PKC-θ [pT538] ABfinity™ Recombinant Rabbit Monoclonal Antibody - Purified

REF Catalog no. 700043

(See product label for lot information)

Clone/PAD: F4H4L1
Isotype: IgG
Gene ID: 5588
Protein Acc. no.: Q04759
Qty: 100 µg
Volume: 200 µl
Concentration: 0.5 mg/ml

Formulation
PBS + 0.09% azide

Immunogen
A peptide corresponding to amino acids 531-539 of Q04759.

Immunogen sequence
LGDAKTN[pT]F

Reactivity
This antibody reacts with human PKC-θ [pT538]. Based on sequence identity and similarity, reactivity to mouse, rat, chimpanzee, bovine, and Xenopus is expected.

Specificity
This antibody is specific for PKC-θ [pT538] and does not recognize non-phosphorylated PKC-θ

Storage
2-8°C for up to 1 mo, -20°C for long term storage. Avoid repeated freezing and thawing.

Expiration Date
Expires one year from date of receipt when stored as instructed.

Validated Applications:

	Species	Test Material	Concentration
Western Blotting	human	Jurkat	2-4 µg/ml
Immunohistochemistry	human	breast carcinoma	4-6 µg/ml
Immunofluorescence	human	HeLa	8-12 µg/ml
Flow Cytometry	human	Jurkat + PMA	0.1-0.5 µg/test

Background
Protein Kinase Cθ (PKCθ) is an 80 kDa member of the novel group (nPKCs: sensitive to diacylglycerol, phosphatidylserine and phorbol esters) of the PKC family of serine/threonine kinases that are involved in a wide range of physiological processes including mitogenesis, cell survival and homeostasis (2). Transgenic mice over-expressing dominant negative PKCθ develop hyperinsulinimia (1). PKCθ is involved in JNK activation and also plays a specialized role in TCR-mediated activation of T and B cells (5,2,6). Through its control of Rap1, PKCθ establishes a threshold for T cell activation and positively regulates cytokine response and adhesive properties (7). The activation of PKCθ in T cells is associated with its recruitment to the membrane, and is mediated by PI3-kinase and Vav. PKCθ is a constitutively competent kinase and is phosphorylated on threonine 538 in the activation loop (3). The phosphorylation of threonine 538 is critical to PKCθ kinase activity, and plays an important role in PKCθ-mediated activation of nuclear factor κB (NF-κB) (3).

References

1. Serra, C., et al. (2003) Transgenic mice with dominant negative PKC-theta in skeletal muscle: A new model of insulin resistance and obesity. J. Cell Physiol. 196:89-97.
2. Dennehy, K.M., et al. (2003) Mitogenic signals through CD28 activate the protein kinase Ctheta-NF-kappaB pathway in primary peripheral T cells. Int. Immunol. 15:655-663.
3. Liu, Y., et al. (2002) Phosphorylation of the protein kinase C-theta activation loop and hydrophobic motif regulates its kinase activity, but only activation loop phosphorylation is critical to in vivo nuclear-factor-kappaB induction. Biochem. J. 361:255-265.
4. Gao, T. and A.C. Newton (2002) The turn motif is a phosphorylation switch that regulates the binding of Hsp70 to protein kinase C. J. Biol. Chem. 277:31585-31592.
5. Villalba, M., et al. (2002) Translocation of PKCθ in T cells is mediated by a nonconventional, PI3-K- and Vav-dependent pathway, but does not absolutely require phospholipase C. J. Cell Biol. 157:253-263.
6. Bauer, B., et al. (2001) Complex formation and cooperation of protein kinase C theta and Akt1/protein kinase B alpha in the NF-kappa B transactivation cascade in Jurkat T cells. J. Biol. Chem. 276:31627-31634.
7. Letschka T, et al. (2008) PKC-theta selectively controls the adhesion-stimulating molecule Rap1. Blood 112: 4617-4627.

