abcam

## Product datasheet

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

Recombinant RabMAb

4 Abreviews 20 References 12 Images

### Overview

| | |
|---|---|
| **Product name** | Anti-PAK1+PAK2+PAK3 (phospho S144 + S154 + S144 + S141) antibody [EP656Y] |
| **Description** | Rabbit monoclonal [EP656Y] to PAK1+PAK2+PAK3 (phospho S144 + S154 + S144 + S141) |
| **Host species** | Rabbit |
| **Specificity** | ab203958 recognises p21-activated kinase 1 (PAK1). |
| **Tested applications** | **Suitable for:** WB, IHC-P, IP, Flow Cyt, ICC/IF |
| **Species reactivity** | **Reacts with:** Mouse, Rat, Human |
| **Immunogen** | Synthetic peptide within Human PAK1 (phospho S144). The exact sequence is proprietary.<br>Database link: Q13153 |
| **Positive control** | WB: MCF7, HeLa, RAW264.7 and C6 cell lysates. IHC: Human liver carcinoma, mouse cerebral cortex, rat cerebral cortex. ICC/IF: HeLa cells. IP: HeLa cell lysate. Flow Cyt: NIH/3T3 cell lysate. |
| **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.<br>**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.**<br>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<br>Preservative: 0.01% Sodium azide<br>Constituents: 40% Glycerol, 0.05% BSA, 59% PBS |
| **Purity** | Protein A purified |

| | |
|---|---|
| **Clonality** | Monoclonal |
| **Clone number** | EP656Y |
| **Isotype** | IgG |

## Applications

Our Abpromise guarantee covers the use of **ab40795** in the following tested applications.

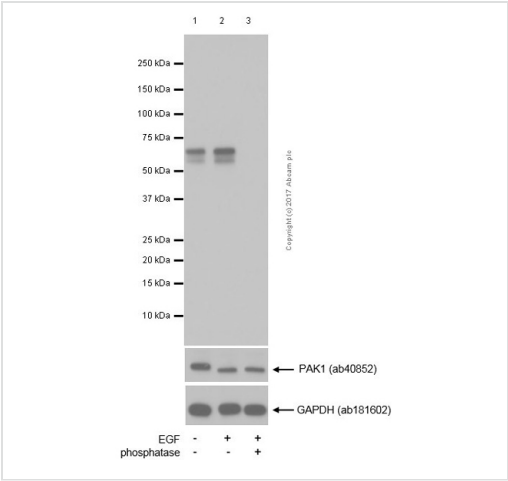
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|---|---|---|
| WB | ★★★★★ | 1/10000 - 1/50000. Detects a band of approximately 66 kDa (predicted molecular weight: 65 kDa). |
| IHC-P | ★★★☆☆ | 1/100 - 1/500. |
| IP | | 1/40. |
| Flow Cyt | | 1/120.<br>ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |
| ICC/IF | ★★★★★ | 1/250 - 1/500. |

## Target

| | |
|---|---|
| **Cellular localization** | PAK1: Cytoplasm. Cell junction > focal adhesion. Recruited to focal adhesions upon activation. PAK1+PAK2+PAK3: 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 |

## Images




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

**All lanes :** Anti-PAK1+PAK2+PAK3 (phospho S144 + S154 + S144 + S141) 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.

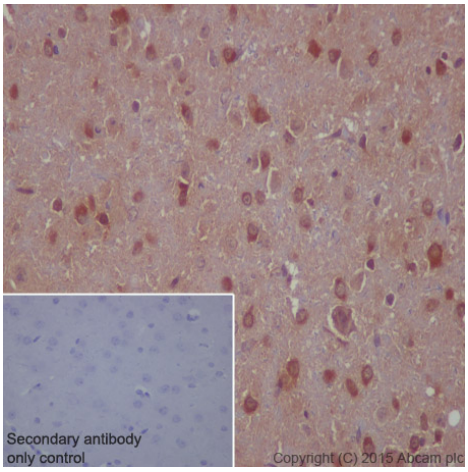
Lysates/proteins at 10 µg per lane.

