abcam

Product datasheet

Anti-PAK2 antibody [EP796Y] ab76293

敲除验证 重组 RabMAb

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概述

产品名称	Anti-PAK2抗体[EP796Y]
描述	兔单克隆抗体[EP796Y] to PAK2
宿主	Rabbit
经测试应用	**适用于:** IP, ICC/IF, WB, IHC-P, Flow Cyt (Intra)
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human PAK2 aa 1-100 (N terminal). The exact sequence is proprietary. Database link: **Q13177**
阳性对照	WB: HeLa, NIH/3T3, RAW 264.7, Wild-type HEK-293T, PAK2 CRISPR-Cas9 edited HEK-293T and C6 cell lysates. IHC-P; Human breast carcinoma tissue IF/ICC: T47D cell line. Flow Cyt (intra): HeLa cells. IP: HeLa and NIH/3T3 cell lysates.
常规说明	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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EP796Y

同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab76293于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

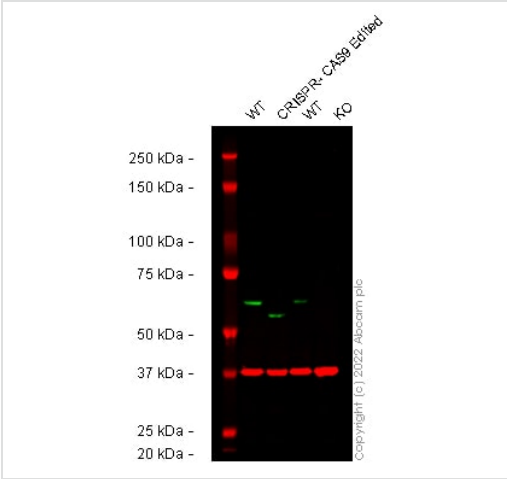
应用	Ab评论	说明
IP		1/30.
ICC/IF	★★★★☆ (1)	1/100 - 1/250.
WB	★★★★☆ (1)	1/5000. Predicted molecular weight: 58 kDa. **For unpurified use at 1/1000 - 1/2000.**
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. This antibody may not be suitable for IHC 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 (Intra)		1/20. For unpurified use at 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

靶标

功能	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.
组织特异性	Ubiquitously expressed. Higher levels seen in skeletal muscle, ovary, thymus and spleen.
序列相似性	Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. STE20 subfamily. Contains 1 CRIB domain. Contains 1 protein kinase domain.
翻译后修饰	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.

细胞定位	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.

图片



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

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

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : PAK2 CRISPR-Cas9 edited HEK-293T cell lysate
Lane 3 : Wild-type HeLa **ab255552** cell lysate
Lane 4 : PAK2 knockout HeLa **ab260287** cell lysate

Lysates/proteins at 20 µg per lane.

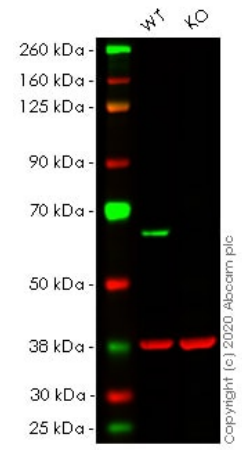
Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 58 kDa
Observed band size: 65 kDa

False colour image of Western blot: Anti-PAK2 antibody [EP796Y] staining at 1/5000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab76293 was shown to bind specifically to PAK2. A band was observed at 65 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in PAK2 CRISPR-Cas9 edited cell line **ab282648** (CRISPR-Cas9 edited cell lysate **ab283047**). The band observed in the CRISPR-Cas9 edited lysate lane below 65 kDa is likely to represent a truncated form of PAK2. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and PAK2 CRISPR-Cas9 edited HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary

antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



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

All lanes : Anti-PAK2 antibody [EP796Y] (ab76293) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PAK2 knockout HeLa cell lysate

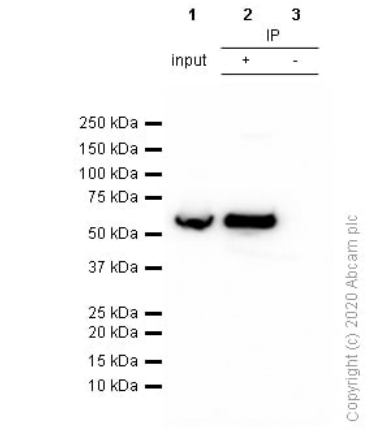
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 58 kDa
Observed band size: 60 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab76293 observed at 60 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab76293 was shown to react with PAK2 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab264814** (knockout cell lysate **ab257573**) was used. Wild-type HeLa and PAK2 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. ab76293 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.



