# abcam

## Product datasheet

## Anti-PKC beta 1 antibody [EPR18512] ab195039

Recombinant RabMAb

6 References 13 Images

Overview

**Product name** Anti-PKC beta 1 antibody [EPR18512]

**Description** Rabbit monoclonal [EPR18512] to PKC beta 1

**Host species** Rabbit

**Tested applications** Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Active human PKC beta 1 full length protein; Human fetal brain whole cell lysate; L-929,

> C2C12, U-87 MG, HeLa, C6 and NIH/3T3 cell lysates; Mouse brain and rat brain lysates. IHC-P: Human colon, Human colon cancer, mouse kidney and rat spleen tissues. ICC/IF: HeLa and K562

cells. Flow Cyt (intra): K562 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 pH: 7.2

Preservative: 0.01% Sodium azide

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

**Purity** Protein A purified

Clonality Monoclonal Clone number EPR18512

**Isotype** IgG

#### **Applications**

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab195039 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)		1/200.
WB		1/2000. Detects a band of approximately 77, 40 kDa (predicted molecular weight: 77 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100.

#### **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.

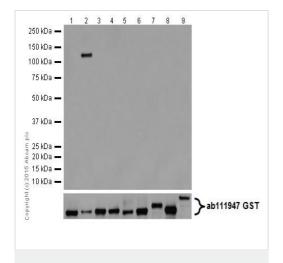
Autophosphorylation on other sites i.e. in the N-terminal and hinge regions have no effect on

enzyme activity.

Cellular localization

Cytoplasm. Nucleus. Membrane.

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Western blot - Anti-PKC beta 1 antibody [EPR18512] (ab195039)

**All lanes :** Anti-PKC beta 1 antibody [EPR18512] (ab195039) 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**

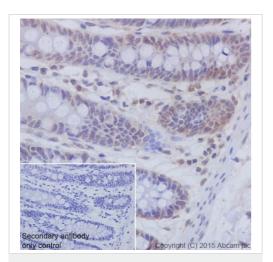
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/50000 dilution

**Predicted band size:** 77 kDa **Observed band size:** 103 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.

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.

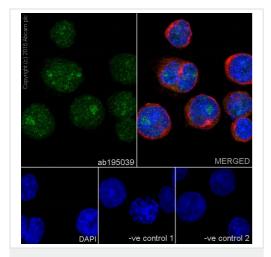


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC beta 1 antibody [EPR18512] (ab195039)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling PKC beta 1 with ab195039 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus and weak cytoplasm staining on epithelial cells of Human colon is observed. Counter stained with Hematoxylin.

Secondary antibody only 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.

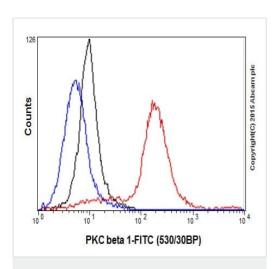


Immunocytochemistry/ Immunofluorescence - Anti-PKC beta 1 antibody [EPR18512] (ab195039)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling PKC beta 1 with ab195039 at 1/100 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and cytoplasm staining on K562 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

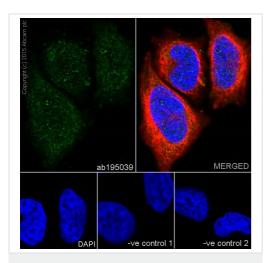
The negative controls are as follows:

-ve control 1: ab195039 at 1/100 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-PKC beta 1 antibody [EPR18512] (ab195039)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling PKC beta 1 with ab195039 at 1/200 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.

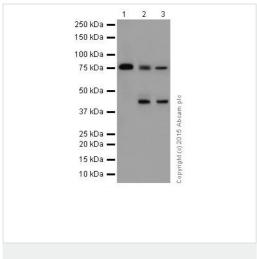


Immunocytochemistry/ Immunofluorescence - Anti-PKC beta 1 antibody [EPR18512] (ab195039)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling PKC beta 1 with ab195039 at 1/100 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and cytoplasm staining on HeLa cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab195039 at 1/100 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.



Western blot - Anti-PKC beta 1 antibody [EPR18512] (ab195039) **All lanes :** Anti-PKC beta 1 antibody [EPR18512] (ab195039) at 1/10000 dilution

Lane 1: Human fetal brain whole cell lysate

Lane 2: L-929 (Mouse connective tissue fibroblast cells) whole cell

lysate

Lane 3: C2C12 (Mouse myoblast cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

All lanes: Anti-Rabbit IgG (HRP), specific to the non-reduced form

of IgG at 1/50000 dilution

**Predicted band size:** 77 kDa **Observed band size:** 40,77 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The peptide immunogen corresponds to the C terminal region of human PKC beta 1. The antibody recognizes the full length (~77KD) and the catalytic domain (~40KD) of PKC beta 1 (Geraldes P and King GL, 2010. Circ Res. 106, 1319-1331.



