# abcam

#### Product datasheet

## Anti-FAK (phospho Y397) antibody [EP2160Y] ab81298

Recombinant RabMAb

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Overview

Product name Anti-FAK (phospho Y397) antibody [EP2160Y]

**Description** Rabbit monoclonal [EP2160Y] to FAK (phospho Y397)

Host species Rabbit

**Specificity** The activation of phosphorylation of FAK is reported to be related to developmental processes in

brain tissue (PMID: 14642275, PMID: 21118706). So expression level of phosphorylated modified FAK in normal brain is quite low causing not easy to be detected. We suggest optimizing experimental protocols (increasing lysate amount, using lower dilution or higher

sensitivity ECL substrate) to improve results.

Tested applications Suitable for: WB, ICC/IF

Unsuitable for: IHC-P

**Species reactivity** Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human, mouse and rat brain tissue, treated NIH/3T3 with 10mM Pervanadate for 60 min and

treated HeLa with 10mM Pervanadate for 60 min cell lysates. ICC/IF: SK-N-SH cell line.

General notes 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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

**Properties** 

Form Liquid

**Storage instructions** Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

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**Purity** Protein A purified

Clonality Monoclonal Clone number EP2160Y

Isotype ΙgG

#### **Applications**

#### Our Abpromise guarantee covers the use of ab81298 in the following tested applications. The Abpromise guarantee

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

Application	Abreviews	Notes
WB	<b>★★★★</b> <u>(1)</u>	1/1000. Predicted molecular weight: 119 kDa.
ICC/IF	<b>★★★☆☆(1)</b>	Use a concentration of 5 µg/ml.

**Application notes** Is unsuitable for IHC-P.

#### **Target**

Function	Non-receptor protein-tyrosine kinase implicated in signaling pathways involved in cell motility, proliferation and apoptosis. Activated by tyrosine-phosphorylation in response to either integrin clustering induced by cell adhesion or antibody cross-linking, or via G-protein coupled receptor (GPCR) occupancy by ligands such as bombesin or lysophosphatidic acid, or via LDL receptor occupancy. Microtubule-induced dephosphorylation at Tyr-397 is crucial for the induction of focal adhesion disassembly. Plays a potential role in oncogenic transformations resulting in increased kinase activity.
Tissue specificity	Expressed in all organs tested, in lymphoid cell lines, but most abundantly in brain.

Sequence similarities Belongs to the protein kinase superfamily. Tyr protein kinase family. FAK subfamily. Contains 1 FERM domain.

Contains 1 protein kinase domain.

**Domain** The first Pro-rich domain interacts with the SH3 domain of CRK-associated substrate (BCAR1)

and CASL.

The carboxy-terminal region is the site of focal adhesion targeting (FAT) sequence which

mediates the localization of FAK1 to focal adhesions.

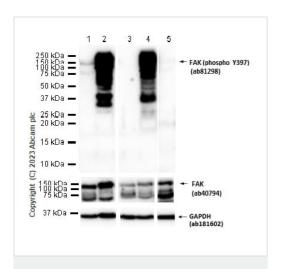
Phosphorylated on 6 tyrosine residues upon activation. Microtubule-induced dephosphorylation at Post-translational modifications

Tyr-397 could be catalyzed by PTPN11 and regulated by ZFYVE21. Dephosphorylated by

PTPN11 upon EPHA2 activation by its ligand EFNA1.

**Cellular localization** Cell junction > focal adhesion. Cell membrane. Constituent of focal adhesions.

### **Images**



Western blot - Anti-FAK (phospho Y397) antibody [EP2160Y] (ab81298)

**All lanes :** Anti-FAK (phospho Y397) antibody [EP2160Y] (ab81298) at 1/1000 dilution

**Lane 1 :** Untreated NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

Lane 2: NIH/3T3 treated with 10mM Pervanadate for 60 min whole cell lysate

**Lane 3 :** Untreated HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 4: HeLa treated with 10mM Pervanadate for 60 min whole cell lysate

Lane 5: Mouse brain tissue lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

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

**Predicted band size:** 119 kDa **Observed band size:** 119 kDa

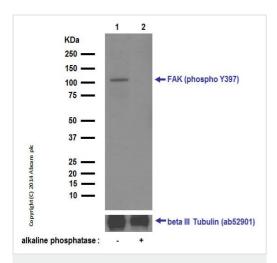
Exposure time: 40 seconds

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

The activation of phosphorylation of FAK is reported to be related to developmental processes in brain tissue (PMID: 14642275, PMID: 21118706). So expression level of phosphorylated modified FAK in normal brain is quite low causing not easy to be detected. We suggest optimizing experimental protocols (increasing lysate amount, using lower dilution or higher sensitivity ECL substrate) to improve results.

ab181602 has been used as a loading control.



