Revision 4

#13643 Store at -20C

PKCθ (E1I7Y) Rabbit mAb

Cell Signaling TECHNOLOGY®

Orders: 877-616-CELL (2355) orders@cellsignal.com

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Web: info@cellsignal.com cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP, IHC-P, IF-IC, FC-FP	H M R	Endogenous	78	Rabbit IgG	#Q04759	5588

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:50
Immunofluorescence (Immunocytochemistry)	1:400
Flow Cytometry (Fixed/Permeabilized)	1:400

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.

For a carrier free (BSA and azide free) version of this product see product #86952.

Specificity / Sensitivity

PKCθ (E1I7Y) Rabbit mAb recognizes endogenous levels of total PKCθ protein.

Species predicted to react based on 100% sequence homology:

Bovine

Source / Purification

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

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).

Background References

1. Nishizuka, Y. (1984) *Nature* 308, 693-8.
2. Keranen, L.M. et al. (1995) *Curr Biol* 5, 1394-403.
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.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

WB: Western Blotting **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin)
IF-IC: Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **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
GP: Guinea Pig **Rab:** rabbit **All:** all species expected

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Limited Uses