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ProductInformation

ANTI-PHOSPHO-PYK2 (pTyr^{881})
Developed in Rabbit, Affinity Isolated Antibody

Product Number **P 6864**

Product Description

Anti-phospho-Pyk2 (pTyr^{881}) (proline-rich/Ca-activated tyrosine kinase) was developed in rabbit using as immunogen a synthetic phosphorylated peptide derived from the region of human Pyk2 that contains tyrosine^{881}. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preabsorbed to remove any reactivity toward the non-tyrosine phosphorylated Pyk2 protein.

Anti-phospho-Pyk2 (pTyr^{881} detects human and rat Pyk2 (pTyr^{881}) (approx. 116 kDa). It has been used in immunoblotting applications.

Protein Tyrosine Kinases (PTKs) are critical components of the signaling pathways that control cell growth, differentiation, apoptosis, metabolism, cell cycle regulation and cytoskeletal function. The Focal Adhesion PTK subfamily consists of two closely related cytoplasmic tyrosine kinases: Fak (Focal Adhesion Kinase, pp^{125FAK}) and Pyk2 (proline-rich kinase 2) also designated CAKβ (cell adhesion kinase β), RAFTK (related adhesion focal tyrosine kinase), Fak2 (focal adhesion kinase 2) and CADTK (calcium-dependent tyrosine kinase).[1-5]

Fak and Pyk2 share about 45% overall sequence identity and 60 % identity in the centrally located catalytic domain.[1,2] Both lack a transmembrane region, myristylation sites and SH2 and SH3 domains. Whereas Fak is rather ubiquitous, Pyk2 is primarily expressed in the central nervous system and in cells derived from hematopoietic lineages. Fak and Pyk2 are coexpressed in mesenchymal, epithelial, endothelial and neural cells. Pyk2 is more prominent than Fak in unseparated peripheral blood leukocytes. It is found as a short isoform Pyk2H in normal circulating monocytes, B, T and NK cells.[6,7].

Pyk2 is expressed in a number of tissues and cells, such as vascular endothelial cells, osteoclasts, neuronal cells, neonatal cardiomyocytes. Subcellular localization of Pyk2 may vary in different cell types. Pyk2 has been detected in cell-cell contacts, at focal adhesion-like structures and podosomes, cytoplasmic perinuclear region, in association with actin filaments and diffusely distributed in the cytoplasm.[2,5,8] Various extracellular stimuli causing increases in the intracellular calcium level and activation of Protein Kinase C may bring about rapid tyrosine phosphorylation and activation of Pyk2. Such stimuli include: cytotoxic agents, drugs, bioactive lipids, neurotransmitters, neuropeptides, reactive oxygen species and growth factors. Integrins and receptors such as the T cell receptor and G protein-coupled receptors may be involved in this phenomenon. Pyk2 phosphorylation is critical for its interaction with SH2-containing signaling molecules and their linkage to signaling pathways that regulate ERK, JNK and p38 kinases.[1,4,5,9,10] Pyk2 has been shown to interact with Src family kinases, the Grb2/Sos complex, p130^{cas}, paxillin, Hic-5, and several other proteins, including inhibitors, to regulate signaling as well as cytoskeletal and morphological changes of cells. Pyk2 has also been implicated in modulation of ion channel function, T and B cell antigen receptor signaling, NK cytotoxicity, cell cycle progression, metastasis, cell death, neuronal short- and long- term responses[10] and bone resorption.

The phosphorylation of Pyk2 at the primary autophosphorylation site (Tyr^{402}), and at the Grb2-binding site (Tyr^{881}) occurs following integrin activation during epithelial-mesenchymal transdifferentiation (EMT).[11,12] Phosphorylated Pyk2 (pTyr^{402}) associates with Src SH2, and induces the formation of a Pyk2/Src/Bcl complex that mediates Src activity, cell adhesion and migration. During cell migration these phosphorylation events are augmented further by the phosphorylation of Pyk2 at Tyr^{580} located within the kinase activation loop. Phosphorylation of tyrosine^{579} and tyrosine^{580} results in maximum Pyk2 activation.

Reagent

Anti-phospho-Pyk2 (pTyr^{881}) is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, containing 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.
Each vial contains approximately 80 µg antibody in 100 µl.

Storage/Stability
Store at –70 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. 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
A recommended working concentration of 1.0 to 1.5 µg/ml is determined by immunoblotting using human pro-B cells treated with 100 ng/ml of SDF-1α. The data demonstrate that only the phosphopeptide corresponding to the region containing tyrosine^{881} blocks the antibody signal, which confirms the specificity of Anti-phospho-Pyk2 (pTyr^{881}) for this phoshosphorylated residue.

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

References

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