Western blot - Anti-FAK (phospho Y397) antibody [EP2160Y] (ab81298)

**All lanes :** Anti-FAK (phospho Y397) antibody [EP2160Y] (ab81298) at 1/2000 dilution (purified)

Lane 1: Untreated mouse brain

Lane 2: Mouse brain treated with alkaline phosphatase

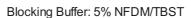
Lysates/proteins at 10 µg per lane.

#### **Secondary**

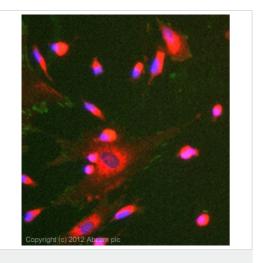
All lanes: HRP conjugated goat anti-rabbit lgG (H+L) at 1/1000

dilution

**Predicted band size:** 119 kDa **Observed band size:** 119 kDa

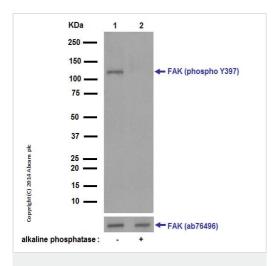


Dilution Buffer: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-FAK (phospho Y397) antibody [EP2160Y] (ab81298)

ICC/IF image of unpurified ab81298 stained SK-N-SH (human neuroblastoma cell line) 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 (ab81298, 10 $\mu$ g/ml) overnight at +4°C in PBS containing 1% BSA and 0.1% tween. The secondary antibody (green) was **ab96899**, DyLight® 488 goat anti-rabbit lgG (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 1.43 $\mu$ M.



Western blot - Anti-FAK (phospho Y397) antibody [EP2160Y] (ab81298)

**All lanes :** Anti-FAK (phospho Y397) antibody [EP2160Y] (ab81298) at 1/1000 dilution (purified)

Lane 1: Untreated rat brain

Lane 2: Rat brain treated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

#### **Secondary**

All lanes: HRP conjugated goat anti-rabbit lgG (H+L) at 1000 μg

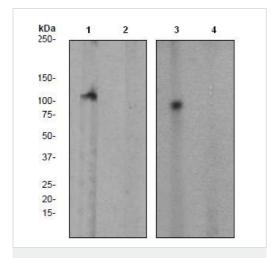
**Predicted band size:** 119 kDa **Observed band size:** 119 kDa

Blocking Buffer: 5% NFDM/TBST

Dilution Buffer: 5% NFDM/TBST

Immunocytochemistry/ Immunofluorescence - Anti-FAK (phospho Y397) antibody [EP2160Y] (ab81298) Unpurified ab81298 staining FAK (phospho Y397) in SK-N-SH (human neuroblastoma cell line) cells treated with anandamide (in water soluble emulsion) (ab120429), by ICC/IF. Increase in FAK (phospho Y397) expression correlates with increased concentration of anandamide (in water soluble emulsion), as described in literature.

The cells were incubated at  $37^{\circ}\text{C}$  for 5 minutes in media containing different concentrations of  $\underline{ab120429}$  (anandamide (in water soluble emulsion)) in water, fixed with 100% methanol for 5 minutes at -20°C and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab81298 (5  $\mu$ g/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody ( $\underline{ab96899}$ ) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue. Membranes are stained in red with WGA.



Western blot - Anti-FAK (phospho Y397) antibody [EP2160Y] (ab81298)

Immunocytochemistry/ Immunofluorescence - Anti-FAK (phospho Y397) antibody [EP2160Y] (ab81298) **All lanes**: Anti-FAK (phospho Y397) antibody [EP2160Y] (ab81298) at 1/2000 dilution (unpurified)

Lane 1: human brain tissue lysates, untreated.

Lane 2: human brain tissue lysates treated with AP.

Lane 3: rat brain tissue lysates, untreated.

Lane 4: rat brain tissue lysates treated with AP.

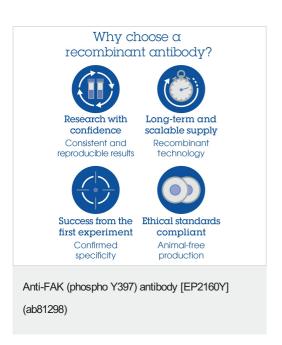
Lysates/proteins at 10 µg per lane.

#### **Secondary**

All lanes: HRP labelled goat anti rabbit. at 1/2000 dilution

**Predicted band size:** 119 kDa **Observed band size:** 119 kDa

Unpurified ab81298 staining FAK (phospho Y397) in SK-N-SH (human neuroblastoma cell line) cells treated with anandamide (ethanol solution) (ab120087), by ICC/IF. Increase in FAK (phospho Y397) expression correlates with increased concentration of anandamide (ethanol solution), as described in literature. The cells were incubated at 37°C for 10 minutes in media containing different concentrations of ab120087 (anandamide (ethanol solution)) in ethanol, fixed with 100% methanol for 5 minutes at -20°C and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab81298 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue. Membranes are stained in red with WGA.



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