abcam

Product datasheet

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

重组 RabMAb

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概述

产品名称	Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154)**抗体**[EP656Y]
描述	**兔单克隆抗体**[EP656Y] to PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154)
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, IHC-P, IP, ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human PAK1 (phospho S144). The exact sequence is proprietary. Database link: **Q13153**
阳性对照	WB: MCF7, HeLa, RAW 264.7 and C6 cell lysates. IHC: Human liver carcinoma, mouse cerebral cortex, rat cerebral cortex. ICC/IF: HeLa cells. IP: HeLa cell lysate. Flow Cyt (intra): NIH/3T3 cell lysate.
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: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS
纯度	Protein A purified

克隆	单克隆
克隆编号	EP656Y
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab40795于以下的经测试应用**

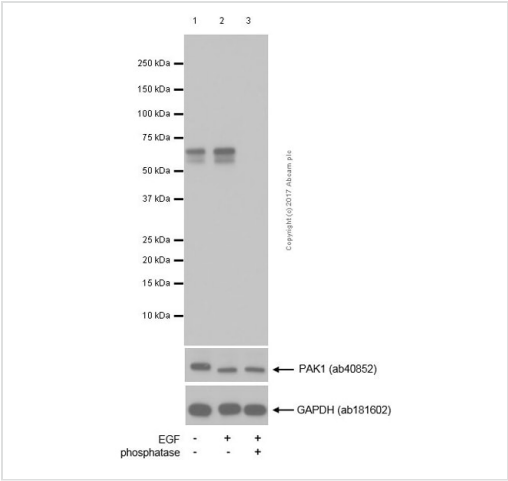
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/120. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (2)	1/10000 - 1/50000. Detects a band of approximately 66 kDa (predicted molecular weight: 65 kDa).
IHC-P	★★★☆☆ (1)	1/100 - 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/40.
ICC/IF	★★★★★ (1)	1/250 - 1/500.

靶标

细胞定位 PAK1: Cytoplasm. Cell junction > focal adhesion. Recruited to focal adhesions upon activation. PAK2: 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. PAK3: Cytoplasmic

图片



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

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

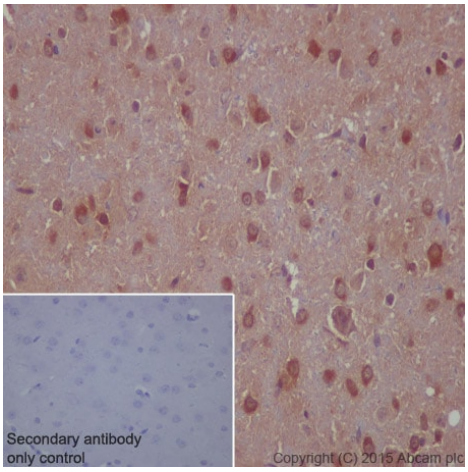
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 65 kDa
Observed band size: 55 kDa

Exposure time: 1 minute

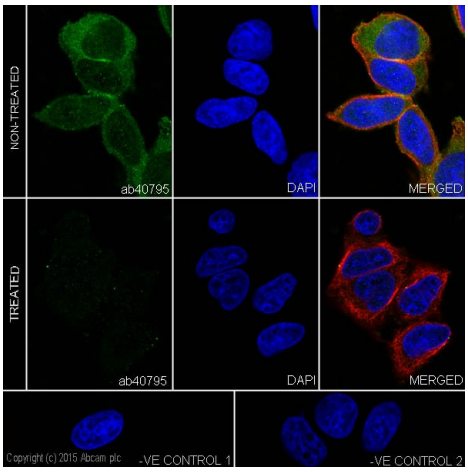
Blocking and dilution buffer: 5% NFDM/TBST.



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

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.

