abcam

Product datasheet

Anti-PKC delta + PKC theta antibody [EPR17074] ab182127

Recombinant RabMAb

10 Images

Overview

Product name Anti-PKC delta + PKC theta antibody [EPR17074]

Description Rabbit monoclonal [EPR17074] to PKC delta + PKC theta

Host species Rabbit

Tested applications Suitable for: ICC/IF, IHC-P, WB Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Active Human PKC delta full length protein and Active Human PKC theta full length protein.

> Mouse and Rat thymus lysates. Molt4, HeLa and Raw264.7 cell lysates; Human fetal brain lysates; Rat and mouse brain lysates. IHC-P: Human tonsil and human breast cancer, mouse kidney, rat

spleen tissues. ICC/IF: HeLa cells.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR17074

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise quarantee covers the use of ab182127 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		1/200.
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/2000. Detects a band of approximately 78 kDa (predicted molecular weight: 78, 82 kDa).

Target

Relevance

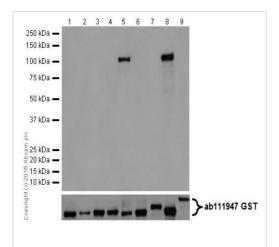
Protein kinase C theta type: Calcium-independent, phospholipid- and diacylglycerol (DAG)dependent serine/threonine-protein kinase that mediates non-redundant functions in T-cell receptor (TCR) signaling, including T-cells activation, proliferation, differentiation and survival, by mediating activation of multiple transcription factors such as NF-kappa-B, JUN, NFATC1 and NFATC2. In TCR-CD3/CD28-co-stimulated T-cells, is required for the activation of NF-kappa-B and JUN, which in turn are essential for IL2 production, and participates to the calcium-dependent NFATC1 and NFATC2 transactivation. Mediates the activation of the canonical NF-kappa-B pathway (NFKB1) by direct phosphorylation of CARD11 on several serine residues, inducing CARD11 association with lipid rafts and recruitment of the BCL10-MALT1 complex, which then activates IKK complex, resulting in nuclear translocation and activation of NFKB1. May also play an indirect role in activation of the non-canonical NF-kappa-B (NFKB2) pathway. In the signaling pathway leading to JUN activation, acts by phosphorylating the mediator STK39/SPAK and may not act through MAP kinases signaling. Plays a critical role in TCR/CD28-induced NFATC1 and NFATC2 transactivation by participating in the regulation of reduced inositol 1,4,5-trisphosphate generation and intracellular calcium mobilization. After costimulation of T-cells through CD28 can phosphorylate CBLB and is required for the ubiquitination and subsequent degradation of CBLB, which is a prerequisite for the activation of TCR. During T-cells differentiation, plays an important role in the development of T-helper 2 (Th2) cells following immune and inflammatory responses, and, in the development of inflammatory autoimmune diseases, is necessary for the activation of LL17-producing Th17 cells. May play a minor role in Th1 response. Upon TCR stimulation, mediates T-cell protective survival signal by phosphorylating BAD, thus protecting T-cells from BAD-induced apoptosis, and by up-regulating BCL-X(L)/BCL2L1 levels through NF-kappa-B and JUN pathways. In platelets, regulates signal transduction downstream of the ITGA2B, CD36/GP4, F2R/PAR1 and F2RL3/PAR4 receptors, playing a positive role in 'outside-in' signaling and granule secretion signal transduction. May relay signals from the activated ITGA2B receptor by regulating the uncoupling of WASP and WIPF1, thereby permitting the regulation of actin filament nucleation and branching activity of the Arp2/3 complex. May mediate inhibitory effects of free fatty acids on insulin signaling by phosphorylating IRS1, which in turn blocks IRS1 tyrosine phosphorylation and downstream activation of the PI3K/AKT pathway. Phosphorylates MSN

