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Product Information

Anti-phospho-FAK (pTyr^{407})
produced in rabbit, affinity isolated antibody

Catalog Number **F8051**

Product Description

Anti-phospho-FAK (pTyr^{407}) was produced in rabbit using as immunogen a synthetic phosphopeptide derived from the region of human FAK that contains tyrosine 407. The sequence is conserved in mouse, rat and chicken. The serum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward: (i) a non-phosphorylated FAK enzyme, (ii) phosphotyrosine, irrespective of the sequence.

Anti-phospho-FAK (pTyr^{407}) recognizes FAK (Focal Adhesion Kinase) phosphorylated at tyrosine 407 (125 kDa). The antibody detects human, mouse, rat, chicken, and frog FAK (pTyr^{407}). Mouse, rat, and chicken FAK are 100% homologous with human FAK and frog FAK is 92% homologous with human FAK. The antibody may be 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.[2-5]

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.[6] 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.[7] Tyr^{576} and Tyr^{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.[8] 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.[9] The mitosis-specific serine phosphorylation causes FAK modification and uncouples signal transduction pathways involving integrin, CAS and c-Src.[10] 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 (amino acids 722, 840, 843, and 910), using phosphorylation-specific antibodies, have shown that Ser^{722} is constitutively phosphorylated during the cell cycle and plays role as a regulator of FAK-CAS interaction. In contrast, Ser^{843} and Ser^{910} are mitosis-specific and exhibit increased phosphorylation during mitosis.[9]

Reagent

Supplied as a solution in Dulbecco's phosphate buffered saline (without magnesium and calcium), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL bovine serum albumin (IgG, protease free) as a carrier and 0.05% sodium azide as a preservative.

Storage/Stability

Store at –20 °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.

Product Profile

Immunoblotting: a starting antibody dilution of 1:1000 is recommended. Chicken embryo fibroblast (CEF) cells expressing FAK protein and plated on fibronectin may be used as a positive control. Data demonstrates that only phosphopeptide corresponding to the region containing tyrosine 407 blocks the antibody signal, which confirms the specificity of the Anti-phospho-FAK ($pTyr^{407}$) for this phosphorylated residue.

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

References

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