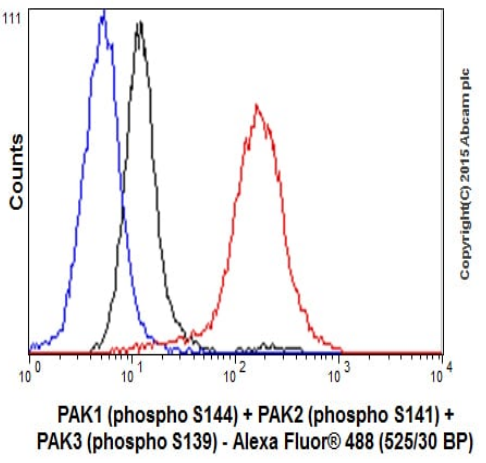
Negative control 1: PBS in place of primary



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

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**)

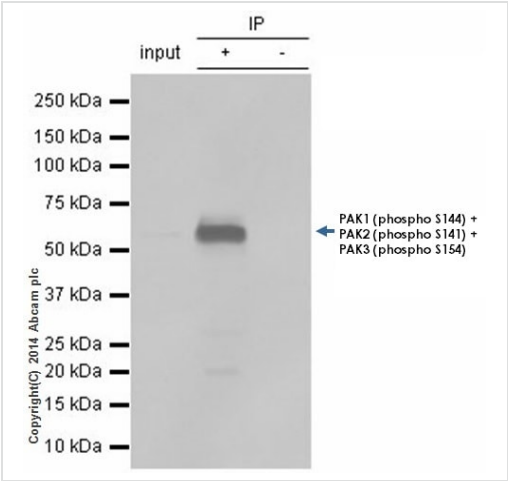


Flow Cytometry (Intracellular) - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

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)

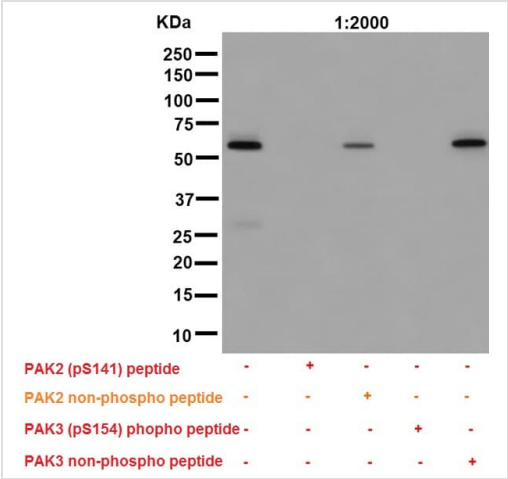
Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



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

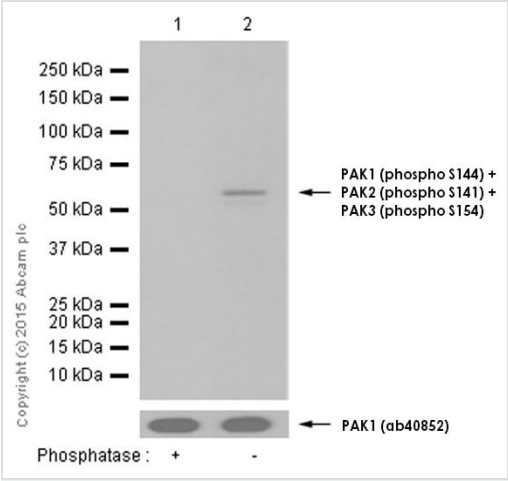


Western blot - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

All lanes : 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

Predicted band size: 65 kDa



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

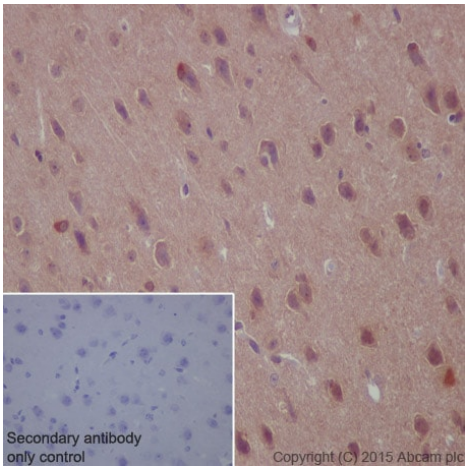
All lanes : Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795) at 1/50000 dilution

Lane 1 : C6 (rat glioma) whole cell lysate - treated with phosphatase
Lane 2 : C6 (rat glioma) whole cell lysate - untreated

Lysates/proteins at 10 µg per lane.

