

Product datasheet

Anti-PAK2 antibody [EP796Y] - BSA and Azide free
ab227990

KO VALIDATED Recombinant RabMAb

3 References 7 Images

Overview

Product name	Anti-PAK2 antibody [EP796Y] - BSA and Azide free
Description	Rabbit monoclonal [EP796Y] to PAK2 - BSA and Azide free
Host species	Rabbit
Tested applications	Suitable for: WB, IHC-P, Flow Cyt, ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide (N terminal).
Positive control	WB: HeLa cytoplasmic lysate IHC-P; Human breast carcinoma tissue IF/ICC: T47D cell line.
General notes	<p>The formulation and the concentration of this product is compatible for metal-conjugation for mass cytometry (CyTOF®).</p> <p>ab227990 is a PBS only buffer version of ab76293, containing no BSA or sodium azide, ideal for antibody labeling. Please refer to ab76293 for information on protocols, dilutions, and image data.</p> <p>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.</p>

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Constituent: PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP796Y
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab227990** in the following tested applications.

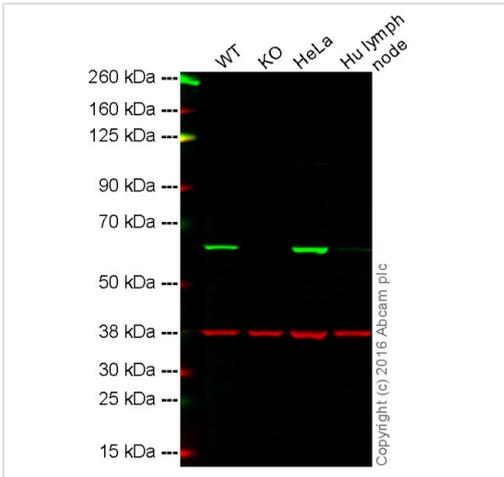
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 58 kDa.
IHC-P		Use at an assay dependent concentration. 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		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.

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



Western blot - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

This WB data was generated using the same anti-PAK2 antibody clone, EP796Y, in a different buffer formulation (cat# [ab76293](#)).

Lane 1: Wild-type HAP1 cell lysate (20 µg)

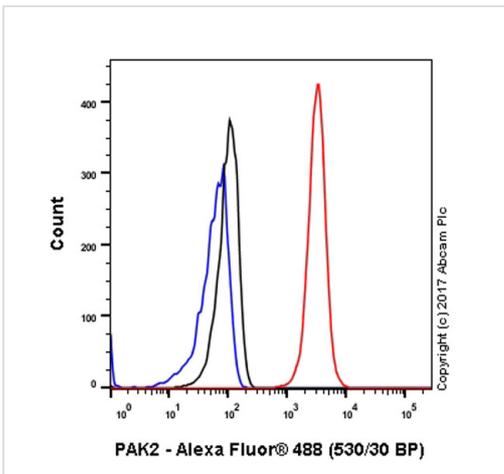
Lane 2: PAK2 knockout HAP1 cell lysate (20 µg)

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.

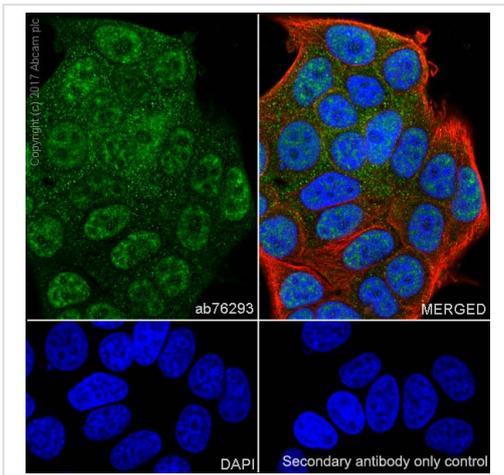
Unpurified [ab76293](#) was shown to specifically react with PAK2 when PAK2 knockout samples were used. Wild-type and PAK2 knockout samples were subjected to SDS-PAGE. [ab76293](#) and [ab8245](#) (loading control to GAPDH) were diluted 1/1000 and 1/10000 respectively and incubated overnight at 4°C.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



Flow Cytometry - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling PAK2 with purified [ab76293](#) at 1:20 dilution (10 µg/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).

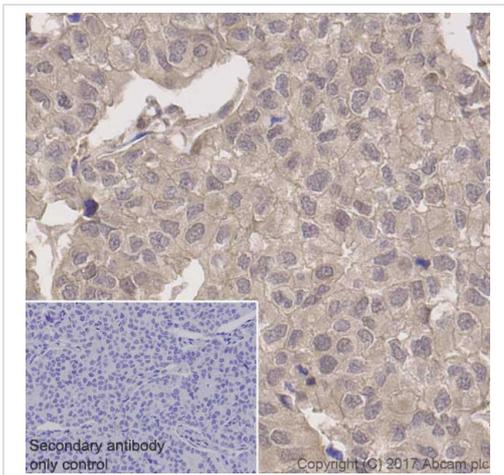
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab76293](#)).



Immunocytochemistry/ Immunofluorescence - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

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.

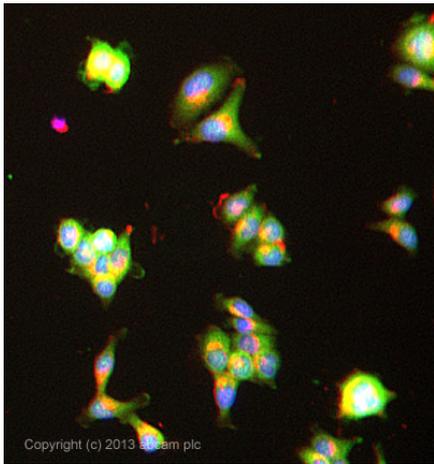
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab76293](#)).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

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.

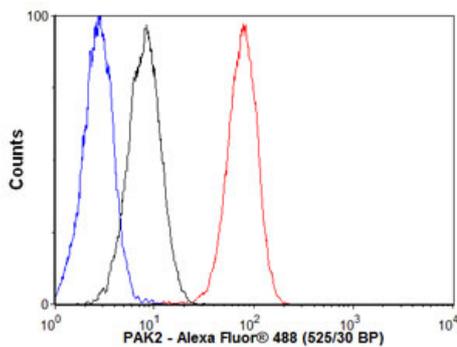
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab76293](#)).



Immunocytochemistry/ Immunofluorescence - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

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.

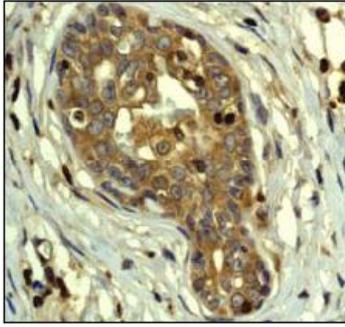
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab76293](#)).



Flow Cytometry - Anti-PAK2 antibody [EP796Y] - BSA and Azide free (ab227990)

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/1x10⁶ 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab76293](#)).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAK2 antibody [EP796Y]
- BSA and Azide free (ab227990)

This IHC data was generated using the same anti-PAK2 antibody clone, EP796Y, in a different buffer formulation (cat# [ab76293](#)).

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

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