abcam

Product datasheet

Anti-PAK1 antibody [EPR20048] - BSA and Azide free ab242421

KO VALIDATED | Recombinant | RabMAb

8 Images

Overview

Product name	Anti-PAK1 antibody [EPR20048] - BSA and Azide free
Description	Rabbit monoclonal [EPR20048] to PAK1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt, ICC/IF, IP, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human PAK1 aa 200-300. The exact sequence is proprietary. Database link: Q13153
Positive control	ICC/IF: SH-SY5Y and HeLa cells. Flow Cyt: SH-SY5Y and HeLa cells. IP: SH-SY5Y whole cell lysate. WB: HeLa and HAP1 cell lysates.
General notes	Ab242421 is the carrier-free version of ab223849. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. ab242421 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *Maxpar® is a trademark of Fluidigm Canada Inc.* 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. Reproducibility is key to advancing scientific discovery and accelerating scientists' next

breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20048
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab242421** in the following tested applications.

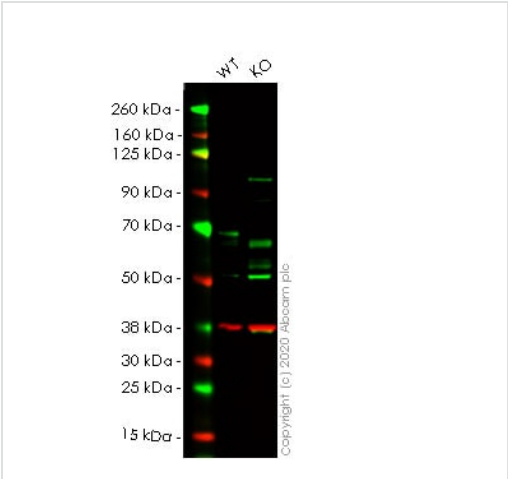
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 61 kDa.

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] - BSA and Azide free (ab242421)

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

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PAK1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

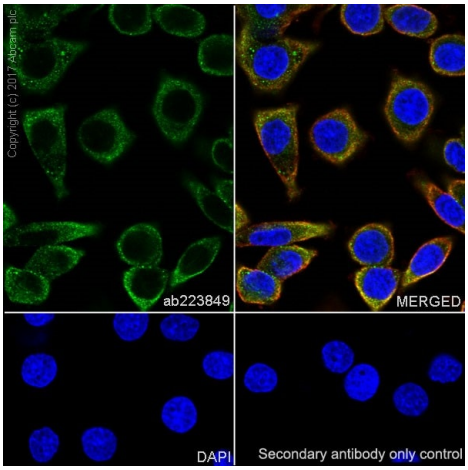
Predicted band size: 61 kDa
Observed band size: 65 kDa
why is the actual band size different from the predicted?

This data was developed using the same antibody clone in a different buffer formulation (ab223849).

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 knockout cell line ab264889 (knockout cell lysate ab257572) lane below 65kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and PAK1 knockout 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.



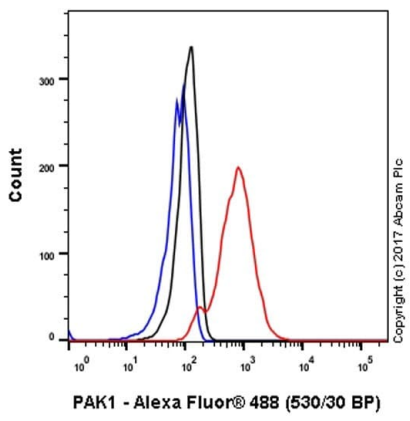
Immunocytochemistry/ Immunofluorescence - Anti-PAK1 antibody [EPR20048] - BSA and Azide free (ab242421)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab223849).

