abcam

ab40795

Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y]

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% 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	EP656Y
Purification technique	Affinity purification Protein A
Concentration	0.664 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/40
Notes	-

Expected

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

WB

Tested

Species	Mouse
Dilution info	1/10000 - 1/50000
Notes	-

Species	Rat
Dilution info	1/10000 - 1/50000
Notes	-

Species	Human
Dilution info	1/10000 - 1/50000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/250 - 1/500
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/120
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Species	Human
Dilution info	1/120
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

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

IHC-P

Tested

Species	Mouse
Dilution info	1/100 - 1/500
Notes	-

Species	Rat
Dilution info	1/100 - 1/500
Notes	-

Species	Human
Dilution info	1/100 - 1/500
Notes	-

Target data

See full target information PAK1 pS144

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

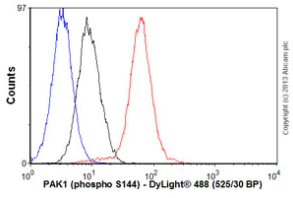
We do not recommend this combination. It is not covered by our product promise.

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.

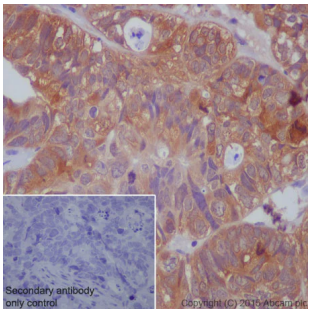
13 product images



Flow Cytometry (Intracellular) - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) Flow Cytometry (Intracellular) staining using rabbit Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody

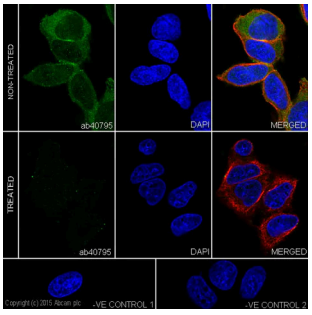
Overlay histogram showing HeLa cells stained with unpurified ab40795 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab40795, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody

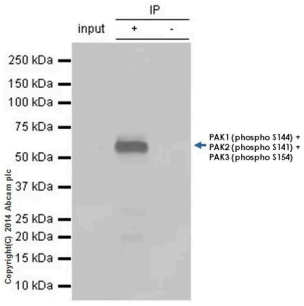
ab40795 staining PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) in human liver carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti-rabbit IgG H&L (HRP) ab97051 was used as the secondary antibody at a dilution of 1/500.
Negative control 1: PBS in place of primary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody

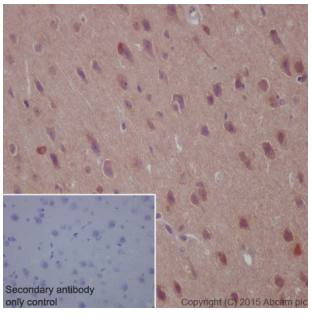
ab40795 staining PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) in HeLa (human cervix adenocarcinoma) cells, treated and untreated with Lambda Protein Phosphtase 31☒ for 5h by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody. ab7291 and ab150120 were used as counterstains for primary antibody ab75748 and secondary antibody ab150077 respectively and DAPI was used as a nuclear counterstain.
Negative control 1: Rabbit primary antibody and anti-mouse secondary antibody (ab150120)
Negative control 2: Mouse primary antibody (ab7291) and anti-rabbit secondary antibody (ab150077)



Immunoprecipitation - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

ab40795 immunoprecipitating PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154). 10µg of HeLa (human cervix adenocarcinoma) whole cell lysate was incubated with primary antibody at a dilution of 1/40 and VeriBlot for IP Detection Reagent (HRP) (ab131366) at a dilution of 1/10000.
Lane 1: HeLa whole cell lysate (10ug)
Lane 2: ab40795 IP in HeLa whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab40795 in HeLa (human cervix adenocarcinoma) whole cell lysate

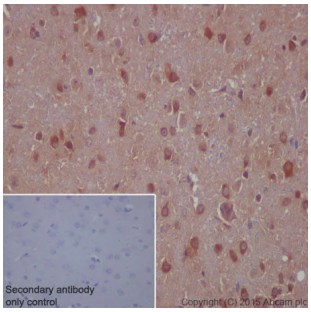
All lanes:
Immunoprecipitation - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody

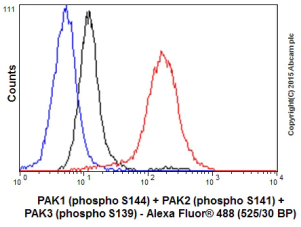
ab40795 staining PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) in mouse cerebral cortex tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti-rabbit IgG H&L (HRP) ab97051 was used as the secondary antibody at a dilution of 1/500.
Negative control 1: PBS in place of primary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody

ab40795 staining PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) in rat cerebral cortex tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti-rabbit IgG H&L (HRP) ab97051 was used as the secondary antibody at a dilution of 1/500.
Negative control 1: PBS in place of primary

