abcam

Product datasheet

Anti-PKC antibody [EPR16898] ab179522

Recombinant RabMAb

8 References 12 Images

Overview

Product name Anti-PKC antibody [EPR16898]

Description Rabbit monoclonal [EPR16898] to PKC

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, ICC/IF, IP

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: HeLa, 293T, SH-SY5Y, K562, A431, L6, Neuro-2a and L929 whole cell lysates; Human fetal

brain, fetal heart and fetal kidney lysates; Rat brain whole cell lysate. ICC/IF: K562 cells. Flow Cyt

(intra): NIH/3T3 cells. IP: Rat brain extract.

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

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

term. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR16898

Isotype ΙgG

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab179522 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
Flow Cyt (Intra)		1/360. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		1/1000. Detects a band of approximately 77 kDa (predicted molecular weight: 77 kDa).
ICC/IF		1/200.
IP		1/100.

Target

Function

Calcium-activated, phospholipid- and diacylglycerol (DAG)-dependent serine/threonine-protein kinase that is involved in positive and negative regulation of cell proliferation, apoptosis, differentiation, migration and adhesion, tumorigenesis, cardiac hypertrophy, angiogenesis, platelet function and inflammation, by directly phosphorylating targets such as RAF1, BCL2, CSPG4, TNNT2/CTNT, or activating signaling cascade involving MAPK1/3 (ERK1/2) and RAP1GAP. Involved in cell proliferation and cell growth arrest by positive and negative regulation of the cell cycle. Can promote cell growth by phosphorylating and activating RAF1, which mediates the activation of the MAPK/ERK signaling cascade, and/or by up-regulating CDKN1A, which facilitates active cyclin-dependent kinase (CDK) complex formation in glioma cells. In intestinal cells stimulated by the phorbol ester PMA, can trigger a cell cycle arrest program which is associated with the accumulation of the hyper-phosphorylated growth-suppressive form of RB1 and induction of the CDK inhibitors CDKN1A and CDKN1B. Exhibits anti-apoptotic function in glioma cells and protects them from apoptosis by suppressing the p53/TP53-mediated activation of IGFBP3, and in leukemia cells mediates anti-apoptotic action by phosphorylating BCL2. During macrophage differentiation induced by macrophage colony-stimulating factor (CSF1), is translocated to the nucleus and is associated with macrophage development. After wounding, translocates from focal contacts to lamellipodia and participates in the modulation of desmosomal adhesion. Plays a role in cell motility by phosphorylating CSPG4, which induces association of CSPG4 with extensive lamellipodia at the cell periphery and polarization of the cell accompanied by increases in cell motility. Is highly expressed in a number of cancer cells where it can act as a tumor promoter and is implicated in malignant phenotypes of several tumors such as gliomas and breast cancers. Negatively regulates myocardial contractility and positively regulates angiogenesis, platelet aggregation and thrombus formation in arteries. Mediates hypertrophic growth of neonatal cardiomyocytes, in part through a MAPK1/3 (ERK1/2)-dependent signaling pathway, and upon PMA treatment, is required to induce cardiomyocyte hypertrophy up to heart failure and death, by increasing protein synthesis, protein-DNA ratio and cell surface area. Regulates cardiomyocyte function by phosphorylating cardiac troponin T (TNNT2/CTNT), which induces significant reduction in actomyosin ATPase activity, myofilament calcium sensitivity and myocardial contractility. In angiogenesis, is required for full endothelial cell migration, adhesion to vitronectin (VTN), and vascular endothelial growth factor A (VEGFA)-dependent regulation of kinase activation and vascular tube formation. Involved in the stabilization of VEGFA mRNA at

post-transcriptional level and mediates VEGFA-induced cell proliferation. In the regulation of calcium-induced platelet aggregation, mediates signals from the CD36/GP4 receptor for granule release, and activates the integrin heterodimer ITGA2B-ITGB3 through the RAP1GAP pathway for adhesion. During response to lipopolysaccharides (LPS), may regulate selective LPS-induced macrophage functions involved in host defense and inflammation. But in some inflammatory responses, may negatively regulate NF-kappa-B-induced genes, through IL1A-dependent induction of NF-kappa-B inhibitor alpha (NFKBIA/IKBA). Upon stimulation with 12-O-tetradecanoylphorbol-13-acetate (TPA), phosphorylates EIF4G1, which modulates EIF4G1 binding to MKNK1 and may be involved in the regulation of EIF4E phosphorylation. Phosphorylates KIT, leading to inhibition of KIT activity. Phosphorylates ATF2 which promotes cooperation between ATF2 and JUN, activating transcription.

