3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Anti-phospho-FAK (pTyr^{925})
Developed in Rabbit, Affinity Isolated Antibody

Product Number **F 9426**

Product Description

Anti-phospho-FAK (pTyr^{925}) was developed in rabbit using as immunogen a synthetic phosphopeptide derived from the region of FAK that contains tyrosine 925. The serum is affinity purified using epitope-specific affinity chromatography. The antibody is preabsorbed to remove any reactivity towards: (i) a non-phosphorylated FAK enzyme and (ii) corresponding region of phosphorylated Pyk2/CAKβ enzyme (a FAK-related enzyme).

Anti-phospho-FAK (pTyr^{925}) specifically recognizes FAK (Focal Adhesion Kinase) phosphorylated at tyrosine 925 (125 kDa). The antibody detects human, mouse, rat, chicken and frog FAK (pTyr^{925}). It has been used in immunoblotting 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.[2,3,4,5]

FAK autophosphorylation is critical for maximum adhesion and migration responses. Integrin-induced autophosphorylation of FAK at Tyr-397 (the major autophosphorylation site) creates a binding site on FAK for Src-family kinases.[8] Src then binds to and phosphorylates Tyr-925, 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.[6] Tyrosine 576 and 577, 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.[7]

While phosphorylation of FAK at Tyr-397 occurs even in sedentary cells and is localized exclusively at cytoplasm, the phosphorylation of Tyr-407 and Tyr-861 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.[7] 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 serine 722 is constitutively phosphorylated during the cell cycle and plays role as a regulator of FAK-CAS interaction. In contrast, serine 843 and 910 are mitosis-specific and exhibit increased phosphorylation during mitosis.[7]

Reagent

Anti-phospho-FAK (pTyr^{925}) is supplied as a solution in phosphate buffered saline, pH 7.3, with no preservatives added.

Storage/Stability

Store at –70 °C. For extended storage, upon initial thawing, freeze in working aliquots. Avoid repeated freezing and thawing to prevent denaturing the antibody. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months when stored appropriately.

Product Profile

A recommended working concentration of 0.1 to 0.5 µg/ml is determined by immunoblotting using NIH3T3 cells treated with PDGF. Data demonstrates that only phosphopeptide corresponding to the region containing tyrosine 925 blocks the antibody signal, which confirms the specificity of the Anti-phospho-FAK (pTyr^{925}) for this phoshosphorylated residue.

References

1. Nakamura, K., et al., Oncogene, **21**, 2626-2635 (2001).
2. Zhao, J.H., et al., J. Cell Biol., **143**, 1997-2008 (1998).
3. Cary, L.A. and Guan, J.L., Front. Biosci., **4**, D102-113 (1999).
4. Owen, J.D., et al., Mol. Cell Biol., **19,** 4806-4818 (1999).
5. Ridyard, M.S. and Sanders, E.J., Anat. Embryol. (Berlin), **199**,1-7 (1999).
6. Schlaepfer, D.D. and Hunter, T., Mol. Cell. Biol., **16,** 5623-5633 (1996).
7. Maa, M.C. and Leu, T.H., Biochem. Biophys. Res. Commun., **251**, 344-349 (1998).
8. Ruest, P.J., at al., Cell Growth Differ., **11**, 41-48 (2000).
9. Yamakita, Y., et al., J. Cell Biol., **144**, 315-324 (1999).

AH 8/01