abcam

ab223849

Anti-PAK1 antibody [EPR20048]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.1% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR20048
Purification technique	Affinity purification Protein A
Concentration	0.461 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	In WB this antibody showed weak staining of PAK1 on HeLa cell lysate

Species	Rat
Dilution info	1/1000
Notes	In WB this antibody showed weak staining of PAK1 on HeLa cell lysate

Species	Human
Dilution info	1/1000
Notes	In WB this antibody showed weak staining of PAK1 on HeLa cell lysate

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information PAK1

Function

Protein kinase involved in intracellular signaling pathways downstream of integrins and receptor-type kinases that plays an important role in cytoskeleton dynamics, in cell adhesion, migration, proliferation, apoptosis, mitosis, and in vesicle-mediated transport processes (PubMed:10551809, PubMed:11896197, PubMed:12876277, PubMed:14585966, PubMed:15611088, PubMed:17726028, PubMed:17989089, PubMed:30290153, PubMed:17420447). Can directly phosphorylate BAD and protects cells against apoptosis (By similarity). Activated by interaction with CDC42 and RAC1 (PubMed:8805275, PubMed:9528787). Functions as a GTPase effector that links the Rho-related GTPases CDC42 and RAC1 to the JNK MAP kinase pathway (PubMed:8805275, PubMed:9528787). Phosphorylates and activates MAP2K1, and thereby mediates activation of downstream MAP kinases (By similarity). Involved in the reorganization of the actin cytoskeleton, actin stress fibers and of focal adhesion complexes (PubMed:9032240, PubMed:9395435). Phosphorylates the tubulin chaperone TBCB and thereby plays a role in the regulation of microtubule biogenesis and organization of the tubulin cytoskeleton (PubMed:15831477). Plays a role in the regulation of insulin secretion in response to elevated glucose levels (PubMed:22669945). Part of a ternary complex that contains PAK1, DVL1 and MUSK that is important for MUSK-dependent regulation of AChR clustering during the formation of the neuromuscular junction (NMJ) (By similarity). Activity is inhibited in cells undergoing apoptosis, potentially due to binding of CDC2L1 and CDC2L2 (PubMed:12624090). Phosphorylates MYL9/MLC2 (By similarity). Phosphorylates RAF1 at 'Ser-338' and 'Ser-339' resulting in: activation of RAF1, stimulation of RAF1 translocation to mitochondria, phosphorylation of BAD by RAF1, and RAF1 binding to BCL2 (PubMed:11733498). Phosphorylates SNAI1 at 'Ser-246' promoting its transcriptional repressor activity by increasing its accumulation in the nucleus (PubMed:15833848). In podocytes, promotes NR3C2 nuclear localization (By similarity). Required for atypical chemokine receptor ACKR2-induced phosphorylation of LIMK1 and cofilin (CFL1) and for the up-regulation of ACKR2 from endosomal compartment to cell membrane, increasing its efficiency in

chemokine uptake and degradation (PubMed:23633677). In synapses, seems to mediate the regulation of F-actin cluster formation performed by SHANK3, maybe through CFL1 phosphorylation and inactivation (By similarity). Plays a role in RUFY3-mediated facilitating gastric cancer cells migration and invasion (PubMed:25766321). In response to DNA damage, phosphorylates MORC2 which activates its ATPase activity and facilitates chromatin remodeling (PubMed:23260667). In neurons, plays a crucial role in regulating GABA(A) receptor synaptic stability and hence GABAergic inhibitory synaptic transmission through its role in F-actin stabilization (By similarity). In hippocampal neurons, necessary for the formation of dendritic spines and excitatory synapses; this function is dependent on kinase activity and may be exerted by the regulation of actomyosin contractility through the phosphorylation of myosin II regulatory light chain (MLC) (By similarity). Along with GIT1, positively regulates microtubule nucleation during interphase (PubMed:27012601). Phosphorylates FXR1, promoting its localization to stress granules and activity (PubMed:20417602). Phosphorylates ILK on 'Thr-173' and 'Ser-246', promoting nuclear export of ILK (PubMed:17420447).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

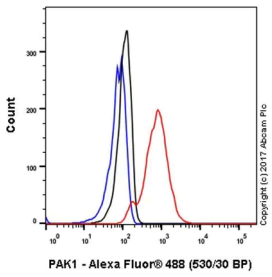
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

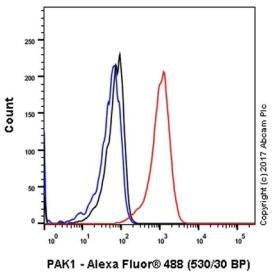
11 product images



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

PAK1 Flow Cytometry (Intracellular) staining using rabbit Anti-PAK1 antibody

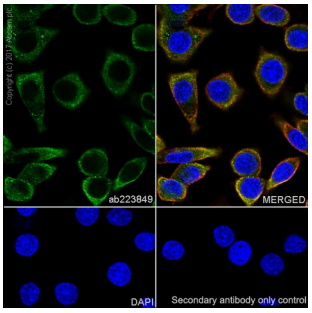
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized SH-SY5Y (human neuroblastoma cell line from bone marrow) cell line labeling PAK1 with ab223849 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and 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



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

PAK1 Flow Cytometry (Intracellular) staining using rabbit Anti-PAK1 antibody

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.

