abcam

Product datasheet

Anti-PAK2 antibody [EP796Y] ab76293

KO VALIDATED Recombinant RabMAb

3 References 9 Images

Overview

Product name	Anti-PAK2 antibody [EP796Y]
Description	Rabbit monoclonal [EP796Y] to PAK2
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human PAK2 aa 1-100 (N terminal). The exact sequence is proprietary. Database link: Q13177
Positive control	WB: HeLa cytoplasmic lysate, NIH/3T3, RAW 264.7, C6 whole cell lysate. IHC-P; Human breast carcinoma tissue IF/ICC: T47D cell line. FC: HeLa cells.
General notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.** This product is a recombinant rabbit monoclonal antibody.

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EP796Y
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab76293** in the following tested applications.

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

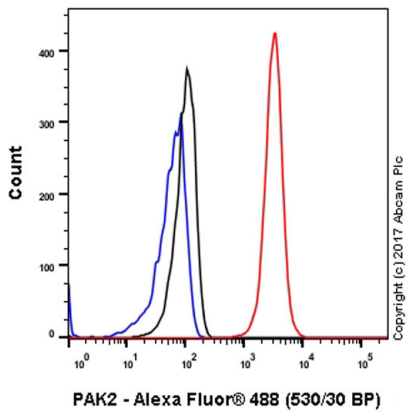
Application	Abreviews	Notes
ICC/IF		1/100 - 1/250.
WB		1/5000. Predicted molecular weight: 58 kDa. **For unpurified use at 1/1000 - 1/2000.**
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. This antibody may not be suitable for IHC with mouse or rat samples Use of HRP conjugated or polymerized HRP secondary antibody is recommended. Stronger signals have been found using the polymerized HRP secondary.
Flow Cyt		1/20. For unpurified use at 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

Function	The activated kinase acts on a variety of targets. Phosphorylates ribosomal protein S6, histone H4 and myelin basic protein. Full length PAK 2 stimulates cell survival and cell growth. The process is, at least in part, mediated by phosphorylation and inhibition of pro-apoptotic BAD. Caspase-activated PAK-2p34 is involved in cell death response, probably involving the JNK signaling pathway. Cleaved PAK-2p34 seems to have a higher activity than the CDC42-activated form.
Tissue specificity	Ubiquitously expressed. Higher levels seen in skeletal muscle, ovary, thymus and spleen.
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	Full length PAK 2 is autophosphorylated when activated by CDC42/p21. Following cleavage, both peptides, PAK-2p27 and PAK-2p34, become highly autophosphorylated, with PAK-2p27 being phosphorylated on serine and PAK-2p34 on threonine residues, respectively. Autophosphorylation of PAK-2p27 can occur in the absence of any effectors and is dependent on phosphorylation of Thr-402, because PAK-2p27 is acting as an exogenous substrate. During apoptosis proteolytically cleaved by caspase-3 or caspase-3-like proteases to yield active PAK-2p34. Ubiquitinated, leading to its proteasomal degradation. PAK-2p34 is myristoylated.
Cellular localization	Cytoplasm and Nucleus. Cytoplasm > perinuclear region. Membrane. Interaction with

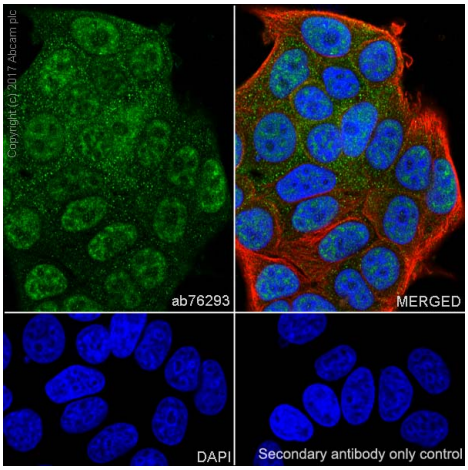
ARHGAP10 probably changes PAK-2p34 location to cytoplasmic perinuclear region. Myristoylation changes PAK-2p34 location to the membrane.

Images



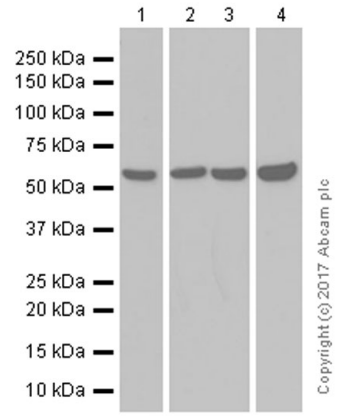
Flow Cytometry - Anti-PAK2 antibody [EP796Y] (ab76293)

Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling PAK2 with purified ab76293 at 1:20 dilution (10 ug/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilized with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1:2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-PAK2 antibody [EP796Y] (ab76293)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling PAK2 with purified ab76293 at 1:100 dilution (2.0µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). ab150077 Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-PAK2 antibody [EP796Y] (ab76293)

