# abcam

## Product datasheet

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

יעלאעבע RabMAb

★★★★ 2 Abreviews 118 References 画像数8

製品の概要

製品名 Anti-FAK (phospho Y397) antibody [EP2160Y]

製品の詳細 Rabbit monoclonal [EP2160Y] to FAK (phospho Y397)

由来種 Rabbit

特異性 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.

アプリケーション 適用あり: WB, ICC/IF

適用なし: IHC-P

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール 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.

特記事項 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.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

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

精製度 Protein A purified

**ポリ/モノ** モノクローナル

**クローン名** EP2160Y

アイソタイプ IgG

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab81298の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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.

追加情報 Is unsuitable for IHC-P.

#### ターゲット情報

機能

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.

組織特異性 Expressed in all organs tested, in lymphoid cell lines, but most abundantly in brain.

配列類似性 Belongs to the protein kinase superfamily. Tyr protein kinase family. FAK subfamily.

Contains 1 FERM domain.

Contains 1 protein kinase domain.

ドメイン 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.

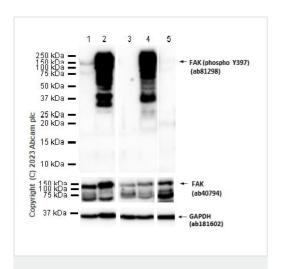
翻訳後修飾 Phosphorylated on 6 tyrosine residues upon activation. Microtubule-induced dephosphorylation at

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

PTPN11 upon EPHA2 activation by its ligand EFNA1.

細胞内局在 Cell junction > focal adhesion. Cell membrane. Constituent of focal adhesions.

#### 画像



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

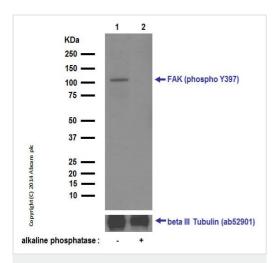
Exposure time: 40 seconds

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

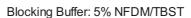
Lysates/proteins at 10 µg per lane.

#### **Secondary**

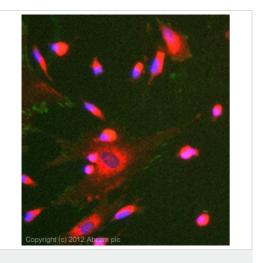
All lanes: HRP conjugated goat anti-rabbit lgG (H+L) at 1/1000

dilution

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

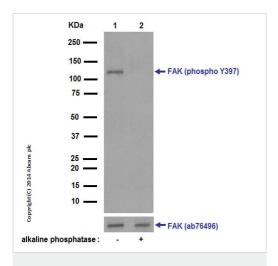


Dilution Buffer: 5% NFDM/TBST



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

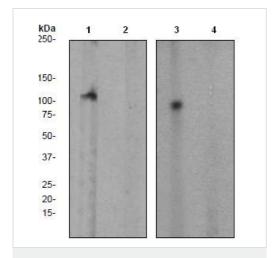
**Predicted band size:** 119 kDa **Observed band size:** 119 kDa

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.

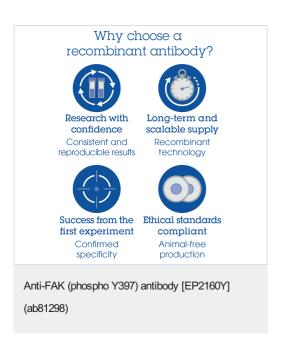
Lysates/proteins at 10 µg per lane.

#### **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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