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ProductInformation

Anti-phospho-Paxillin [pTyr^{31}]
Developed in Rabbit, Affinity isolated antibody

Product Number **P 6368**

Product Description

Anti-phospho-Paxillin [pTyr^{31}] is developed in rabbit using as immunogen a synthetic phosphorylated peptide derived from the region of paxillin that contains a phosphate on tyrosine 31. This region is conserved between human and chick. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preabsorbed to remove any reactivity towards a non-phosphorylated paxillin. Anti-phospho-Paxillin [pTyr^{31}] specifically recognizes endogenous and expressed forms of human wild type paxillin (68 kDa) phosphorylated on tyrosine 31. It does not crossreact with recombinant Y31F mutant recombinant proteins. It has been used in immunoblotting and immunocytochemistry applications.[1-4]

Paxillin is a cytoskeletal component found in the focal adhesions at the ends of actin stress fibers, but not in adherens junctions of the cells. Paxillin interacts with several proteins including members of the *src* family of tyrosine kinases, the transforming protein *v-cr*k, the cytoskeletal protein vinculin, and the tyrosine kinase, focal adhesion kinase (FAK). This interaction has suggested a function for paxillin as a molecular adaptor, responsible for the recruitment of structural and signaling molecules to focal adhesions. The paxillin molecule has a single binding site for vinculin, and at least two binding sites for FAK, that are separated by an intervening sequence of 100 amino acids.[5]

Phosphorylation of multiple tyrosines in paxillin is necessary for the proper function of paxillin and is involved in the temporospatial regulation of focal adhesion formation and actin cytoskeletal organization in motile cells. Formation of a complex between paxillin and Fak appears to be required for maximal phosphorylation in response to cell adhesion in fibroblasts, although Fak may not be the sole tyrosine kinase that phosphorylates paxillin. It may be that Fak directs paxillin phosphorylation by recruiting Src family kinases. Pyk2, another tyrosine kinase acting in integrin signaling, has also been shown to associate with paxillin and becomes highly phosphorylated and activated during epithelial-mesenchymal trans-differentiation (EMT).[4,6,7] Pyk2 may be responsible for the phosphorylation of Tyr-31 and Tyr-118 of paxillin.[1]

The function of different tyrosine phosphorylation sites in paxillin has been investigated using the phospho-specific antibodies. Tyrosines 31 and 181 are phosphorylated after the cell adhesion to the fibronectin matrix, and this phosphorylation is regulated in cell adhesion-dependent manner.[8] Recent studies indicate that phosphopeptides with pY-*X*-*X*-P (where pY indicates the phosphorylated tyrosine residue) motifs can bind to Crk-SH2 domains. Human paxillin has three of these motifs at tyrosine positions 31, 118, and 181. Decreased binding of CrkL-SH2 to paxillin was observed with mutant tyrosines at positions 31 and 118, but not 181. CrkL may link BCR/ABL and paxillin and thereby contribute to the adhesion defects of chronic myeloid leukemia progenitor cells.[9]

Reagent

Anti-phospho-Paxillin [pTyr^{31}] is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 with 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide. The amount of the reagent is sufficient for 10 blots.

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 –70 °C. For extended storage, upon initial thawing, freeze in working aliquots. Do not store in frost-free freezers. 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 0.3 to 1.5 µg/ml is determined by immunoblotting using NMµMG cells treated with TGFβ or chick embryo fibroblasts.

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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