All lanes : Anti-PAK2 antibody [EP796Y] (ab76293) at 1/5000 dilution (purified)

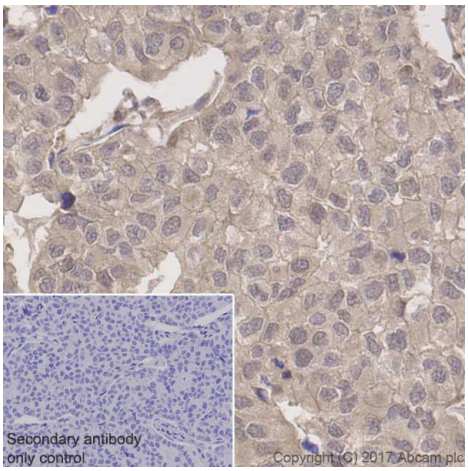
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 3 : RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysates
Lane 4 : C6 (Rat glial tumor glial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

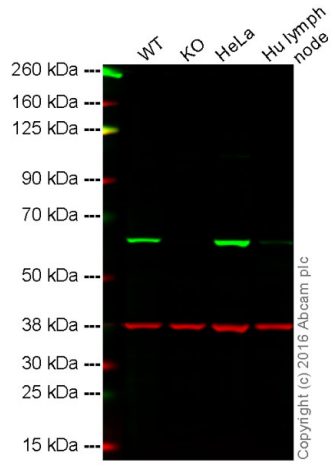
Predicted band size: 58 kDa

Blocking and diluting buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAK2 antibody [EP796Y] (ab76293)

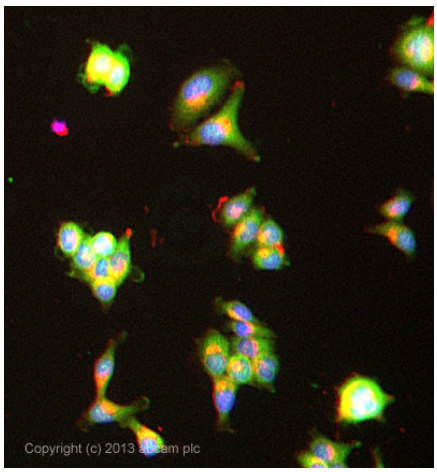
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling PAK2 with Purified ab76293 at 1:100 dilution (2.02 µg/ml). Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-PAK2 antibody [EP796Y] (ab76293)

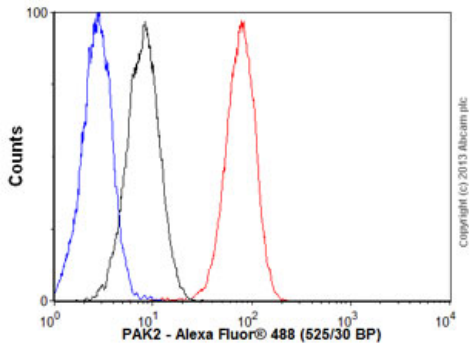
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: PAK2 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Human lymph node tissue lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab76293 observed at 60 kDa. Red - loading control, ab8245, observed at 37 kDa.

Unpurified ab76293 was shown to specifically react with PAK2 when PAK2 knockout samples were used. Wild-type and PAK2 knockout samples were subjected to SDS-PAGE. ab76293 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/10000 respectively and incubated overnight at 4°C. 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 1/10000 dilution for 1 h at room temperature before imaging.



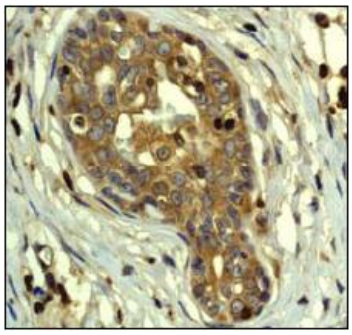
Immunocytochemistry/ Immunofluorescence - Anti-PAK2 antibody [EP796Y] (ab76293)

ICC/IF image of unpurified ab76293 stained T47D cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab76293, 1µg/ml) overnight at +4°C. The secondary antibody (green) was ab96899, DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



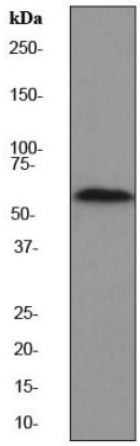
Flow Cytometry - Anti-PAK2 antibody [EP796Y] (ab76293)

Overlay histogram showing HeLa cells stained with unpurified ab76293 (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 (ab76293, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 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-PAK2 antibody [EP796Y] (ab76293)

Unpurified ab76293, at a 1/100 dilution, staining PAK2 in paraffin embedded human breast carcinoma tissue by Immunohistochemistry.



Western blot - Anti-PAK2 antibody [EP796Y] (ab76293)

Anti-PAK2 antibody [EP796Y] (ab76293) at 1/2000 dilution (unpurified) + HeLa cell lysate at 10 µg

Secondary

HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 58 kDa
Observed band size: 61 kDa

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