Anti-FAK Antibody [SR46-04] - BSA and Azide free

HA750039

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 119 kDa
Clone number:	SR46-04

Description: Focal adhesion kinase was initially identified as a major substrate for the intrinsic protein tyrosine kinase activity of Src encoded pp60. The deduced amino acid sequence of FAK p125 has shown it to be a cytoplasmic protein tyrosine kinase whose sequence and structural organization are unique as compared to other proteins described to date. Localization of p125 by immunofluorescence suggests that it is primarily found in cellular focal adhesions leading to its designation as focal adhesion kinase (FAK). FAK is concentrated at the basal edge of only those basal keratinocytes that are actively migrating and rapidly proliferating in repairing burn wounds and is activated and localized to the focal adhesions of spreading keratinocytes in culture. Thus, it has been postulated that FAK may have an important in vivo role in the reepithelialization of human wounds. FAK protein tyrosine kinase activity has also been shown to increase in cells stimulated to grow by use of mitogenic neuropeptides or neurotransmitters acting through G protein coupled receptors.

Immunogen: Synthetic peptide within human FAK aa 700-740.

Positive control: A431 cell lysate, U-87 MG cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, C6 cell lysate, C2C12, PANC-1, SH-SY5Y, human brain tissue, mouse brain tissue, rat brain tissue, rat kidney tissue, rat spleen tissue.

Subcellular location: Cytoplasm, Nucleus, Cell membrane, Cell junction.

Database links: SwissProt: Q05397 Human | P34152 Mouse | O35346 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:50-1:100
IF-Tissue	1:50
IHC-P	1:200-1:1,000
FC	1:50
IP	Use at an assay dependent concentration.

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

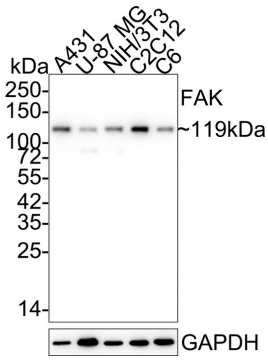

Fig1: Western blot analysis of FAK on different lysates with Rabbit anti-FAK antibody (HA750039) at 1/5,000 dilution.

Lane 1: A431 cell lysate (15 µg/Lane)
Lane 2: U-87 MG cell lysate (15 µg/Lane)
Lane 3: NIH/3T3 cell lysate (15 µg/Lane)
Lane 4: C2C12 cell lysate (15 µg/Lane)
Lane 5: C6 cell lysate (15 µg/Lane)

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

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750039) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

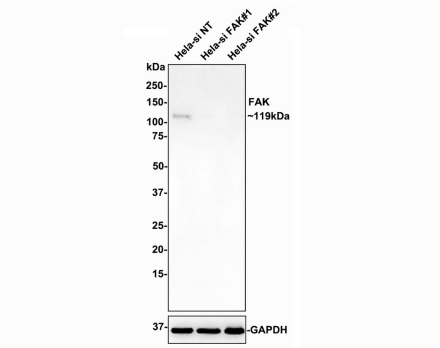

Fig2: Western blot analysis of FAK on different lysates with Rabbit anti-FAK antibody (HA750039) at 1/1,000 dilution.

Lane 1: Hela-si NT cell lysate (10 µg/Lane)
Lane 2: Hela-si FAK#1 cell lysate (10 µg/Lane)
Lane 3: Hela-si FAK#2 cell lysate (10 µg/Lane)

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

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

ET1602-25 was shown to specifically react with FAK in Hela-si NT cells. No bands were observed when Hela-si FAK samples were tested. Hela-si NT and Hela-si FAK samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1602-25, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

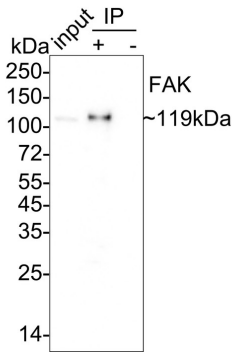

Fig3: FAK was immunoprecipitated in 0.2mg A431 cell lysate with HA750039 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA750039 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: A431 cell lysate (input)
Lane 2: HA750039 IP in A431 cell lysate
Lane 3: Rabbit IgG instead of HA750039 in A431 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 minutes; ECL: K1801

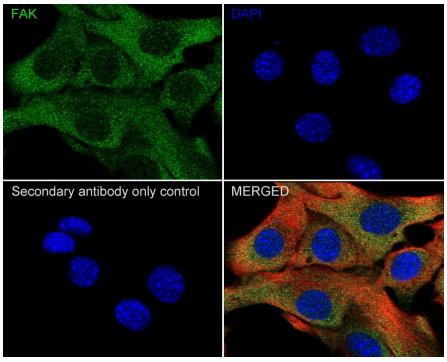

Fig4: Immunocytochemistry analysis of C2C12 cells labeling FAK with Rabbit anti-FAK antibody (HA750039) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-FAK antibody (HA750039) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

