

## Product Information

### Anti-phospho-FAK (pTyr<sup>577</sup>)

Developed in Rabbit, Affinity Isolated Antibody

Product Number **F 8926**

#### Product Description

Anti-phospho-FAK (pTyr<sup>577</sup>) was developed in rabbit using a synthetic phosphopeptide derived from the region of FAK that contains tyrosine 577 as immunogen. The serum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity towards the non-tyrosine phosphorylated FAK protein.

Anti-phospho-FAK (pTyr<sup>577</sup>) specifically recognizes FAK (Focal Adhesion Kinase) phosphorylated at tyrosine 577 (125 kDa). The antibody detects human, mouse and frog FAK (pTyr<sup>577</sup>). It has been used in immunoblotting applications.<sup>1</sup>

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.<sup>1,2</sup> 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.<sup>2,3,4,5</sup>

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.<sup>8</sup> 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.<sup>6</sup> 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.<sup>7</sup> 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.<sup>1</sup>

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.<sup>7</sup> The mitosis-specific serine phosphorylation causes FAK modification and uncouples signal transduction pathways involving integrin, CAS and c-Src.<sup>9</sup> 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.<sup>7</sup>

#### Reagent

Anti-phospho-FAK (pTyr<sup>577</sup>) is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg<sup>2+</sup> and Ca<sup>2+</sup>), 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. 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 amount of reagent supplied is sufficient for 10 immunoblots.

A recommended working dilution 1:1000 is determined by immunoblotting using NIH3T3 cells treated with PDGF. Data demonstrates that only phosphopeptide corresponding to the region containing tyrosine 577 blocks the antibody signal, which confirms the specificity of the Anti-phospho-FAK (pTyr<sup>577</sup>) for this phosphorylated residue.

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

### References

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