitment of PKCθ to the immunological synapse following T cell receptor stimulation plays an important role in the activation and proliferation of conventional T cells (9). Conversely, PKCθ negatively regulates the suppressive function of regulatory T cells and is excluded from regulatory T cell immunological synapses (10).



Western blot analysis of extracts from various cell lines using PKCθ (E1I7Y) Rabbit mAb (upper) and β-Actin (D6A8) Rabbit mAb #8457 (lower).

Specificity/Sensitivity: PKCθ (E1I7Y) Rabbit mAb recognizes endogenous levels of total PKCθ protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro632 of human PKCθ protein.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. 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
Immunohistochemistry (Paraffin)	1:50†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

Immunofluorescence (IF-IC)	1:400
IF Protocol:	Methanol Permeabilization Required
Flow Cytometry	1:400

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

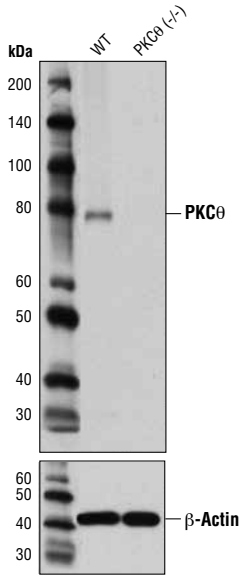
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

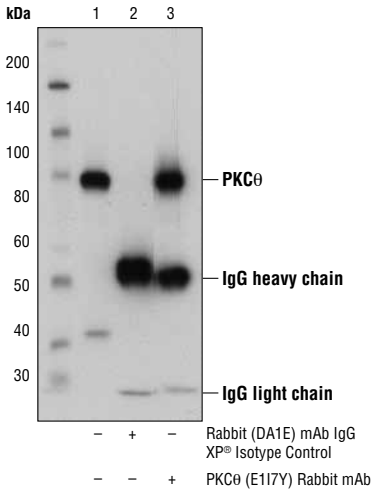
(1) Nishizuka, Y. (1984) *Nature* 308, 693-8.
(2) Keranen, L.M. et al. (1995) *Curr Biol* 5, 1394-1403.
(3) Mellor, H. and Parker, P.J. (1998) *Biochem J* 332 (Pt 2), 281-92.
(4) Ron, D. and Kazanietz, M.G. (1999) *FASEB J* 13, 1658-76.
(5) Moscat, J. and Diaz-Meco, M.T. (2000) *EMBO Rep* 1, 399-403.
(6) Baron, C.L. and Malhotra, V. (2002) *Science* 295, 325-8.
(7) Flynn, P. et al. (2000) *J Biol Chem* 275, 11064-70.
(8) Baier, G. et al. (1993) *J Biol Chem* 268, 4997-5004.
(9) Monks, C.R. et al. (1997) *Nature* 385, 83-6.
(10) Zanin-Zhorov, A. et al. (2010) *Science* 328, 372-6.

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.

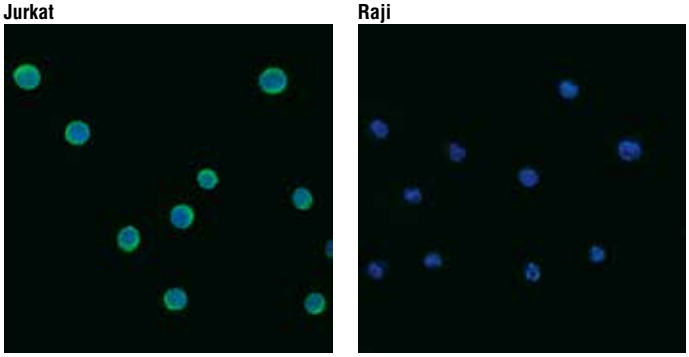
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **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.