(moesin) in the presence of phosphatidylqlycerol or phosphatidylinositol. Phosphorylates PDPK1 at 'Ser-504' and 'Ser-532' and negatively regulates its ability to phosphorylate PKB/AKT1. Protein kinase C delta type: Calcium-independent, phospholipid- and diacylglycerol (DAG)-dependent serine/threonine-protein kinase that plays contrasting roles in cell death and cell survival by functioning as a pro-apoptotic protein during DNA damage-induced apoptosis, but acting as an anti-apoptotic protein during cytokine receptor-initiated cell death, is involved in tumor suppression as well as survival of several cancers, is required for oxygen radical production by NADPH oxidase and acts as positive or negative regulator in platelet functional responses. Negatively regulates B cell proliferation and also has an important function in self-antigen induced B cell tolerance induction. Upon DNA damage, activates the promoter of the death-promoting transcription factor BCLAF1/Btf to trigger BCLAF1-mediated p53/TP53 gene transcription and apoptosis. In response to oxidative stress, interact with and activate CHUK/IKKA in the nucleus, causing the phosphorylation of p53/TP53. In the case of ER stress or DNA damage-induced apoptosis, can form a complex with the tyrosine-protein kinase ABL1 which trigger apoptosis independently of p53/TP53. In cytosol can trigger apoptosis by activating MAPK11 or MAPK14, inhibiting AKT1 and decreasing the level of X-linked inhibitor of apoptosis protein (XIAP), whereas in nucleus induces apoptosis via the activation of MAPK8 or MAPK9. Upon ionizing radiation treatment, is required for the activation of the apoptosis regulators BAX and BAK, which trigger the mitochondrial cell death pathway. Can phosphorylate MCL1 and target it for degradation which is sufficient to trigger for BAX activation and apoptosis. Is required for the control of cell cycle progression both at G1/S and G2/M phases. Mediates phorbol 12-myristate 13-acetate (PMA)-induced inhibition of cell cycle progression at G1/S phase by up-regulating the CDK inhibitor CDKN1A/p21 and inhibiting the cyclin CCNA2 promoter activity. In response to UV irradiation can phosphorylate CDK1, which is important for the G2/M DNA damage checkpoint activation. Can protect glioma cells from the apoptosis induced by TNFSF10/TRAIL, probably by inducing increased phosphorylation and subsequent activation of AKT1. Is highly expressed in a number of cancer cells and promotes cell survival and resistance against chemotherapeutic drugs by inducing cyclin D1 (CCND1) and hyperphosphorylation of RB1, and via several pro-survival pathways, including NF-kappa-B, AKT1 and MAPK1/3 (ERK1/2). Can also act as tumor suppressor upon mitogenic stimulation with PMA or TPA. In N-formyl-methionyl-leucylphenylalanine (fMLP)-treated cells, is required for NCF1 (p47-phox) phosphorylation and activation of NADPH oxidase activity, and regulates TNF-elicited superoxide anion production in neutrophils, by direct phosphorylation and activation of NCF1 or indirectly through MAPK1/3 (ERK1/2) signaling pathways. May also play a role in the regulation of NADPH oxidase activity in eosinophil after stimulation with IL5, leukotriene B4 or PMA. In collagen-induced platelet aggregation, acts a negative regulator of filopodia formation and actin polymerization by interacting with and negatively regulating VASP phosphorylation. Downstream of PAR1, PAR4 and CD36/GP4 receptors, regulates differentially platelet dense granule secretion; acts as a positive regulator in PAR-mediated granule secretion, whereas it negatively regulates CD36/GP4-mediated granule release. Phosphorylates MUC1 in the C-terminal and regulates the interaction between MUC1 and beta-catenin. The catalytic subunit phosphorylates 14-3-3 proteins (YWHAB, YWHAZ and YWHAH) in a sphingosine-dependent fashion.

Cellular localization

Cytoplasm. Membrane; Peripheral membrane protein

Images



Western blot - Anti-PKC delta + PKC theta antibody [EPR17074] (ab182127)

All lanes : Anti-PKC delta + PKC theta antibody [EPR17074] (ab182127) at 1/2000 dilution

Lane 1: Active human PKC alpha full length protein

Lane 2: Active human PKC beta 1 full length protein

Lane 3: Active human PKC beta 2 full length protein

Lane 4: Active human PKC gamma full length protein

Lane 5: Active human PKC delta full length protein

Lane 6: Active human PKC eta full length protein

Lane 7: Active human PKC epsilon full length protein

Lane 8 : Active human PKC theta full length protein

Lane 9: Active human PKC mu full length protein

Lysates/proteins at 0.02 µg per lane.

Secondary

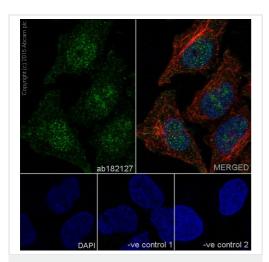
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 78,82 kDa

Exposure time: 5 seconds

5% NFDM/TBST: Blocking and diluting buffer.

Active human PKC alpha full length protein (ab55672) contains aa1-672 with GST-tag; Active human PKC beta 1 full length protein (ab60840) contains aa1-671 with GST-tag; Active human PKC beta 2 full length protein (ab60841) contains aa1-673 with GST-tag; Active human PKC gamma full length protein (ab60842) contains aa1-677 with GST-tag; Active human PKC delta full length protein (ab60844) contains aa1-676 with GST-tag; Active human PKC eta full length protein (ab60849) contains aa1-683 with GST-tag; Active human PKC epsilon full length protein (ab60847) contains aa1-737 with GST-tag; Active human PKC theta full length protein (ab56641) contains aa1-706 with GST-tag; Active human PKC mu full length protein (ab60873) contains aa1-912 with GST-tag.