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

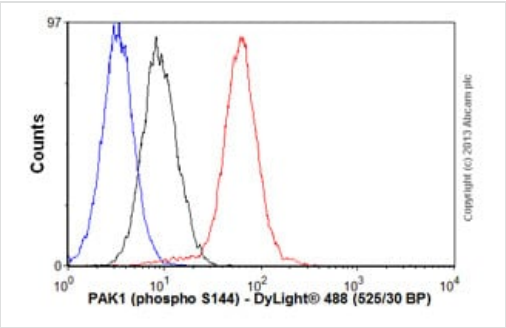
Predicted band size: 65 kDa



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

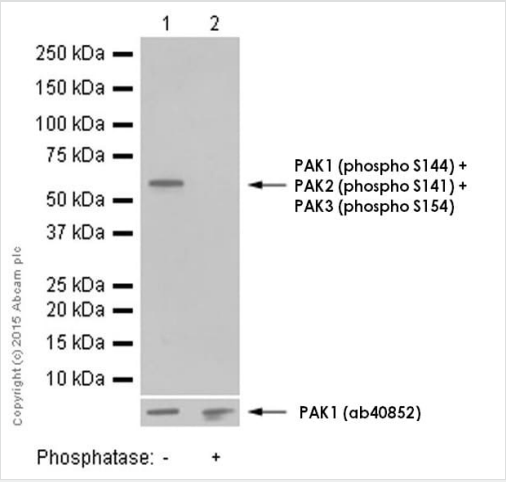
ab40795 staining PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) in mouse cerebral cortex tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA 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.



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

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.



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

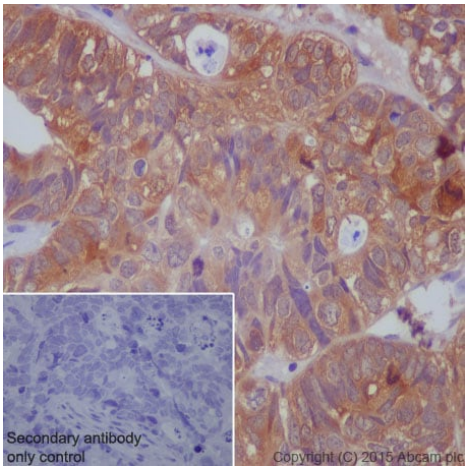
All lanes : Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795) at 1/10000 dilution

Lane 1 : HeLa whole cell lysate - untreated
Lane 2 : HeLa whole cell lysate - treated with phosphatase

Lysates/proteins at 10 µg per lane.

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

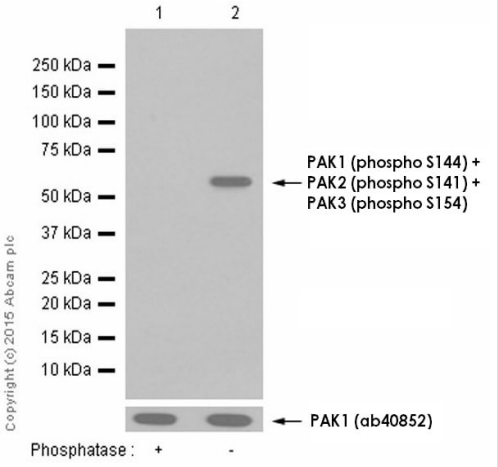
Predicted band size: 65 kDa



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

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.



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

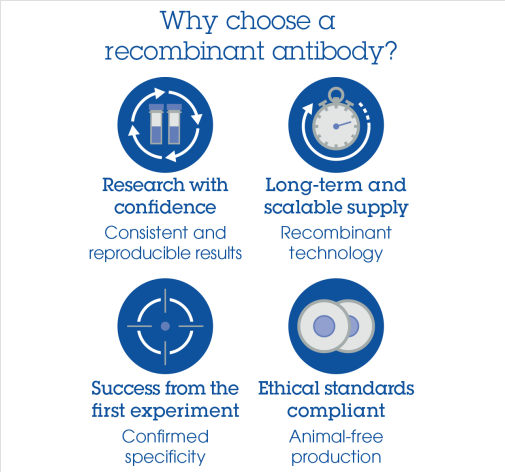
All lanes : Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795) at 1/10000 dilution

Lane 1 : RAW264.7 (mouse abelson murine leukemia virus-induced tumor) whole cell lysate - treated with phosphatase
Lane 2 : RAW264.7 (mouse abelson murine leukemia virus-induced tumor) whole cell lysate - untreated

Lysates/proteins at 10 µg per lane.

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

Predicted band size: 65 kDa



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

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