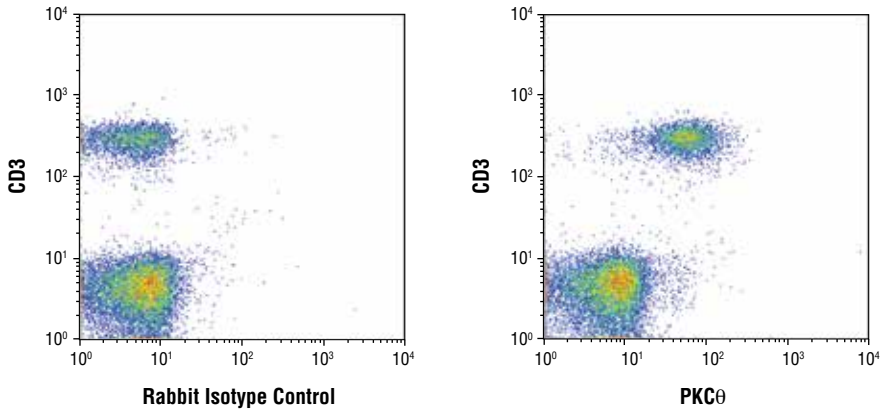
Western blot analysis of extracts from wild-type and PKCθ (-/-) mouse splenocytes using PKCθ (E1I7Y) Rabbit mAb (upper) and β-Actin (D6A8) Rabbit mAb #8457 (lower). Extracts from wild-type and PKCθ (-/-) mouse splenocytes were kindly provided by Dr. Morgan Huse (Memorial Sloan-Kettering Cancer Center).



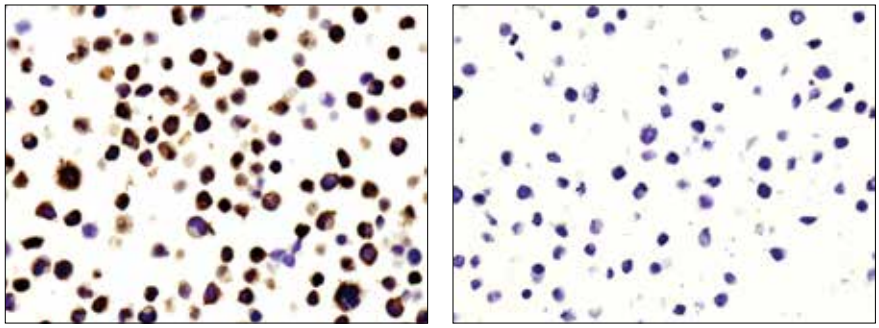
Immunoprecipitation of PKCθ from Jurkat cell extracts using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or PKCθ (E1I7Y) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using PKCθ (E1I7Y) Rabbit mAb.



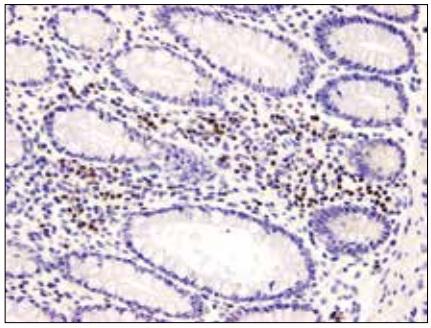
Confocal immunofluorescent analysis of Jurkat cells (positive, left) and Raji cells (negative, right) cells using PKCθ (E1I7Y) Rabbit mAb (green). Blue pseudocolor= DRAQ5® #4084 (fluorescent DNA dye).



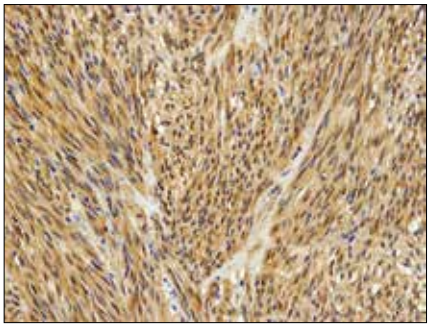
Flow cytometric analysis of mouse splenocytes using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (left) and PKCθ (E1I7Y) Rabbit mAb (right). Splenocytes were co-stained with anti-CD3 APC and the Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (PE Conjugate) #8885 was used as a secondary antibody.



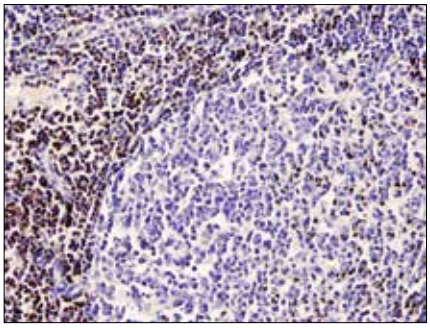
Immunohistochemical analysis of paraffin-embedded Jurkat (positive, left) or RL7 (negative, right) cell pellets using PKCθ (E1I7Y) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human colon using PKCθ (E1I7Y) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human gastrointestinal stromal tumor (GIST) using PKCθ (E1I7Y) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human lymph node using PKCθ (E1I7Y) Rabbit mAb.