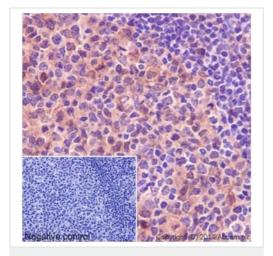


Immunocytochemistry/ Immunofluorescence - Anti-PKC delta + PKC theta antibody [EPR17074] (ab182127)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling PKC delta + PKC theta with ab182127 at 1/200 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/400 dilution (green). Confocal image showing both cytoplasm and nuclear staining on HeLa cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/500 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

1. ab182127 at 1/200 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/400 dilution.

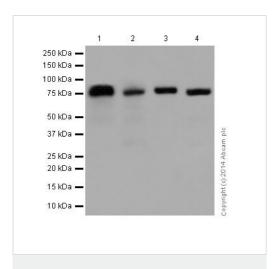


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC delta + PKC theta antibody [EPR17074] (ab182127)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling PKC delta + PKC theta with ab182127 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Cytoplasmic and nuclear staining on lymphocytes on germinal center of Human tonsil is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-PKC delta + PKC theta antibody [EPR17074] (ab182127) **All lanes :** Anti-PKC delta + PKC theta antibody [EPR17074] (ab182127) at 1/2000 dilution

Lane 1: Mouse thymus lysates

Lane 2: Rat thymus lysates

Lane 3: Molt4 (Human lymphoblastic leukemia cell line) whole cell

lysates

Lane 4: HeLa (Human epithelial cells from cervix

adenocarcinoma) whole cell lysates

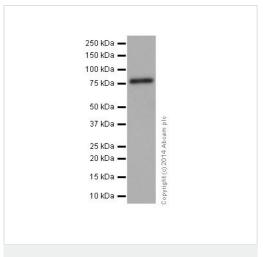
Lysates/proteins at 20 µg per lane.

Secondary

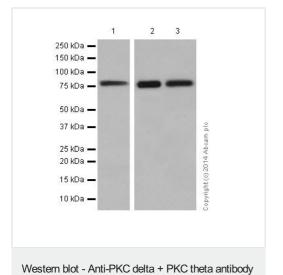
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 78, 82 kDa **Observed band size:** 78 kDa

5% NFDM/TBST: Blocking and diluting buffer.



Western blot - Anti-PKC delta + PKC theta antibody [EPR17074] (ab182127)



[EPR17074] (ab182127)

Anti-PKC delta + PKC theta antibody [EPR17074] (ab182127) at 1/2000 dilution + Human fetal brain lysate at 10 μ g

Secondary

Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/1000 dilution

Predicted band size: 78, 82 kDa **Observed band size:** 78 kDa

5% NFDM/TBST: Blocking and diluting buffer.

All lanes : Anti-PKC delta + PKC theta antibody [EPR17074] (ab182127) at 1/10000 dilution

Lane 1 : Raw264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysates

Lane 2 : Rat brain tissue lysate

Lane 3 : Mouse brain tissue lysate

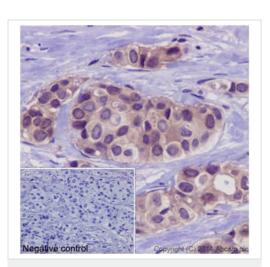
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 78, 82 kDa **Observed band size:** 78 kDa

5% NFDM/TBST: Blocking and diluting buffer.

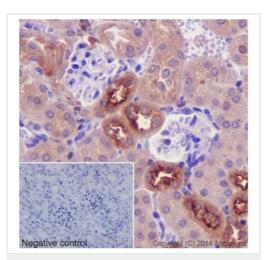


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC delta + PKC theta antibody [EPR17074] (ab182127)

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling PKC delta + PKC theta with ab182127 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Cytoplasmic and nuclear staining on cancer cells of Human breast carcinoma is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

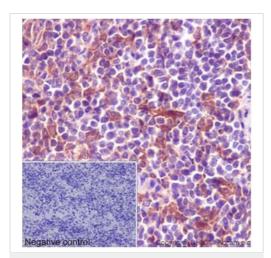


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC delta + PKC theta antibody [EPR17074] (ab182127)

Immunohistochemical analysis of paraffin-embedded mouse kidney cancer tissue labeling PKC delta + PKC theta with ab182127 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Cytoplasmic staining on mouse kidney tubules is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

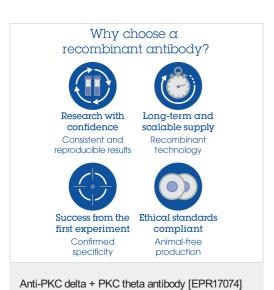


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC delta + PKC theta antibody [EPR17074] (ab182127)

Immunohistochemical analysis of paraffin-embedded rat spleen cancer tissue labeling PKC delta + PKC theta with ab182127 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Cytoplasmic and nuclear staining on monocytes and macrophages on rat spleen is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



(ab182127)

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