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.

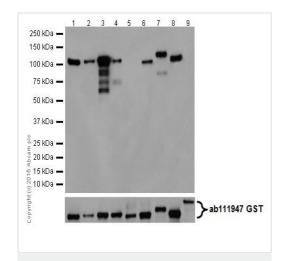
Contains 2 phorbol-ester/DAG-type zinc fingers.

Contains 1 protein kinase domain.

Cellular localization

Cytoplasm. Cell membrane. Mitochondrion membrane. Nucleus.

Images



Western blot - Anti-PKC antibody [EPR16898] (ab179522)

All lanes : Anti-PKC antibody [EPR16898] (ab179522) 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) (ab97051)

Predicted band size: 77 kDa

Exposure time: 2 seconds

Active human PKC alpha full length protein (Catalog#ab55672)

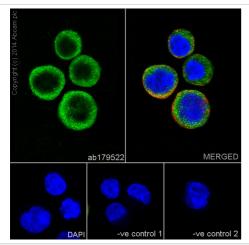
contains aa1-672 with GST-tag; Active human PKC beta 1 full length protein(Catalog#ab60840) contains aa1-671 with GST-tag; Active human PKC beta 2 full length protein(Catalog#ab60841) contains aa1-673 with GST-tag; Active human PKC gamma full length protein(Catalog#ab60842) contains aa1-677 with GST-tag;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

Blocking/Diluting buffer 5% NFDM/TBST

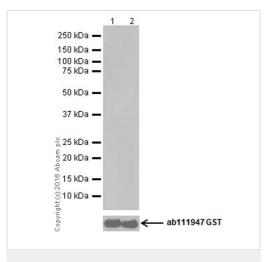
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling PKC alpha + beta + gamma with ab179522 at 1/200 dilution, followed by Goat antirabbit IAlexa Fluor® 488 (IgG) (ab150077) secondary antibody at 1/400 dilution (green). Confocal image shows cytoplasmic staining on K562 cell line. The nuclear counterstain is DAPI (blue). Tubulin is ab179522 detected with ab7291 (anti-Tubulin mouse mAb) at 1/500 dilution and ab150120 (goat anti-mouse AlexaFluor®594 secondary antibody) at 1/500 dilution (red).

The negative controls are as follows:-

-ve control 1: - ab179522 at 1/200 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution. -ve control 2: - ab7291 (anti-Tubulin mouse mAb) at 1/500 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/400 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-PKC antibody [EPR16898] (ab179522)



Western blot - Anti-PKC antibody [EPR16898] (ab179522)

All lanes : Anti-PKC antibody [EPR16898] (ab179522) at 1/2000 dilution

Lane 1: Active human PKC zeta full length protein

Lane 2: Active human PKC iota full length protein

Lysates/proteins at 0.02 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) ($\underline{ab97051}$) at 1/50000

dilution

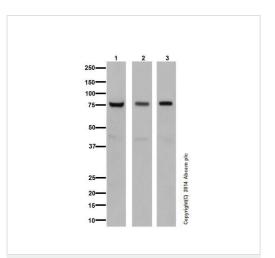
Predicted band size: 77 kDa

Exposure time: 3 minutes

Active human PKC zeta full length protein (Catalog# <u>ab60848</u>) contains aa1-592 with GST-tag; Active human PKC iota full length protein (Catalog#<u>ab60850</u>) contains aa1-596 with GST-tag.

Blocking/Diluting buffer 5% NFDM/TBST

Recombinant PKC isoforms were tested for reactivity by western blot, and are marked as positive (+) or negative (-).



