abcam

Product datasheet

Anti-PKN1 antibody [EPR18808] ab195264





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Overview

Product name Anti-PKN1 antibody [EPR18808]

Description Rabbit monoclonal [EPR18808] to PKN1

Host species Rabbit

Tested applications Suitable for: IP, WB

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: Jurkat, LNCaP, MDA-MB-231, K562, HEK-293, MCF7, C6, RAW 264.7, PC-12 and

NIH/3T3 whole cell lysates; Rat brain and spleen lysates. IP: Jurkat whole cell lysate.

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 (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR18808

Isotype lgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab195264 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
IP		1/40.
WB	****(1)	1/1000. Detects a band of approximately 120 kDa (predicted molecular weight: 104 kDa).

Target

Function

PKC-related serine/threonine-protein kinase involved in various processes such as regulation of the intermediate filaments of the actin cytoskeleton, cell migration, tumor cell invasion and transcription regulation. Regulates the cytoskeletal network by phosphorylating proteins such as VIM and neurofilament proteins NEFH, NEFL and NEFM, leading to inhibit their polymerization. Phosphorylates 'Ser-575', 'Ser-637' and 'Ser-669' of MAPT/Tau, lowering its ability to bind to microtubules, resulting in disruption of tubulin assembly. Acts as a key coactivator of androgen receptor (ANDR)-dependent transcription, by being recruited to ANDR target genes and specifically mediating phosphorylation of 'Thr-11' of histone H3 (H3T11ph), a specific tag for epigenetic transcriptional activation that promotes demethylation of histone H3 'Lys-9' (H3K9me) by KDM4C/JMJD2C. Phosphorylates HDAC5, HDAC7 and HDAC9, leading to impair their import in the nucleus. Phosphorylates 'Thr-38' of PPP1R14A, 'Ser-159', 'Ser-163' and 'Ser-170' of MARCKS, and GFAP. Able to phosphorylate RPS6 in vitro.

Tissue specificity

Found ubiquitously. Expressed in heart, brain, placenta, lung, skeletal muscle, kidney and pancreas. Expressed in numerous tumor cell lines, especially in breast tumor cells.

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 1 protein kinase domain. Contains 3 REM (Hr1) repeats.

Domain

The C1 domain does not bind the diacylglycerol (DAG).

Post-translational

Autophosphorylated; preferably on serine. Phosphorylated during mitosis.

modifications

Activated by limited proteolysis with trypsin.

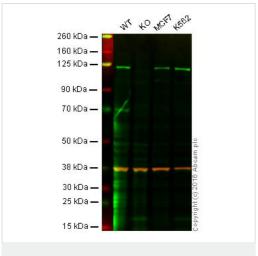
Cellular localization

Cytoplasm. Nucleus. Endosome. Cell membrane. Cleavage furrow. Midbody. Associates with chromatin in a ligand-dependent manner. Localization to endosomes is mediated via its interaction with RHOB. Association to the cell membrane is dependent on Ser-374

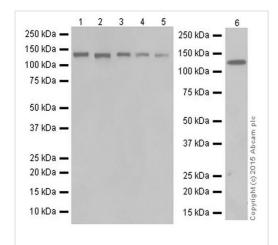
phosphorylation. Accumulates during telophase at the cleavage furrow and finally concentrates

around the midbody in cytokinesis.

Images



Western blot - Anti-PKN1 antibody [EPR18808] (ab195264)



Western blot - Anti-PKN1 antibody [EPR18808] (ab195264)

Lane 1: Wild-type HAP1 cell lysate (40 µg)

Lane 2: PKN1 knockout HAP1 cell lysate (40 µg)

Lane 3: MCF7 cell lysate (20 µg)

Lane 4: K562 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab195264 observed at 125 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab195264 was shown to specifically react with PKN1 when PKN1 knockout samples were used. Wild-type and PKN1 knockout samples were subjected to SDS-PAGE. Ab195264 and <u>ab8245</u> (loading control to GAPDH) were diluted at 1/1000 and 1/10000 dilution respectively and incubated overnight at 4C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preadsorbed <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preadsorbed <u>ab216776</u> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

All lanes : Anti-PKN1 antibody [EPR18808] (ab195264) at 1/1000 dilution

Lane 1 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lane 2: LNCaP (Human prostate cancer cell line) whole cell lysate

Lane 3: MDA-MB-231 (Human breast adenocarcinoma cell line) whole cell lysate

Lane 4: K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate

Lane 5 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 6 : MCF-7 (Human breast adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 104 kDa Observed band size: 120 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
9d weedy 50 25 kDa —
90 kDa —
100 kDa —

Immunoprecipitation - Anti-PKN1 antibody [EPR18808] (ab195264)

PKN1 was immunoprecipitated from 1mg of Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate with ab195264 at 1/40 dilution.

Western blot was performed from the immunoprecipitate using ab195264 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

Lane 1: Jurkat whole cell lysate, 10µg (Input).

Lane 2: ab195264 IP in Jurkat whole cell lysate.

Lane 3: Rabbit lgG,monoclonal-lsotype Control (ab172730) instead of ab195264 in Jurkat whole cell lysate.

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

Exposure time: 3 minutes.

1 2 3 4

250 KDa —

150 KDa —

100 KDa —

75 KDa —

37 KDa —

25 KDa —

20 KDa —

20 KDa —

10 KDa —

110 KDa —

110 KDa —

120 KDa —

130 KDa —

140 KDa —

150 KDa —

150 KDa —

160 KDa —

170 KDa —

180 KDa

Western blot - Anti-PKN1 antibody [EPR18808] (ab195264)

All lanes : Anti-PKN1 antibody [EPR18808] (ab195264) at 1/1000 dilution

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

Lane 2: RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate

Lane 3: PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lane 4 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

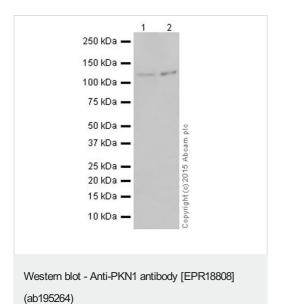
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 104 kDa **Observed band size:** 120 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



All lanes : Anti-PKN1 antibody [EPR18808] (ab195264) at 1/1000 dilution

Lane 1 : Rat brain lysate

Lane 2 : Rat spleen lysate

Lysates/proteins at 10 µg per lane.

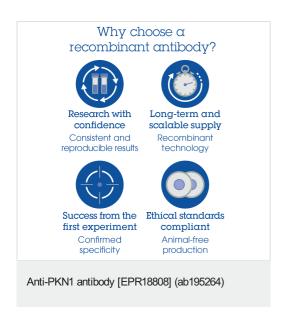
Secondary

All lanes: Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at

1/100000 dilution

Predicted band size: 104 kDa **Observed band size:** 120 kDa

Exposure time: 3 minutes



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