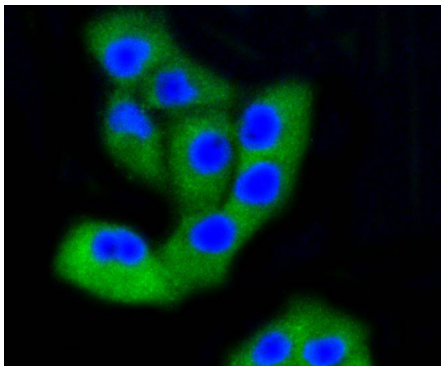

Fig5: ICC staining of FAK in PANC-1 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750039, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

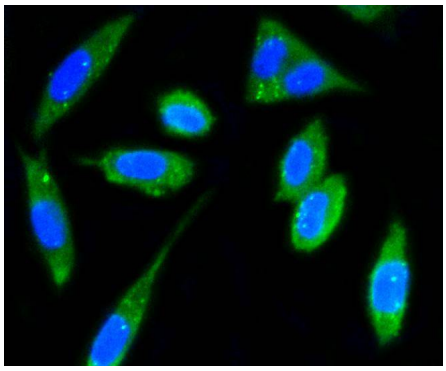

Fig6: ICC staining of FAK in SH-SY5Y cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750039, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

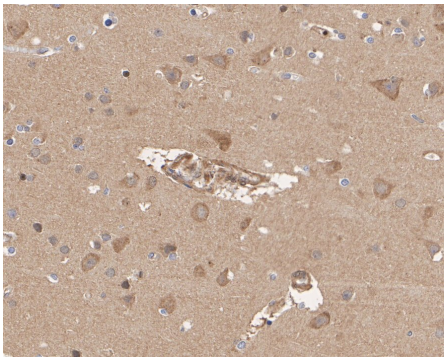

Fig7: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-FAK antibody (HA750039) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750039) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

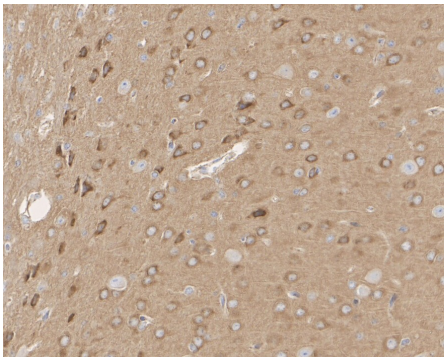

Fig8: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-FAK antibody (HA750039) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750039) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

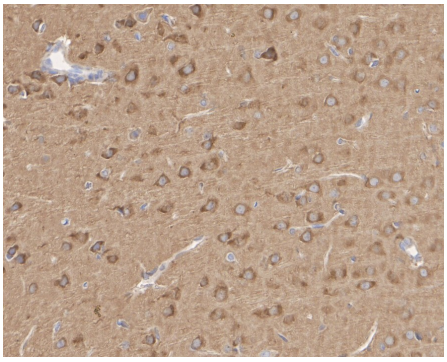

Fig9: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-FAK antibody (HA750039) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750039) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

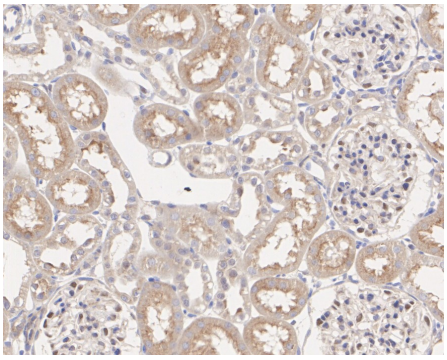

Fig10: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-FAK antibody (HA750039) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750039) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

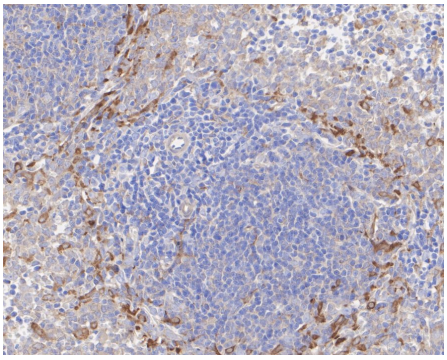

Fig11: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-FAK antibody (HA750039) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750039) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

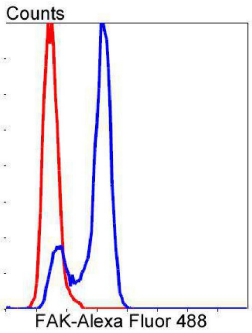

Fig12: Flow cytometric analysis of FAK was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (HA750039, 1/50) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; red).

Background References

1. Guckenberger DJ et al. High-density self-contained microfluidic KOALA kits for use by everyone. J Lab Autom 20:146-53 (2015).
2. Zhang Z et al. Upregulated periostin promotes angiogenesis in keloids through activation of the ERK 1/2 and focal adhesion kinase pathways, as well as the upregulated expression of VEGF and angiopoietin-1. Mol Med Rep 11:857-64 (2015).