abcam

Product datasheet

Anti-PAK1 antibody [EPR20048] ab223849

KO VALIDATED Recombinant RabMAb

11 References 11 Images

Overview

Product name	Anti-PAK1 antibody [EPR20048]
Description	Rabbit monoclonal [EPR20048] to PAK1
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IP, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: His-tagged human PAK1 (aa1-250) recombinant protein; HeLa, SK-OV-3, SH-SY5Y, HEK-293T, NIH/3T3 and PC-12 whole cell lysates; Human fetal brain lysate; Mouse and rat brain lysates. ICC/IF: SH-SY5Y and HeLa cells. Flow Cyt (intra): SH-SY5Y and HeLa cells. IP: SH-SY5Y 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: 0.1% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20048

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab223849 in the following tested applications.

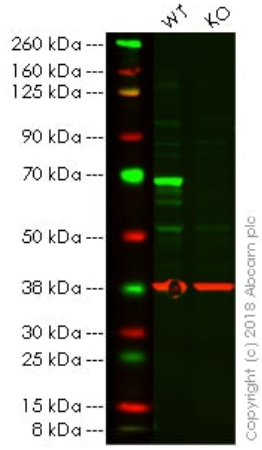
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 60 kDa (predicted molecular weight: 61 kDa). In WB this antibody showed weak staining of PAK1 on HeLa cell lysate
ICC/IF		1/100.
IP		1/30.
Flow Cyt (Intra)		1/50.

Target

Function	The activated kinase acts on a variety of targets. Likely to be the GTPase effector that links the Rho-related GTPases to the JNK MAP kinase pathway. Activated by CDC42 and RAC1. Involved in dissolution of stress fibers and reorganization of focal complexes. Involved in regulation of microtubule biogenesis through phosphorylation of TBCB. Activity is inhibited in cells undergoing apoptosis, potentially due to binding of CDC2L1 and CDC2L2.
Sequence similarities	Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. STE20 subfamily. Contains 1 CRIB domain. Contains 1 protein kinase domain.
Post-translational modifications	Autophosphorylated when activated by CDC42/p21 and RAC1.
Cellular localization	Cytoplasm. Cell junction > focal adhesion. Recruited to focal adhesions upon activation.

Images



Western blot - Anti-PAK1 antibody [EPR20048] (ab223849)

All lanes : Anti-PAK1 antibody [EPR20048] (ab223849) at 1/1000 dilution

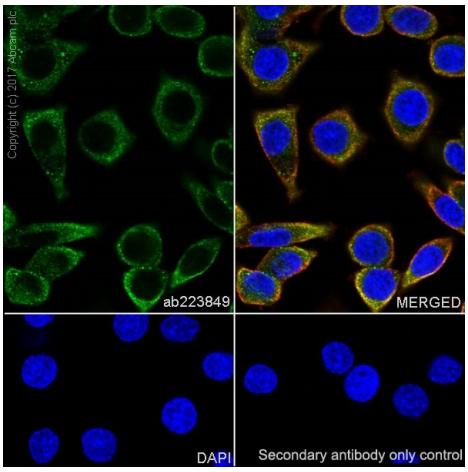
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : PAK1 knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 61 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab223849 observed at 61 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab223849 was shown to specifically react with PAK1 in wild-type HAP1 cells as signal was lost in PAK1 knockout cells. Wild-type and PAK1 knockout samples were subjected to SDS-PAGE. Ab223849 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

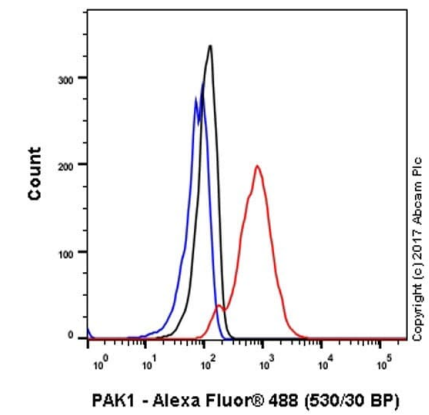


Immunocytochemistry/ Immunofluorescence - Anti-PAK1 antibody [EPR20048] (ab223849)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SH-SY5Y (human neuroblastoma cell line from bone marrow) cells labeling PAK1 with ab223849 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on SH-SY5Y cell line.

