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:** ICC/IF, IP, WB, 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	ICC/IF: SH-SY5Y and HeLa cells. Flow Cyt (intra): 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**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. 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. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. 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**.

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

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

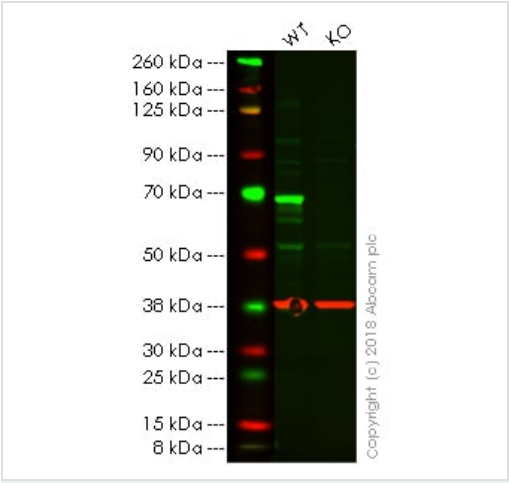
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
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.
Flow Cyt (Intra)		Use at an assay dependent concentration.

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 HAP1 whole cell lysate
Lane 2 : PAK1 knockout HAP1 whole cell lysate

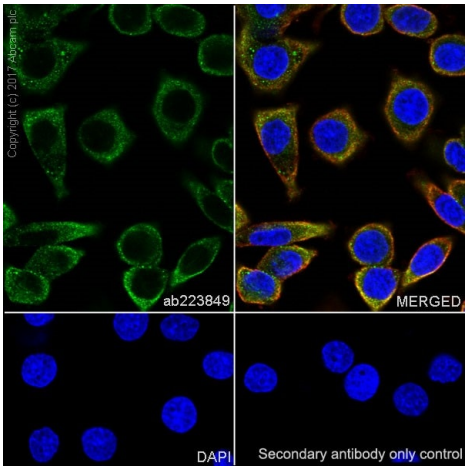
Lysates/proteins at 20 µg per lane.

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.



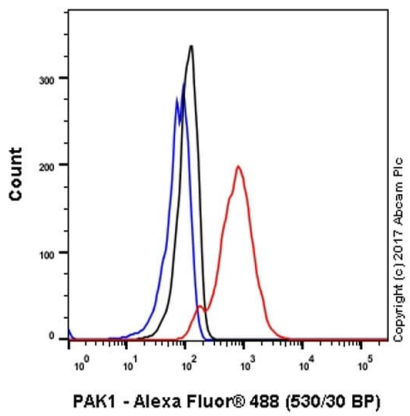
Immunocytochemistry/ Immunofluorescence - Anti-PAK1 antibody [EPR20048] - BSA and Azide free (ab242421)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized 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**).

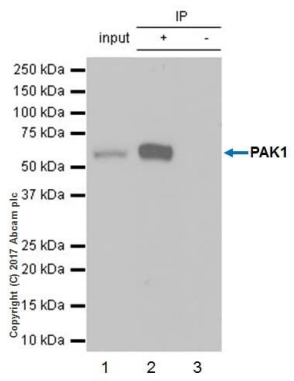


Flow Cytometry (Intracellular) - Anti-PAK1 antibody [EPR20048] - BSA and Azide free (ab242421)

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 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).

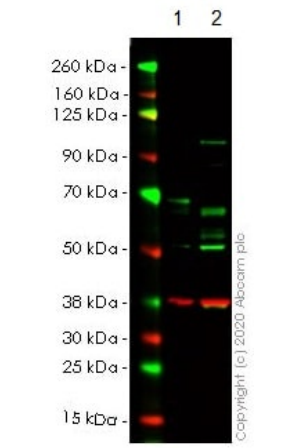
Lane 2: **ab223849** IP in SH-SY5Y whole cell lysate.

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 HeLa cell lysate
Lane 2 : PAK1 CRISPR/Cas9 edited HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

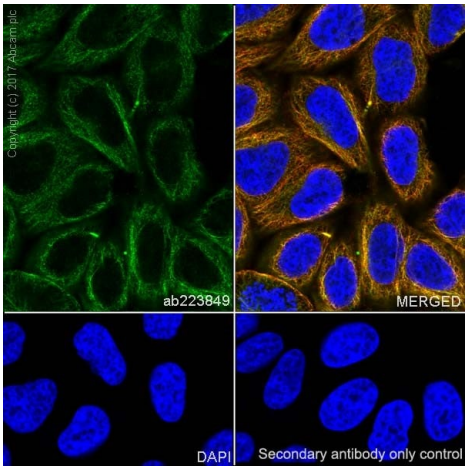
Predicted band size: 61 kDa
Observed band size: 65 kDa

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 CRISPR/Cas9 edited cell line **ab264889** (CRISPR/Cas9 edited cell 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.



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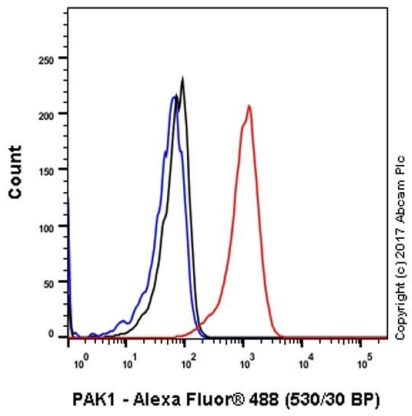
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab223849**).



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

Intracellular 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.

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

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

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