Western blot - Anti-PKC beta 1 antibody [EPR18512] (ab195039) **All lanes :** Anti-PKC beta 1 antibody [EPR18512] (ab195039) at 1/10000 dilution

**Lane 1 :** U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) whole cell lysate

Lane 2: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 10 µg per lane.

### **Secondary**

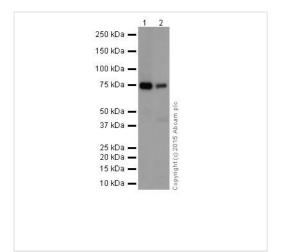
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

**Predicted band size:** 77 kDa **Observed band size:** 40,77 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The peptide immunogen corresponds to the C terminal region of human PKC beta 1. The antibody recognizes the full length (~77KD) and the catalytic domain (~40KD) of PKC beta 1 (Geraldes P and King GL, 2010. Circ Res. 106, 1319-1331.



Western blot - Anti-PKC beta 1 antibody [EPR18512] (ab195039) **All lanes :** Anti-PKC beta 1 antibody [EPR18512] (ab195039) at 1/2000 dilution

Lane 1 : Mouse brain lysate

Lane 2 : Rat brain lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

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

**Predicted band size:** 77 kDa **Observed band size:** 40,77 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The peptide immunogen corresponds to the C terminal region of human PKC beta 1. The antibody recognizes the full length (~77KD) and the catalytic domain (~40KD) of PKC beta 1 (Geraldes P and King GL, 2010. Circ Res. 106, 1319-1331.

1 2 250 KDa — 150 KDa — 75 KDa — 37 KDa — 25 KDa — 20 KDa — 15 KDa — 10 KDa — 10 KDa —

Western blot - Anti-PKC beta 1 antibody

[EPR18512] (ab195039)

**All lanes :** Anti-PKC beta 1 antibody [EPR18512] (ab195039) at 1/2000 dilution

Lane 1: C6 (Rat glial tumor cells) whole cell lysate

Lane 2: NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

#### **Secondary**

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

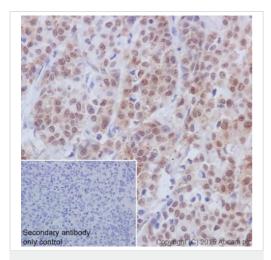
dilution

**Predicted band size:** 77 kDa **Observed band size:** 40,77 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The peptide immunogen corresponds to the C terminal region of human PKC beta 1. The antibody recognizes the full length (~77KD) and the catalytic domain (~40KD) of PKC beta 1 (Geraldes P and King GL, 2010. Circ Res. 106, 1319-1331.

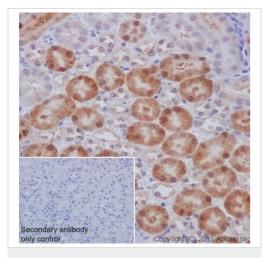


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC beta 1 antibody [EPR18512] (ab195039)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labeling PKC beta 1 with ab195039 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus and cytoplasm staining on cancer cells of colon cancer is observed. Counter stained with Hematoxylin.

Secondary antibody only 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.

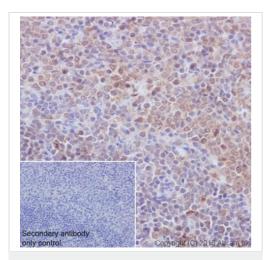


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC beta 1 antibody [EPR18512] (ab195039)

Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling PKC beta 1 with ab195039 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus and cytoplasm staining on epithelial cells of mouse kidney is observed. Counter stained with Hematoxylin.

Secondary antibody only 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.

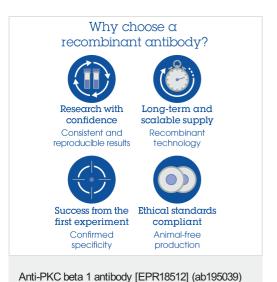


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PKC beta 1 antibody
[EPR18512] (ab195039)

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling PKC beta 1 with ab195039 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus and cytoplasm staining on lymphocytes of rat spleen is observed. Counter stained with Hematoxylin.

Secondary antibody only 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.



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