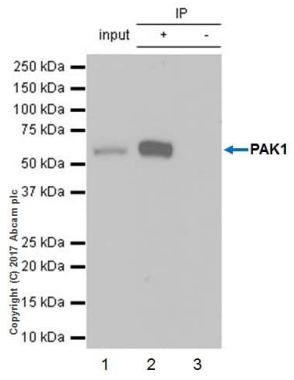
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-PAK1 antibody [EPR20048] (ab223849)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized SH-SY5Y (human neuroblastoma cell line from bone marrow) cell line labeling PAK1 with ab223849 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary



Immunoprecipitation - Anti-PAK1 antibody [EPR20048] (ab223849)

PAK1 was immunoprecipitated from 0.35 mg SH-SY5Y (human neuroblastoma cell line from bone marrow) whole cell lysate with ab223849 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab223849 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

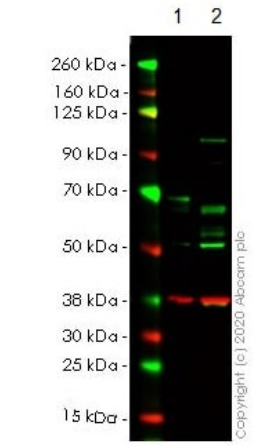
Lane 1: SH-SY5Y whole cell lysate 10 µg (Input).

Lane 2: ab223849 IP in SH-SY5Y whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab223849 in SH-SY5Y whole cell lysate.

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

Exposure time: 1 second.



Western blot - Anti-PAK1 antibody [EPR20048] (ab223849)

All lanes : Anti-PAK1 antibody [EPR20048] (ab223849) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PAK1 CRISPR/Cas9 edited HeLa cell lysate

Lysates/proteins at 20 µg per lane.

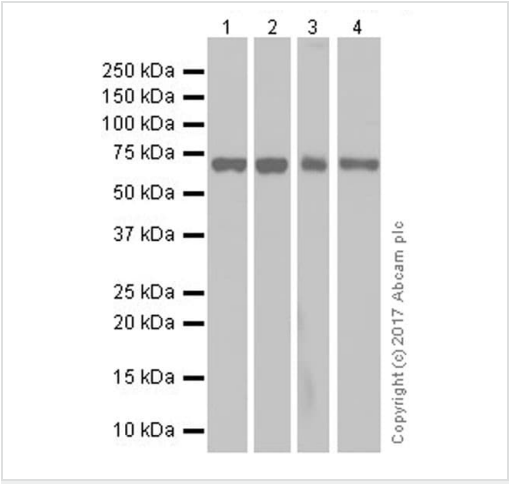
Performed under reducing conditions.

Predicted band size: 61 kDa
Observed band size: 65 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab223849 observed at 65 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab223849 was shown to react with PAK1 in wild-type HeLa cells in western blot. The band observed in CRISPR/Cas9 editedcell line **ab264889** (CRISPR/Cas9 editedcell lysate **ab257572**) lane below 65kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and PAK1

CRISPR/Cas9 edited HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab223849 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-PAK1 antibody [EPR20048] (ab223849)

All lanes : Anti-PAK1 antibody [EPR20048] (ab223849) at 1/1000 dilution

Lane 1 : SK-OV-3 (human ovarian cancer epithelial cell line) whole cell lysate
Lane 2 : NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate
Lane 3 : PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate
Lane 4 : Human fetal brain lysate

Lysates/proteins at 20 µg per lane.

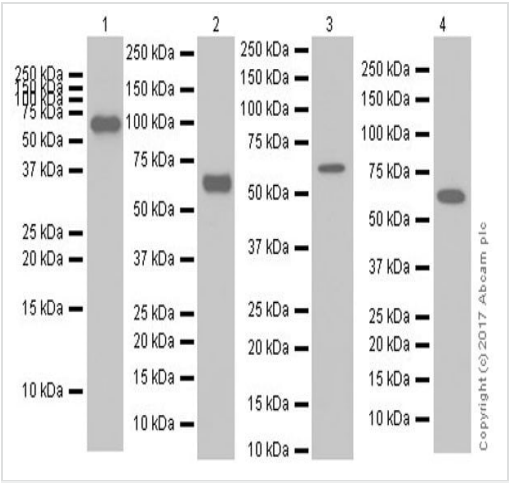
Secondary
Lanes 1-3 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution
Lane 4 : VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/4000 dilution

Developed using the ECL technique.