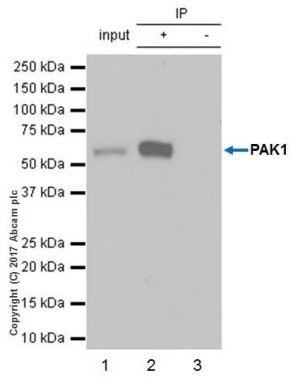


Flow Cytometry - Anti-PAK1 antibody [EPR20048] - BSA and Azide free (ab242421)

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 antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab223849).



Immunoprecipitation - Anti-PAK1 antibody [EPR20048] - BSA and Azide free (ab242421)

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).

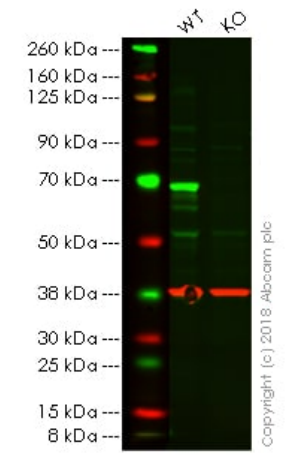
Lane 2: ab223849 IP in SH-SY5Y whole cell lysate.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab223849).



Western blot - Anti-PAK1 antibody [EPR20048] - BSA and Azide free (ab242421)

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.

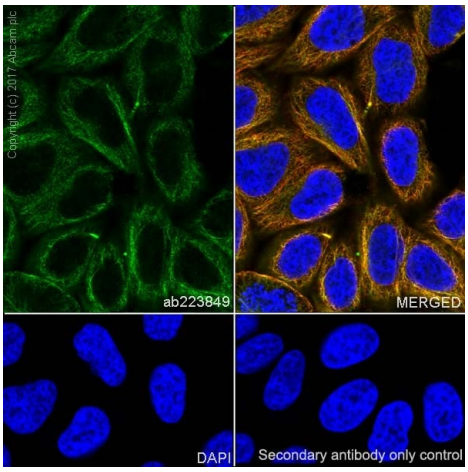
Predicted band size: 61 kDa

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.

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.



Immunocytochemistry/ Immunofluorescence - Anti-PAK1 antibody [EPR20048] - BSA and Azide free (ab242421)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) 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 HeLa 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.

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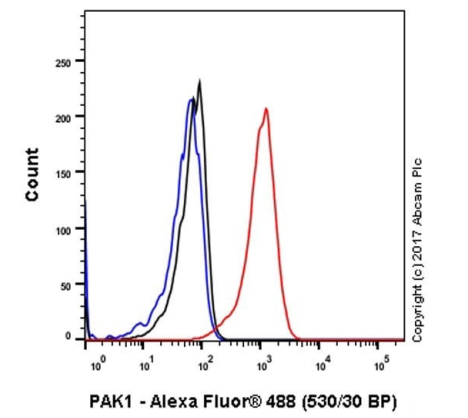

Flow Cytometry - Anti-PAK1 antibody [EPR20048] - BSA and Azide free (ab242421)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling PAK1 with ab223849 at 1/50 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 antibody.

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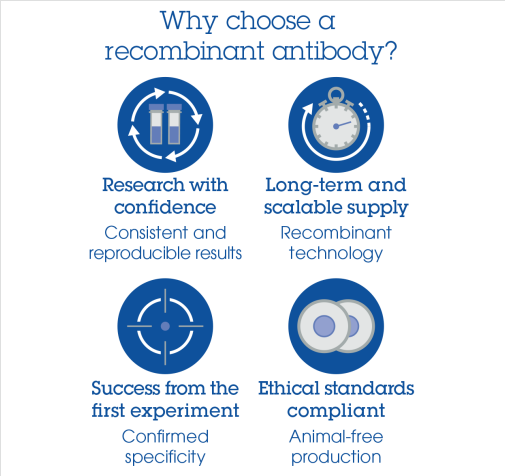

Anti-PAK1 antibody [EPR20048] - BSA and Azide free (ab242421)

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