**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
why is the actual band size different from the predicted?

**Exposure time:** 1 minute

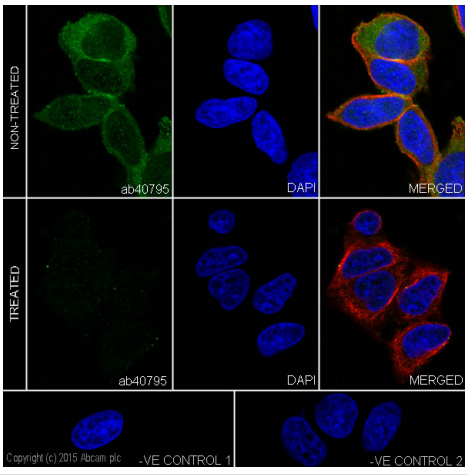
Blocking and dilution buffer: 5% NFDM/TBST.




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.

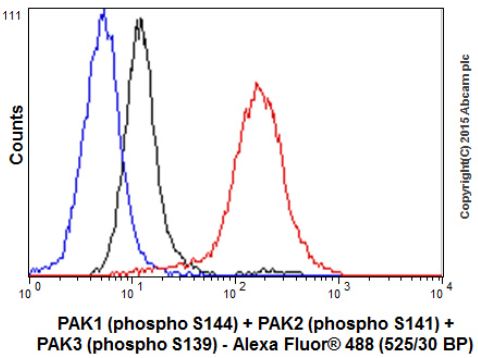
**Negative control 1:** PBS in place of primary




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 31I 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)

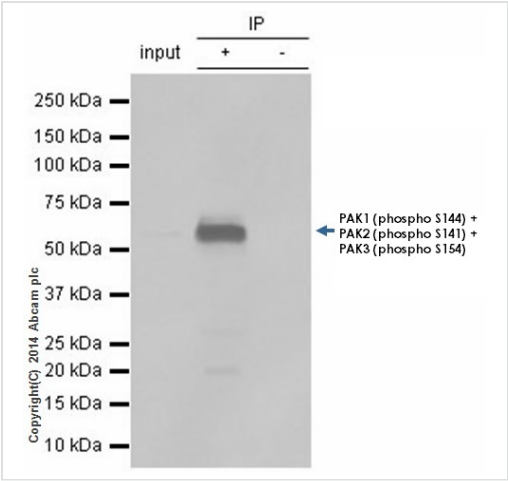



Flow Cytometry - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

ab40795 staining PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) in the human cell line NIH/3T3 (mouse embryo) by 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 Fluor® 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)



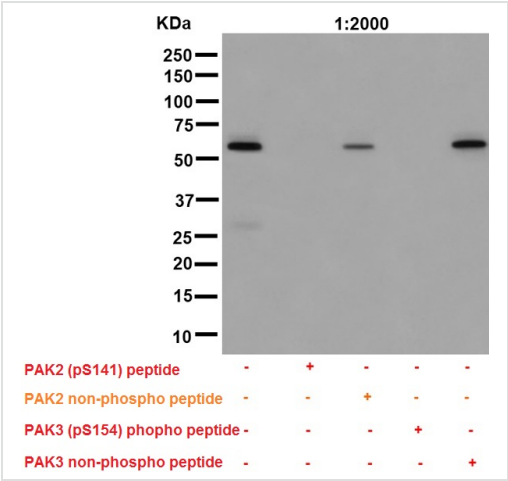

Immunoprecipitation - Anti-PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154) antibody [EP656Y] (ab40795)

ab40795 immunoprecipitating PAK1 (phospho S144) + PAK2 (phospho S141) + PAK3 (phospho S154). 10µg of 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 (human cervix adenocarcinoma) whole cell lysate (10ug)

**Lane 2:** HeLa (human cervix adenocarcinoma) 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+PAK2+PAK3 (phospho S144 + S154 + S144 + S141) antibody [EP656Y] (ab40795) at 1/2000 dilution

**Lane 1 :** HeLa cell lysate with None

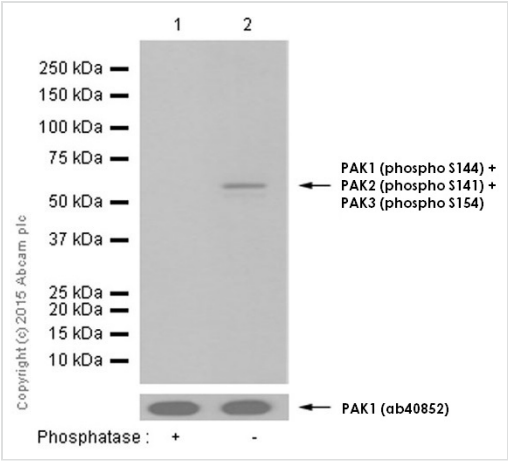
**Lane 2 :** HeLa cell lysate with PAK2 (pS141)