Immunoprecipitation - Anti-PAK2 antibody [EP796Y] (ab76293)

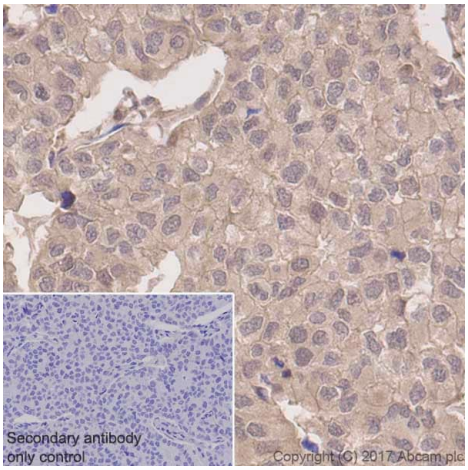
PAK2 was immunoprecipitated from 0.35 mg NIH/3T3 (Mouse embryonic fibroblast) cell lysate 10 µg with ab76293 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab76293 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.
Lane 1: NIH/3T3 (Mouse embryonic fibroblast) cell lysate 10 µg

Lane 2: ab76293 IP in NIH/3T3 cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab76293 in HeLa cell lysate

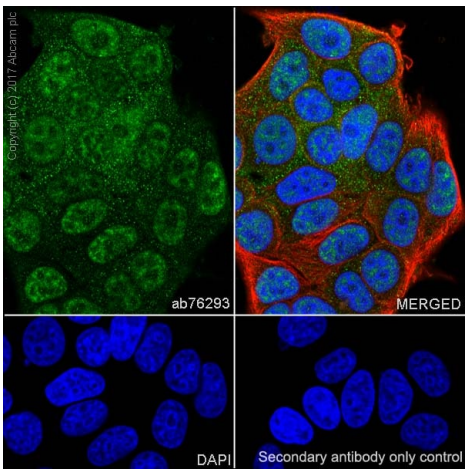
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 7 seconds



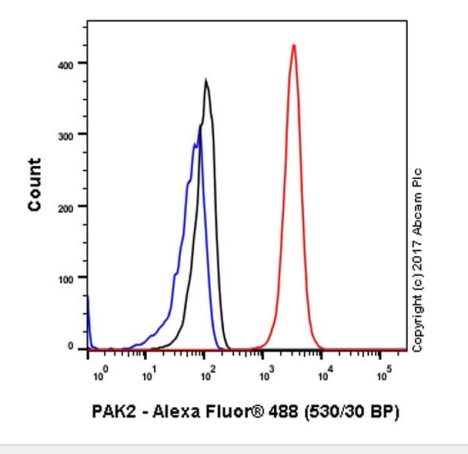
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAK2 antibody [EP796Y] (ab76293)

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.



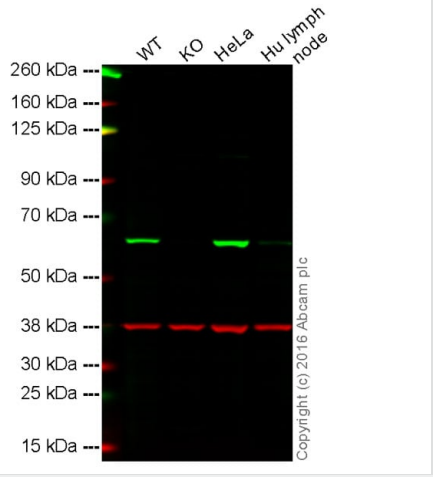
Immunocytochemistry/ Immunofluorescence - Anti-PAK2 antibody [EP796Y] (ab76293)

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.



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

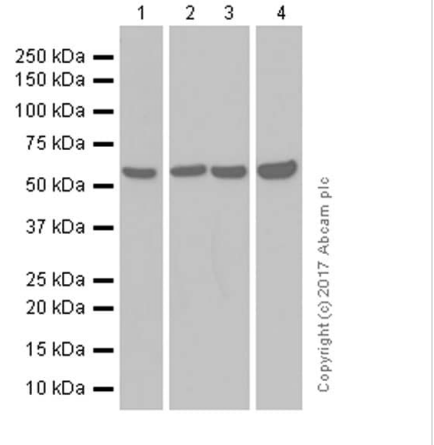
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling PAK2 with purified ab76293 at 1/20 dilution (10 ug/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilized with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488) secondary 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).



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.



