

Product Information

Anti-phospho-FAK (pTyr⁸⁶¹)

Developed in Rabbit, Affinity Isolated Antibody

Product Number **F 9176**

Product Description

Anti-phospho-FAK (pTyr⁸⁶¹) was developed in rabbit using a synthetic phosphopeptide derived from the region of human FAK that contains tyrosine 861 as immunogen. The serum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity towards non-tyrosine phosphorylated FAK.

Anti-phospho-FAK (pTyr⁸⁶¹) specifically recognizes FAK (Focal Adhesion Kinase) phosphorylated at tyrosine 861 (125 kDa). The antibody detects human, mouse, and chicken FAK [pTyr⁸⁶¹]. Rat and frog FAK (100% homologous) have not been tested, but are expected to react. It has been used in immunoblotting and immunohistochemistry applications.

Integrins, adhesion receptors for extracellular matrix proteins, are involved in cell proliferation, apoptosis, migration and spreading. Integrin signaling is activated during epithelial-mesenchymal transdifferentiation (EMT) and cell migration, processes serving as models for carcinogenesis.^{1,2} Focal Adhesion Kinase (FAK) is a cytoplasmic protein tyrosine kinase involved in several integrin-mediated signaling pathways. These signaling cascades are initiated when an integrin interacts with components of the extracellular matrix triggering phosphorylation of FAK at multiple sites. Specifically FAK regulates cell differentiation, adhesion, migration and acceleration of the G1 to S phase transition of the cell cycle.^{2,5}

FAK autophosphorylation is critical for maximum adhesion and migration responses. Integrin-induced autophosphorylation of FAK at Tyr³⁹⁷ (the major autophosphorylation site) creates a binding site on FAK for Src-family kinases.⁶ Src then binds to and phosphorylates Tyr⁹²⁵, localized in the paxillin binding domain. This creates a Grb2 SH2-domain binding site and provides a link to the activation of the Ras signal transduction pathway.⁷ Tyr⁵⁷⁶ and Tyr⁵⁷⁷, located in the activation loop of the kinase domain of FAK, are also phosphorylated by Src. FAK's catalytic activity may be increased by phosphorylation of these residues.⁸ While phosphorylation of FAK at Tyr³⁹⁷ occurs even in sedentary cells and is localized exclusively at

cytoplasm, the phosphorylation of Tyr⁴⁰⁷ and Tyr⁸⁶¹ is induced during EMT and further augmented during cell migration.¹

In addition to the multiple tyrosine phosphorylation events involved in integrin signaling, FAK becomes heavily phosphorylated on serine residues when cells enter mitosis. At this time, tyrosine sites become dephosphorylated and inactivated.⁸ The mitosis-specific serine phosphorylation causes FAK modification and uncouples signal transduction pathways involving integrin, CAS and c-Src.⁹ FAK remains in an inactive state until post-mitosis, and the cells are able to detach from the extracellular matrix until cell division is complete. Studies of four major sites of serine phosphorylation (at amino acids 722, 840, 843 and 910), using phosphorylation-specific antibodies, have shown that Ser⁷²² is constitutively phosphorylated during the cell cycle and plays a role as a regulator of FAK-CAS interaction. In contrast, Ser⁸⁴³ and Ser⁹¹⁰ are mitosis-specific and exhibit increased phosphorylation during mitosis.⁸

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg²⁺ and Ca²⁺), pH 7.3, with 50% glycerol, 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

Store at -20 °C. Due to the presence of 50% glycerol the antibody will remain in solution. For extended storage, centrifuge the vial briefly before opening and prepare working aliquots. To ensure accurate dilutions mix gently, remove excess solution from pipette tip with clean absorbent paper, pipette slowly. The antibody is stable for at least six months when stored appropriately. Working dilutions should be discarded if not used within 12 hours.

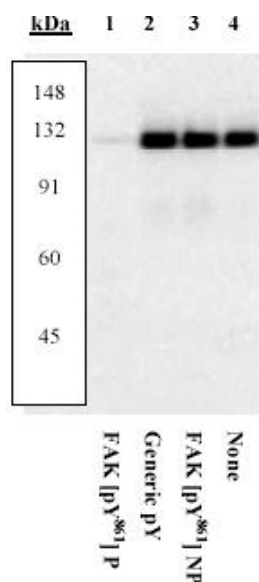
Product Profile

The supplied antibody is sufficient for 10 immunoblots. A recommended working dilution of 1:1000 is determined by immunoblotting.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Peptide Competition

1. Extracts prepared from CEF cells expressing FAK and plated on fibronectin were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:
Lane 1 immunogen
Lane 2 a generic phosphotyrosine containing peptide
Lane 3 non phosphorylated peptide corresponding to the immunogen
Lane 4 no peptide
4. After preincubation membranes were incubated with Fak [pTyr⁸⁶¹] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')₂ anti-rabbit IgG alkaline phosphatase and signals were detected using the Pierce SuperSignal[®] method.



The data shows that only the peptide corresponding to the immunogen blocks the antibody signal, which confirms the specificity of the antibody.

References

1. Nakamura, K., et al., Different modes and qualities of tyrosine phosphorylation of Fak and Pyk2 during epithelial-mesenchymal transdifferentiation and cell migration: analysis of specific phosphorylation events using site-directed antibodies., *Oncogene*, **21**, 2626-2635 (2001).
2. Zhao, J.H., et al., Regulation of the cell cycle by focal adhesion kinase., *J. Cell Biol.*, **143**, 1997-2008 (1998).
3. Cary, L.A. and Guan, J.L., Focal adhesion kinase in integrin-mediated signaling., *Front. Biosci.*, **4**, D102-113 (1999).
4. Owen, J.D., et al., Induced focal adhesion kinase (FAK) expression in FAK-null cells enhances cell spreading and migration requiring both auto- and activation loop phosphorylation sites and inhibits adhesion-dependent tyrosine phosphorylation of Pyk2., *Mol. Cell Biol.*, **19**, 4806-4818 (1999).
5. Ridyard, M.S. and Sanders, E.J., Potential roles for focal adhesion kinase in development., *Anat. Embryol. (Berlin)*, **199**, 1-7 (1999).
6. Ruest, P.J., et al., Phosphospecific antibodies reveal focal adhesion kinase activation loop phosphorylation in nascent and mature focal adhesions and requirement for the autophosphorylation site., *Cell Growth Differ.*, **11**, 41-48 (2000).
7. Schlaepfer, D.D., and Hunter, T., Evidence for in vivo phosphorylation of the Grb2 SH2-domain binding site on focal adhesion kinase by Src-family protein-tyrosine kinases., *Mol. Cell. Biol.*, **16**, 5623-5633 (1996).
8. Maa, M.C. and Leu, T.H., Vanadate-dependent FAK activation is accomplished by the sustained FAK Tyr-576/577 phosphorylation., *Biochem. Biophys. Res. Commun.*, **251**, 344-349 (1998).
9. Yamakita, Y., et al., Dissociation of FAK/p130(CAS)/c-Src complex during mitosis: role of mitosis-specific serine phosphorylation of FAK., *J. Cell Biol.*, **144**, 315-324 (1999).

SuperSignal is a registered trademark of Pierce Biotechnology, Inc.

AH,PHC 04/05-1

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.