Anti-Phospho-FAK (Y397) Antibody [SC54-07] - BSA and Azide free

HA750212

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 119 kDa
Clone number:	SC54-07

Description: Activation of integrins in the extracellular matrix (ECM) of eukaryotic cells promotes the formation of membrane adhesion complexes, known as focal adhesions, which can include cytoskeletal proteins and protein tyrosine kinases, such as focal adhesion kinase (FAK). Phosphorylation events occurring within focal adhesions influence numerous processes that include mitogenic signaling, cell survival, and cell motility. FAK is a non-receptor tyrosine kinase that is ubiquitously expressed and highly conserved between species. FAK is recruited by Integrin clusters and variably phosphorylated depending on the effector molecules present in the focal adhesion. Phosphorylation of FAK Tyr 397 decreases during serum starvation, contact inhibition, and cell cycle arrest, all conditions under which activating FAK Tyr 407 phosphorylation increases.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Tyr397 of human FAK.

Positive control: Hela cell lysate, PC-3 cell lysate, Hela, N2A, NIH/3T3, mouse spleen tissue, rat spleen tissue.

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

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

Recommended Dilutions:

WB	1:500-1:1,000
IF-Cell	1:50-1:100
IF-Tissue	1:50-1:100
IHC-P	1:200

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

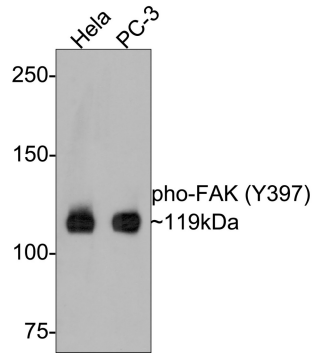

Fig1: Western blot analysis of Phospho-FAK (Y397) on different lysates with Rabbit anti-Phospho-FAK (Y397) antibody (HA750212) at 1/500 dilution.

Lane 1: Hela cell lysate
Lane 2: PC-3 cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 2 minutes;

6% 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 (HA750212) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

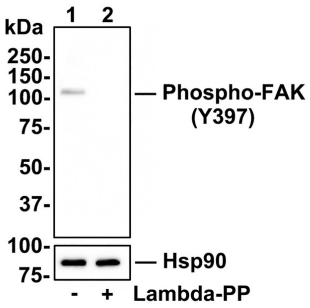

Fig2: Western blot analysis of Phospho-FAK (Y397) on Hela cell lysates.

Lane 1: Hela cells, whole cell lysate, 10ug/lane
Lane 2: Hela cells treated with 2.8ug/ul lambda-PP for 30 minutes, whole cell lysates, 10ug/lane

All lanes :
Anti-Phospho-FAK (Y397) antibody (HA750212) at 1:500 dilution. Anti-Hsp90 antibody (ET1605-56) at 1:10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

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

Blocking and diluting buffer: 5% BSA.

Exposure time: 30 seconds

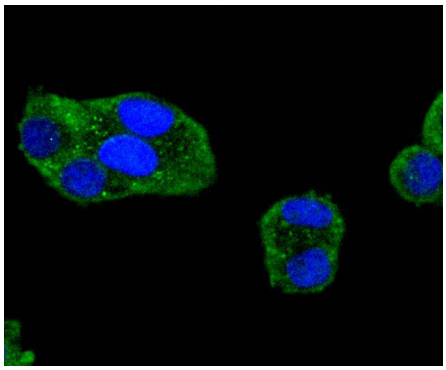

Fig3: ICC staining of Phospho-FAK (Y397) in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750212, 1/50) for 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).

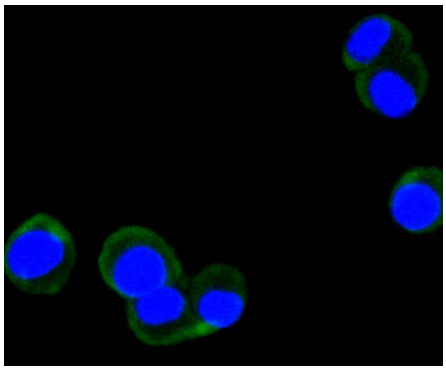

Fig4: ICC staining of Phospho-FAK (Y397) in N2A cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750212, 1/50) for 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).

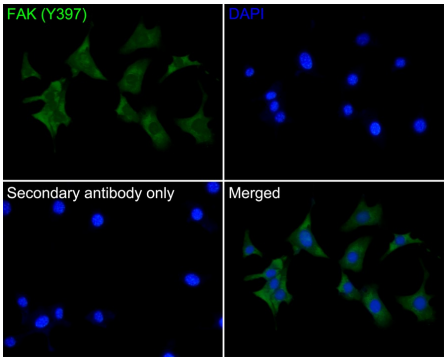

Fig5: Immunocytochemistry analysis of NIH/3T3 cells labeling Phospho-FAK (Y397) with Rabbit anti-Phospho-FAK (Y397) antibody (HA750212) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Phospho-FAK (Y397) antibody (HA750212) at 1/100 dilution in 2% negative goat serum 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.

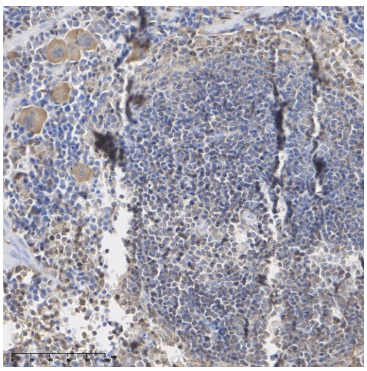

Fig6: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-Phospho-FAK (Y397) antibody (HA750212) at 1/200 dilution.

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 (HA750212) 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.

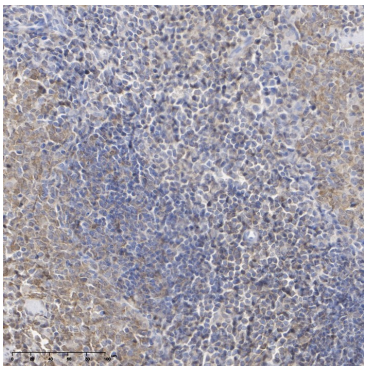

Fig7: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-Phospho-FAK (Y397) antibody (HA750212) at 1/200 dilution.

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 (HA750212) 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.

Background References

1. Kuo SW et al. Regulation of the fate of human mesenchymal stem cells by mechanical and stereo-topographical cues provided by silicon nanowires. Biomaterials 33:5013-22 (2012).
2. Lu H et al. IGFBP2/FAK pathway is causally associated with dasatinib resistance in non-small cell lung cancer cells. Mol Cancer Ther 12:2864-73 (2013).