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

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

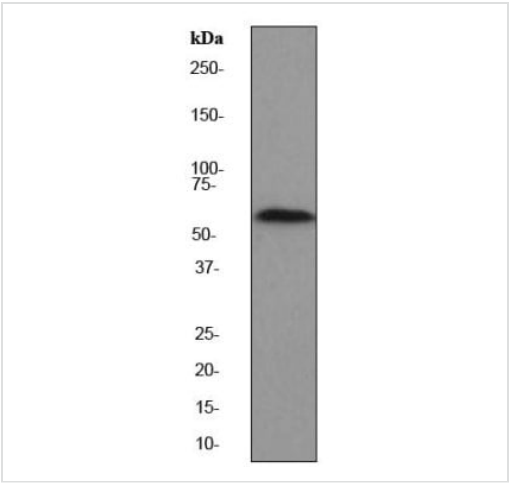
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 3 : RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysates
Lane 4 : C6 (Rat glial tumor glial cell) whole cell lysates

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



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

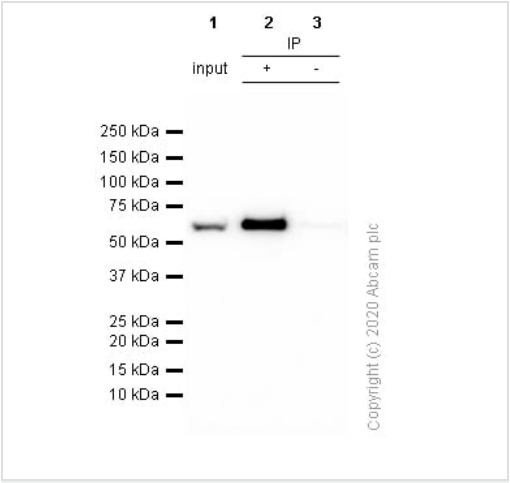
Anti-PAK2 antibody [EP796Y] (ab76293) at 1/2000 dilution (unpurified) + HeLa cell lysate at 10 µg

Secondary

HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 58 kDa

Observed band size: 61 kDa



Immunoprecipitation - Anti-PAK2 antibody [EP796Y] (ab76293)

PAK2 was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) cell lysate 10 µg with ab76293 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab76293 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

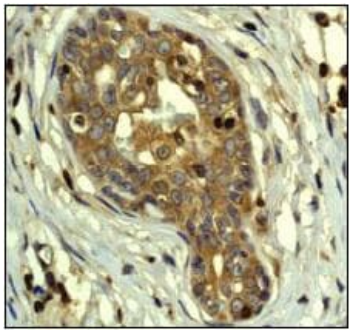
Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) cell lysate 10 µg

Lane 2: ab76293 IP in HeLa cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab76293 in HeLa cell lysate

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

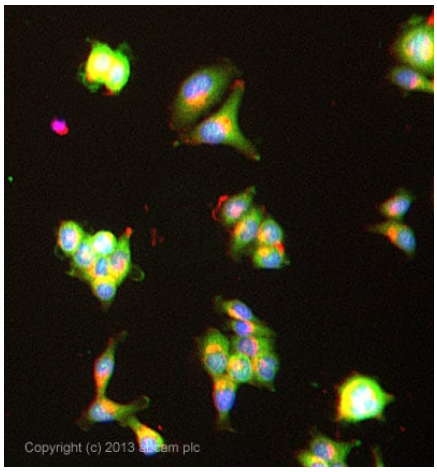
Exposure time: 7 seconds



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

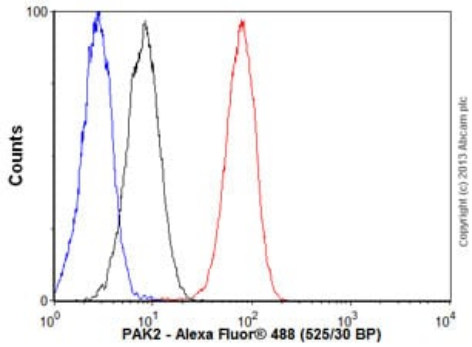
Unpurified ab76293, at a 1/100 dilution, staining PAK2 in paraffin embedded human breast carcinoma tissue by Immunohistochemistry.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



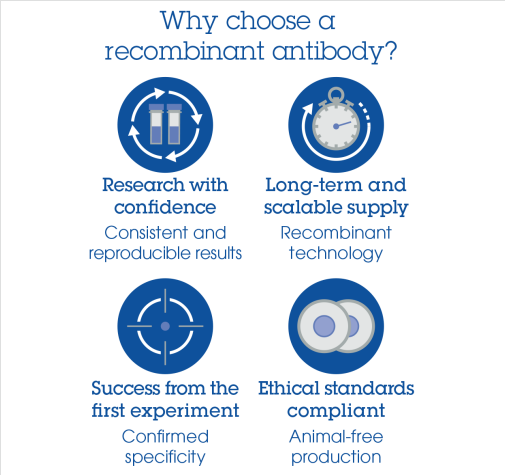
Immunocytochemistry/ Immunofluorescence - Anti-PAK2 antibody [EP796Y] (ab76293)

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 (Intracellular) - 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.



Anti-PAK2 antibody [EP796Y] (ab76293)

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