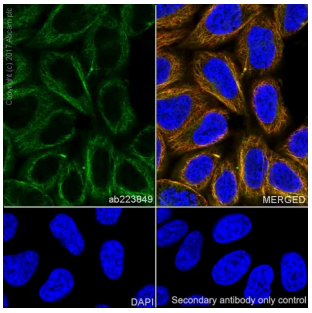


Immunocytochemistry/ Immunofluorescence - Anti-PAK1 antibody [EPR20048] (ab223849)

PAK1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PAK1 antibody

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.

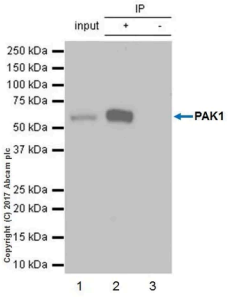


Immunocytochemistry/ Immunofluorescence - Anti-PAK1 antibody [EPR20048] (ab223849)

PAK1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PAK1 antibody

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.

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.



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.

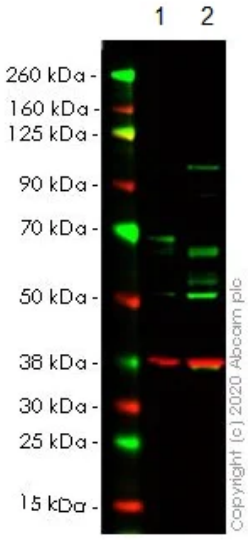
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab223849 in SH-SY5Y whole cell lysate.

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

Exposure time: 1 second.

All lanes:
Immunoprecipitation - Anti-PAK1 antibody [EPR20048] (ab223849)

Predicted band size: 61 kDa



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

PAK1 Western blot staining using rabbit Anti-PAK1 antibody

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.

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

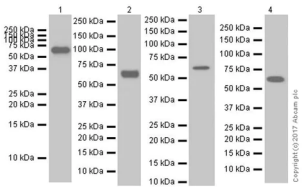
Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
PAK1 CRISPR/Cas9 edited HeLa cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 61 kDa

Observed band size: 65 kDa



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

PAK1 Western blot staining using rabbit Anti-PAK1 antibody

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

Blocking/Dilution buffer: 5% NFDM/TBST.

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

Lane 1:
Mouse brain lysate at 10 µg

Lane 2:
Rat brain lysate at 10 µg

Lane 3:
SH-SY5Y (human euroblastoma cell line from bone marrow) whole cell lysate at 10 µg

Lane 4:
HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate at 10 µg

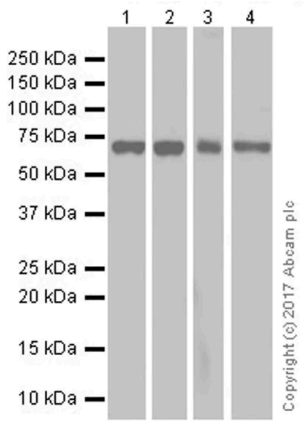
Secondary

All lanes:
Western blot - 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



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

PAK1 Western blot staining using rabbit Anti-PAK1 antibody

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

Blocking/Dilution buffer: 5% NFDM/TBST.

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

Lane 1:
SK-OV-3 (human ovarian cancer epithelial cell line) whole cell lysate at 20 µg

Lane 2:
NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate at 20 µg

Lane 3:
PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate at 20 µg

Lane 4:
Human fetal brain lysate at 20 µg

Secondary

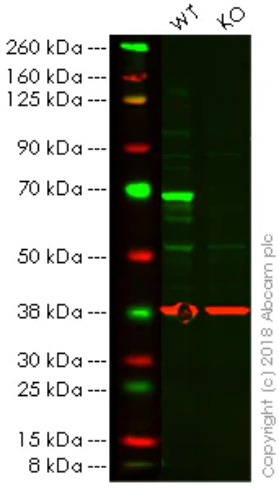
Lanes 1 - 3:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Lane 4:
Western blot - 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



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

PAK1 Western blot staining using rabbit Anti-PAK1 antibody

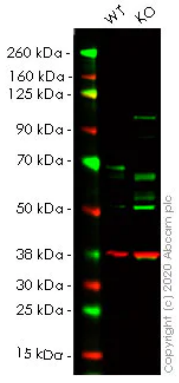
Lanes 1 - 2: Merged signal (red and green). Green - ab223849 observed at 61 kDa. Red - loading control, ab9484, observed at 37 kDa.
ab223849 was shown to specifically react with PAK1 in wild-type HAP1 cells as signal was lost in PAK1 knockout cells. Wild-type and PAK1 knockout samples were subjected 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.

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

Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

Lane 2:
PAK1 knockout HAP1 whole cell lysate at 20 µg

Predicted band size: 61 kDa



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

PAK1 Western blot staining using rabbit Anti-PAK1 antibody

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.

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

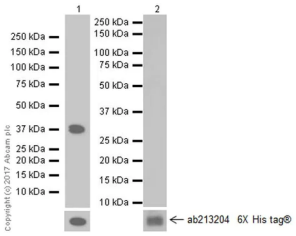
Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
PAK1 knockout HeLa cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 61 kDa

Observed band size: 65 kDa



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

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

Blocking/Dilution buffer: 5% NFDM/TBST.

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

Lane 1:
His-tagged human PAK1 (aa1-250) recombinant protein at 0.01 µg

Lane 2:
His-tagged human PAK2 (aa1-250) recombinant protein at 0.01 µg

Secondary

All lanes:
Western blot - 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

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.