abcam

Product datasheet

Anti-PKC beta 1 + PKC beta 2 (phospho T500) antibody ab5817

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Overview

Product name	Anti-PKC beta 1 + PKC beta 2 (phospho T500) antibody
Description	Rabbit polyclonal to PKC beta 1 + PKC beta 2 (phospho T500)
Host species	Rabbit
Specificity	This antibody cross-reacts with PKC alpha [pT497] (88% homologous) and partially cross-reacts with PKC gamma [pT514] (63% homologous) and epsilon [pT566] (75% homologous), as determined by peptide competition experiments.
Tested applications	**Suitable for:** ICC/IF, WB
Species reactivity	**Reacts with:** Rat, Human **Predicted to work with:** Mouse
Immunogen	Synthetic phosphopeptide derived from a region of human PKC beta 1 & 2 that contains threonine 500.
Positive control	K562 cells treated with PMA, a phorbol ester.
General notes	Protein Kinase C beta (PKC beta) is an 80 kDa member of the conventional group (cPKCs: sensitive to diacylglycerol, phosphotidylserine 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, transcriptional regulation and tumor promotion. PKC beta has been implicated in diabetes and carcinogenesis. PKC beta isoforms 1 & 2 are phosphorylated on three sites, threonine 500 in the activation loop, beta 1 threonine 642 (beta 2 641) in the turn loop and beta 1 serine 661 (beta 2 660) in the hydrophobic loop. Phosphorylation of PKC beta 1 & 2 on threonine 500 by PDK1 is a prerequisite for its autophosphoylation and catalytic competence.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.30 Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol, 0.1% BSA

Purity	Immunogen affinity purified
Purification notes	The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated PKC beta. The final product is generated by affinity chromatography using a PKC beta-derived peptide that is phosphorylated at threonine 500.
Primary antibody notes	Protein Kinase C beta (PKC beta) is an 80 kDa member of the conventional group (cPKCs: sensitive to diacylglycerol, phosphotidylserine 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, transcriptional regulation and tumor promotion. PKC beta has been implicated in diabetes and carcinogenesis. PKC beta isoforms 1 & 2 are phosphorylated on three sites, threonine 500 in the activation loop, beta 1 threonine 642 (beta 2 641) in the turn loop and beta 1 serine 661 (beta 2 660) in the hydrophobic loop. Phosphorylation of PKC beta 1 & 2 on threonine 500 by PDK1 is a prerequisite for its autophosphoylation and catalytic competence.
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab5817** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

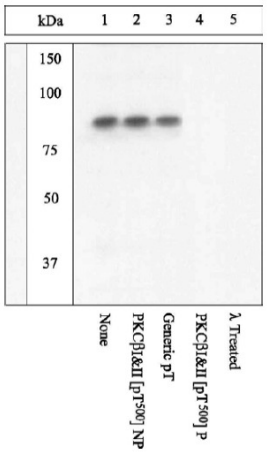
Application	Abreviews	Notes
ICC/IF	★★★★☆	1/1000. Punctate staining observed.
WB	★★★★☆	1/1000. Detects a band of approximately 80 kDa.

Target

Function	Calcium-activated and phospholipid-dependent serine/threonine-protein kinase involved in various processes such as regulation of the B-cell receptor (BCR) signalosome, apoptosis and transcription regulation. Plays a key role in B-cell activation and function by regulating BCR-induced NF-kappa-B activation and B-cell suvival. Required for recruitment and activation of the IKK kinase to lipid rafts and mediates phosphorylation of CARD11/CARMA1 at 'Ser-559', 'Ser-644' and 'Ser-652', leading to activate the NF-kappa-B signaling. Involved in apoptosis following oxidative damage: in case of oxidative conditions, specifically phosphorylates 'Ser-36' of isoform p66Shc of SHC1, leading to mitochondrial accumulation of p66Shc, where p66Shc acts as a reactive oxygen species producer. Acts as a coactivator of androgen receptor (ANDR)-dependent transcription, by being recruited to ANDR target genes and specifically mediating phosphorylation of 'Thr-6' of histone H3 (H3T6ph), a specific tag for epigenetic transcriptional activation that prevents demethylation of histone H3 'Lys-4' (H3K4me) by LSD1/KDM1A. Also involved in triglyceride homeostasis. Serves as the receptor for phorbol esters, a class of tumor promoters.
Sequence similarities	Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. PKC subfamily. Contains 1 AGC-kinase C-terminal domain. Contains 1 C2 domain. Contains 2 phorbol-ester/DAG-type zinc fingers.

	Contains 1 protein kinase domain.
Post-translational modifications	Phosphorylation on Thr-500 within the activation loop renders it competent to autophosphorylate. Subsequent autophosphorylation of Thr-642 maintains catalytic competence, and autophosphorylation on Ser-661 appears to release the kinase into the cytosol. Autophosphorylation on other sites i.e. in the N-terminal and hinge regions have no effect on enzyme activity.
Cellular localization	Cytoplasm. Nucleus. Membrane.

Images



Western blot - Anti-PKC beta 1 + PKC beta 2 (phospho T500) antibody (ab5817)

Peptide Competition and Phosphatase Treatment: Lysates prepared from K562 cells stimulated with PMA were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF. Membranes were either left untreated (1-4) or treated with lambda phosphatase (5), blocked with a 5% BSA-TBST buffer for one hour at room temperature, and incubated with ab5817 antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 5), the non-phosphopeptide corresponding to the immunogen (2), a generic phosphothreonine-containing peptide (3), or, the phosphopeptide immunogen (4). After washing, membranes were incubated with goat F(ab' 2 anti-rabbit IgG HRP conjugate and bands were detected using the Pierce SuperSignalTM method. The data show that only the peptide corresponding to PKC beta 1 & 2 [pT500] blocks the antibody signal. The data also show that phosphatase stripping eliminates the signal, verifying that the antibody is phospho-specific.

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