# PKCθ (E1I7Y) Rabbit mAb



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### For Research Use Only. Not for Use in Diagnostic Procedures.

<b>Applications:</b> WB, IP, IHC-P, IF-IC, FC-FP	Reactivity: H M R	Sensitivity: Endogenous	<b>MW (kDa):</b> 78	Source/Isotype: Rabbit IgG	<b>UniProt ID:</b> #Q04759	Entrez-Gene Id: 5588	
Product Usage Information	Ap	plication		Dilution			
	We	Western Blotting				1:1000	
	Im	Immunoprecipitation				1:100	
	Im	Immunohistochemistry (Paraffin)				1:50	
	Im	Immunofluorescence (Immunocytochemistry)				1:400	
	Flo	Flow Cytometry (Fixed/Permeabilized)				1:400	
Storage		Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 $\mu$ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at $-20$ °C. Do not aliquot the antibody.					
	For	For a carrier free (BSA and azide free) version of this product see product #86952.					
Specificity / Sensit	ivity PKC	PKCθ (E1I7Y) Rabbit mAb recognizes endogenous levels of total PKCθ protein.					
Species predicted react based on 100 sequence homolog	1%	ine					

#### 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. Phosphorvlation 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).

PKC $\theta$  is a novel protein kinase C that is predominantly expressed in T cells (8). Recruitment of PKC $\theta$  to the immunological synapse following T cell receptor stimulation plays an important role in the activation and proliferation of conventional T cells (9). Conversely, PKC $\theta$  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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