Western blot - Anti-PKC antibody [EPR16898] (ab179522)

All lanes : Anti-PKC antibody [EPR16898] (ab179522) at 1/10000 dilution

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 2 : 293T (Human epithelial cells from embryonic kidney) whole cell lysate at 10 μ g

Lane 3: SH-SY5Y (Human neuroblastoma from bone marrow cells) whole cell lysate at 10 μg

Secondary

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

Predicted band size: 77 kDa **Observed band size:** 77 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Western blot - Anti-PKC antibody [EPR16898] (ab179522)

All lanes : Anti-PKC antibody [EPR16898] (ab179522) at 1/1000 dilution

Lane 1 : K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate at 20 μg

Lane 2 : A431 (Human epidermoid carcinoma) whole cell lysate at 10 μg

Secondary

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

Predicted band size: 77 kDa **Observed band size:** 77 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

KDa

250—
150—
150—
100—
75—
50—
37—
25—
20—
15—
10—
10—

Western blot - Anti-PKC antibody [EPR16898] (ab179522)

All lanes : Anti-PKC antibody [EPR16898] (ab179522) at 1/1000 dilution

Lane 1 : Human fetal brain tissue lysate
Lane 2 : Human fetal heart tissue lysate
Lane 3 : Human fetal kidney tissue lysate

Lysates/proteins at 10 μg per lane.

Secondary

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

Predicted band size: 77 kDa **Observed band size:** 77 kDa

KDa 1 2 3 250 — 1 160 — 100 — 75 — 101 unayy yili (O)Hillihida 25 — 25 — 20 — 101 unayy yili (O)Hillihida 25 — 25 — 20 — 101 unayy yili (O)Hillihida 25 — 25 — 20 — 101 unayy yili (O)Hillihida 25 — 25 — 20 — 101 unayy yili (O)Hillihida 25 — 25 — 20 — 101 unayy yili (O)Hillihida 25 — 25 — 20 — 101 unayy yili (O)Hillihida 25 — 101

Western blot - Anti-PKC antibody [EPR16898] (ab179522)

Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes : Anti-PKC antibody [EPR16898] (ab179522) at 1/5000 dilution

Lane 1: L6 (Rat skeletal muscle cell line) whole cell lysate

Lane 2 : Neuro-2a (Mouse neuroblastoma cells) whole cell lysate

Lane 3: L-929 (Mouse connective tissue fibroblast cells) whole cell

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: 77 kDa **Observed band size:** 77 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

250 — 150 — 100 — 75 — 50 — 37 — 3rd unsary 1107 (h) unburkdon 10 — 15 — 15 — 10 — 6h) unburkdon

Western blot - Anti-PKC antibody [EPR16898] (ab179522)

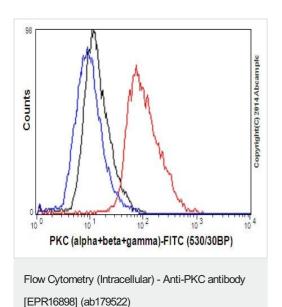
Anti-PKC antibody [EPR16898] (ab179522) at 1/1000 dilution + Rat brain whole tissue lysate at 10 µg

Secondary

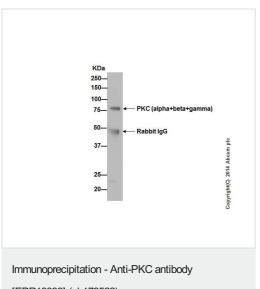
Goat Anti-Rabbit lgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 77 kDa **Observed band size:** 77 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



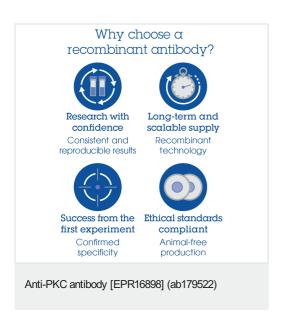
Intracellular flow cytometric analysis of 2% paraformal dehyde-fixed NIH/3T3 (Mouse embyro fibroblast cells) cells labeling PKC alpha + beta + gamma with ab179522 at 1/360 dilution (red) compared with a rabbit monoclonal IgG isotype control (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit lgG (FITC) at 1/150 dilution was used as the secondary antibody.



PKC alpha + beta + gamma was immunoprecipitated from 1mg of Rat brain extract with ab179522 at 1/100 dilution. Western blot was performed from the immunoprecipitate using ab179522 at 1/1000 dilution. Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated antibody was used as secondary antibody at 1/1000 dilution.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

[EPR16898] (ab179522)



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