Predicted band size: 61 kDa
Observed band size: 60 kDa

Exposure time : Lane 1: 3 minutes; Lane 2: 30 seconds; Lane 3: 3 seconds; Lane 4: 15 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-PAK1 antibody [EPR20048] (ab223849)

All lanes : Anti-PAK1 antibody [EPR20048] (ab223849) at 1/1000 dilution

Lane 1 : Mouse brain lysate
Lane 2 : Rat brain lysate
Lane 3 : SH-SY5Y (human euroblastoma cell line from bone marrow) whole cell lysate
Lane 4 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lysates/proteins at 10 µg per lane.

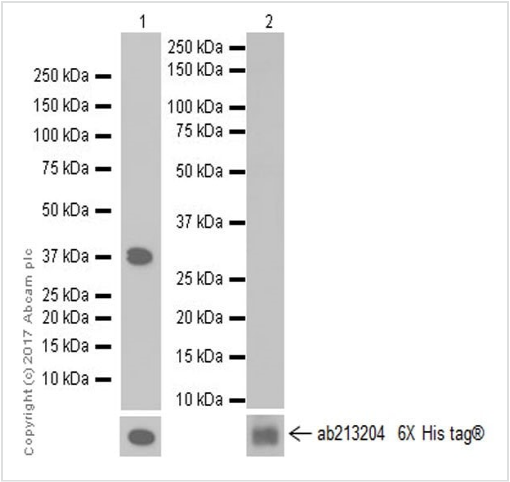
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 61 kDa
Observed band size: 60 kDa

Exposure time : Lane 1: 5 seconds; Lane 2: 3 seconds; Lane 3: 3 minutes; Lane 4: 1 minutes.

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-PAK1 antibody [EPR20048] (ab223849)

All lanes : Anti-PAK1 antibody [EPR20048] (ab223849) at 1/1000 dilution

Lane 1 : His-tagged human PAK1 (aa1-250) recombinant protein
Lane 2 : His-tagged human PAK2 (aa1-250) recombinant protein

Lysates/proteins at 0.01 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

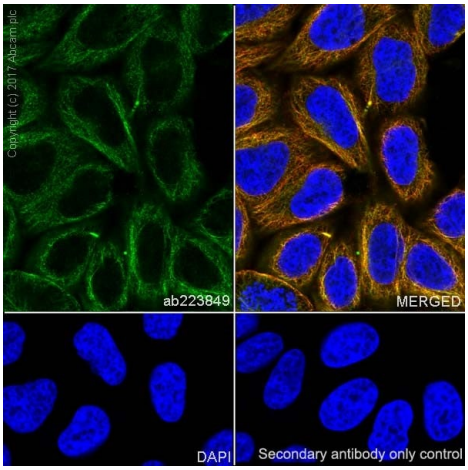
Developed using the ECL technique.

Predicted band size: 61 kDa

Observed band size: 37 kDa

Exposure time : Lane 1: 1 minute; Lane 2: 3 minutes.

Blocking/Dilution buffer: 5% NFDM/TBST.



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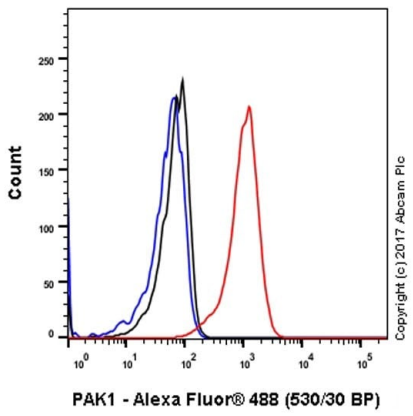

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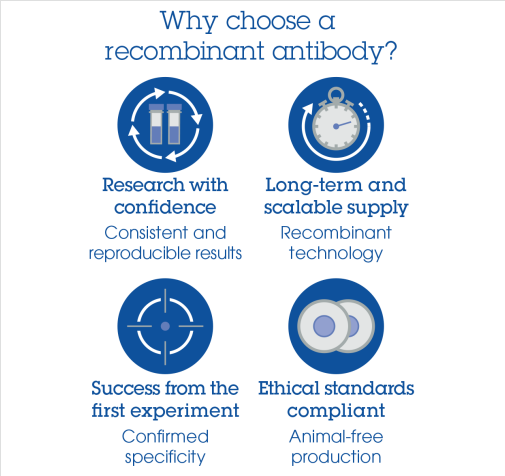

Anti-PAK1 antibody [EPR20048] (ab223849)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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