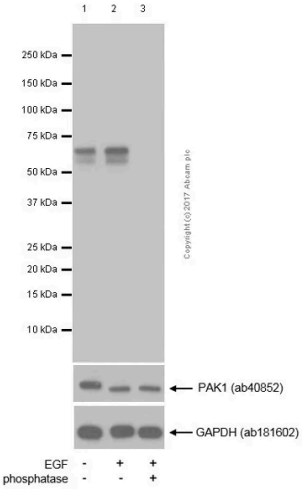


Flow Cytometry (Intracellular) - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) Flow Cytometry (Intracellular) staining using rabbit Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody

ab40795 staining PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) in the human cell line NIH/3T3 (mouse embryo) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/120. A goat anti rabbit IgG (Alexa Fluorr® 488) at a dilution of 1/500 was used as the secondary antibody. Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Western blot - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) Western blot staining using rabbit Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody

Blocking and dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795) at 1/1000 dilution

Lane 1:
MCF7, grown in serum-free media overnight, whole cell lysate at 10 µg

Lane 2:
MCF7, grown in serum-free media overnight, then treated with EGF 1µg/ml for 10min, whole cell lysate at 10 µg

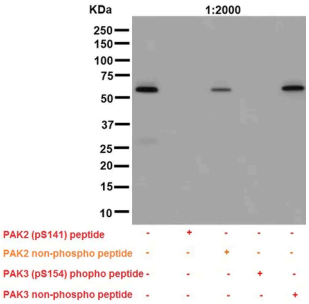
Lane 3:
MCF7, grown in serum-free media overnight, then treated with EGF 1µg/ml for 10min whole cell lysate, The membrane was incubated with phosphatase at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 55 kDa

Exposure time: 1min



Western blot - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

All lanes:
Western blot - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795) at 1/2000 dilution

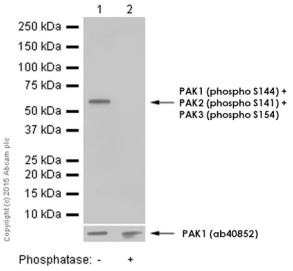
Lane 1:
HeLa cell lysate with None

Lane 2:
HeLa cell lysate with PAK2 (pS141)

Lane 3:
HeLa cell lysate with PAK2 non-phospho

Lane 4:
HeLa cell lysate with PAK3 (pS154)

Lane 5:
HeLa cell lysate with PAK3 non-phospho



Western blot - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

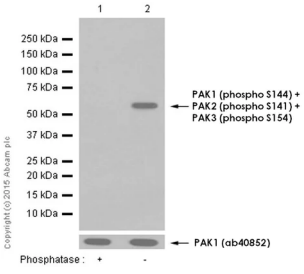
All lanes:
Western blot - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795) at 1/10000 dilution

Lane 1:
HeLa whole cell lysate - untreated at 10 µg

Lane 2:
HeLa whole cell lysate - treated with phosphatase at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution



Western blot - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

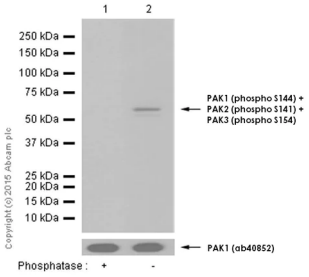
All lanes:
Western blot - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795) at 1/10000 dilution

Lane 1:
RAW264.7 (mouse abelson murine leukemia virus-induced tumor) whole cell lysate - treated with phosphatase at 10 µg

Lane 2:
RAW264.7 (mouse abelson murine leukemia virus-induced tumor) whole cell lysate - untreated at 10 µg

Secondary

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



Western blot - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

All lanes:
Western blot - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795) at 1/50000 dilution

Lane 1:
C6 (rat glioma) whole cell lysate - treated with phosphatase at 10 µg

Lane 2:
C6 (rat glioma) whole cell lysate - untreated at 10 µg

Secondary

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

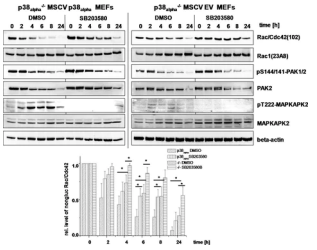


Image collected and cropped by CiteAb under a CC-BY license from the publication

Western blot - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) Western blot staining using rabbit Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody

PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) western blot using anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] ab40795. Publication image and figure legend from Schelle, I., Bruening, J., et al., 2016, Toxins (Basel), PubMed 28025502.

ab40795 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab40795 please see the product overview.

Effects of genetic deletion of p38alpha and of SB203580 treatment on TcsL-catalyzed Rac/Cdc42 glucosylation (time-dependency). p38alpha-/- MSCV p38alpha MEFs and p38alpha-/- MSCV empty vector (EV) MEFs were treated with TcsL (1 µg/mL) in the presence of SB203580 (10 µM) or DMSO alone for the indicated times. The cellular levels of non-glucosylated Rac/Cdc42, total Rac1, pS144/141-PAK1/2, PAK2, pT222-MAPKAPK2, MAPKAPK2, and beta-actin were analyzed by immunoblot using the indicated antibodies. Quantifications of immunoblots were performed using Kodak software and relative amounts of non-glucosylated Rac/Cdc42 versus the total levels of Rac1, respectively, are expressed as mean ± SD of three independent experiments. * indicates significant differences, $p < 0.05$, as analyzed using Student's t-test.

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