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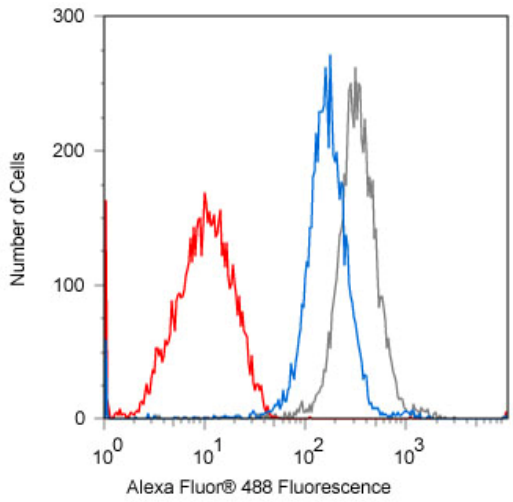
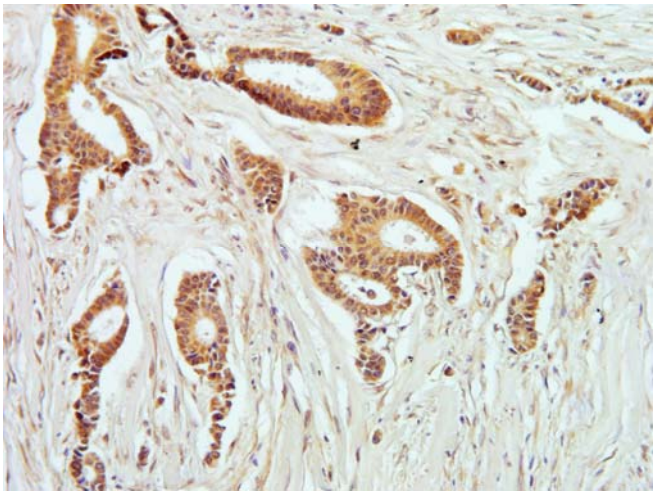


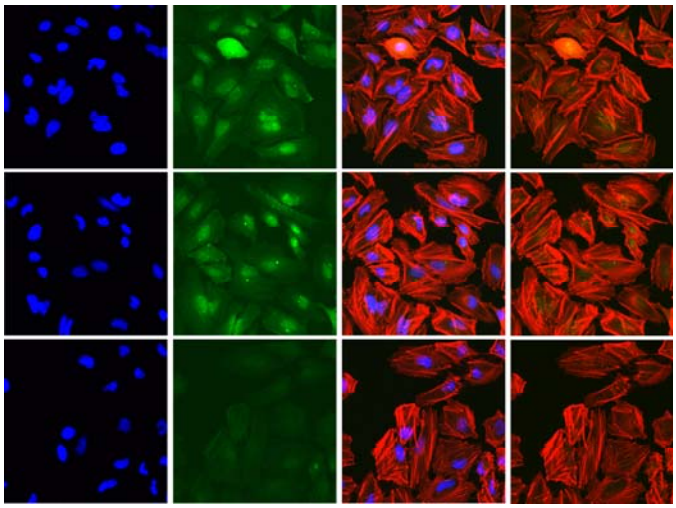
Flow cytometry of Jurkat cells labeled with rabbit anti-PKC-θ [pT538] (Cat. No. 700043).

Jurkat cells incubated with 100 µM PMA for 1 h prior to being fixed and permeabilized using FIX & PERM® (Cat. No. GAS004) reagents. Cells were then stained with (gray trace) 0.1 µg anti-PKC-θ [pT538] followed by Alexa Fluor® 488 goat anti-rabbit Ig (Cat. No. A11008). Pre-incubation with the immunogenic phosphopeptide decreased the signal (red trace), whereas incubation with the non-phosphopeptide did not (blue trace).



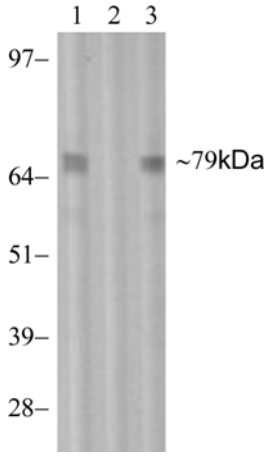
Immunohistochemistry of human breast carcimona tissue labeled with rabbit anti-PKC-θ [pT538] (Cat. No. 700043).

FFPE human breast carcinoma tissue was labeled with rabbit anti-PKC-θ [pT538] (5 µg/ml). Tissues were detected with SuperPicTure™ Polymer DAB (Cat. No.87-8963). Images were taken at 20x magnification. Note cytoplasmic staining in tumor cells.



Immunocytochemistry of HeLa cells labeled with rabbit anti-PKC-θ [pT538] (Cat. No. 700043).

HeLa cells labeled with rabbit anti-PKC-θ [pT538] (10 µg/ml) in the absence of peptides (top panels), and presence of phosphopeptide used as immunogen (bottom panels) or non-phosphopeptide (middle panels). Alexa Fluor® 488 goat anti-rabbit (Cat. No. A11008) at 1:1000 was used as secondary antibody. Actin was stained with Alexa Fluor® 568 Phalloidin (Cat. No. A12380). Hoechst only (left), PKC-θ [pT538] (AF488) signal only (left center), composite image with Phalloidin (right center), and composite image without Hoechst (right).



Western blot of Jurkat lysates labeled with rabbit anti-PKC-θ [pT538] (Cat. No. 700043).

Rabbit anti-PKC-θ [pT538] (3 µg/mL) was used to label PKC-θ [pT538] in Jurkat lysates stimulated with 100ng/mL PMA for 1 h (lane 1). Pre-incubation with the phosphopeptide used for immunization resulted in loss of signal (lane 2) whereas pre-incubation with the non-phosphopeptide did not (lane 3).