**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)

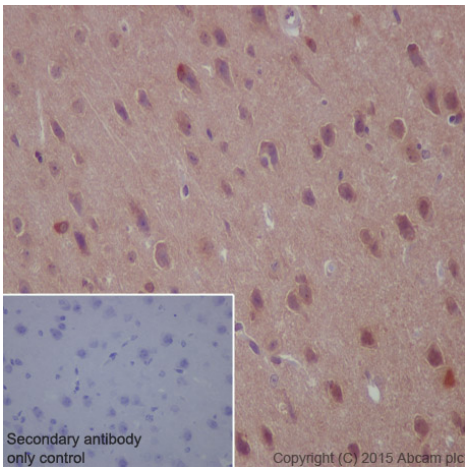
**All lanes :** Anti-PAK1+PAK2+PAK3 (phospho S144 + S154 + S144 + S141) antibody [EP656Y] (ab40795) at 1/50000 dilution

**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

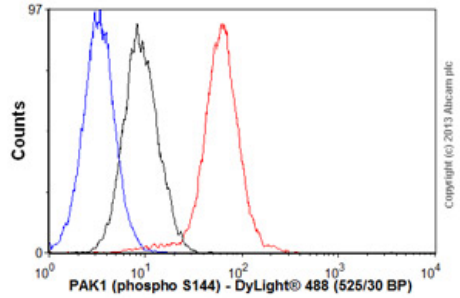
**Predicted band size:** 65 kDa




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.

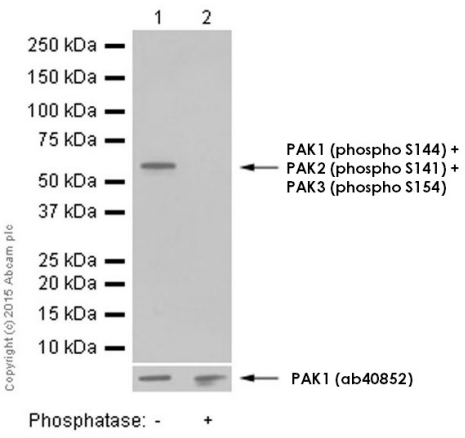
**Negative control 1:** PBS in place of primary antibody.




Flow Cytometry - 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/1x$10^6$ 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)

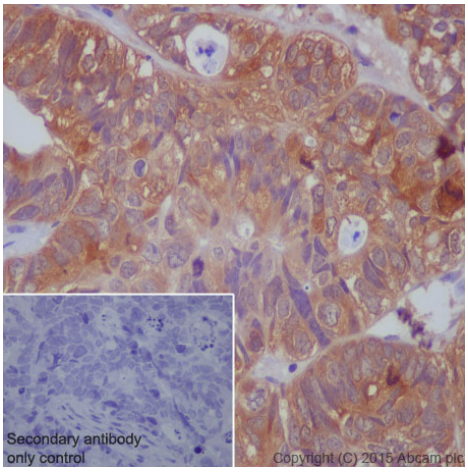
**All lanes :** Anti-PAK1+PAK2+PAK3 (phospho S144 + S154 + S144 + S141) antibody [EP656Y] (ab40795) at 1/10000 dilution

**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

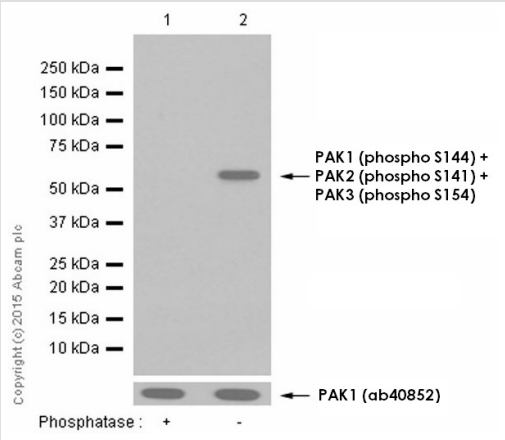
**Predicted band size:** 65 kDa




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+PAK2+PAK3 (phospho S144 + S154 + S144 + S141) 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



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