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PKC θ (E117Y) Rabbit mAb (PE Conjugate)

#86495

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New 09/15

For Research Use Only. Not For Use In Diagnostic Procedures.**Applications**
F
Endogenous**Species Cross-Reactivity***
H, M, R, (B)**Isotype**
Rabbit IgG

Description: This Cell Signaling Technology antibody is conjugated to phycoerythrin (PE) and tested in-house for direct flow cytometry analysis in mouse cells. This antibody is expected to exhibit the same species cross-reactivity as the unconjugated PKC θ (E117Y) Rabbit mAb #13643.

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 PKC μ (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: PKC θ (E117Y) Rabbit mAb (PE Conjugate) recognizes endogenous levels of total PKC θ protein.

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

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.

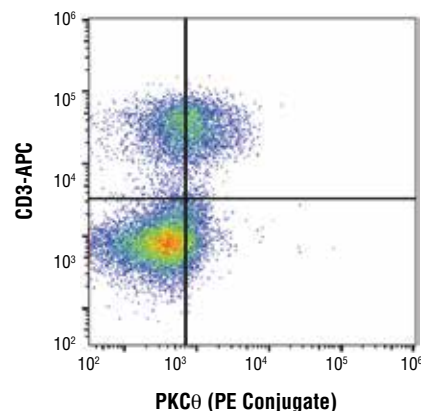
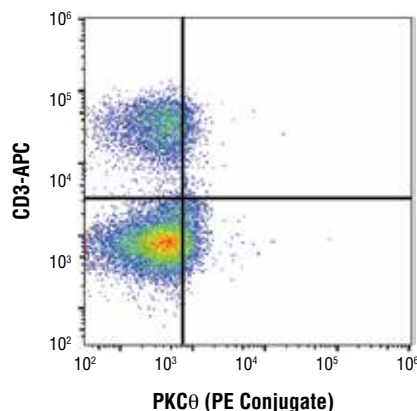
Storage: Supplied in PBS (pH 7.2), less than 0.1% sodium azide and 2 mg/ml BSA. Store at 4°C. Do not aliquot the antibody. Protect from light. Do not freeze.

***Species cross-reactivity is determined by western blot using the unconjugated antibody.**

Recommended Antibody Dilutions:

Flow Cytometry 1:50

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com



Flow cytometric analysis of mouse splenocytes using Rabbit (DA1E) mAb IgG XP[®] Isotype Control (PE Conjugate) #5742 (left) and PKC θ (E117Y) Rabbit mAb (PE Conjugate) (right). Splenocytes were co-stained with anti-CD3 conjugated to APC.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide **Species Cross-Reactivity:** 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.*