

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-PYK2 (CAKß)

Developed in Rabbit, Affinity Isolated Antibody

Product Number P 3902

Product Description

Anti-Pyk2 is developed in rabbit using a synthetic peptide corresponding to amino acid residues 991-1009 of human Pyk2, conjugated to KLH with glutaraldehyde, as immunogen. This sequence is highly conserved in rat and mouse (1 amino acid substitution). The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-Pyk2 specifically recognizes Pyk2 by immuno-blotting and immunoprecipitation (110-116 kDa). Staining of Pyk2 by immunoblotting is inhibited with the immunizing peptide. Also, the antibody may be used for detection of Pyk2 by immunohistochemistry. The epitope(s) recognized by the antibody is compatible with routine formalin-fixation and paraffin-embedding. The antibody reacts with Pyk2 of human, rat and mouse origin.

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 CAKß (cell adhesion kinase ß), RAFTK (related adhesion focal tyrosine kinase), Fak2 (focal adhesion kinase 2) and CADTK (calcium-dependent tyrosine kinase). 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}

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 cell short and long term responses 10 and bone resorption.

Reagent

Anti-Pyk2 is supplied as an affinity isolated antibody in 10 mM phosphate buffered saline, pH 7.4, containing 1 % bovine serum albumin and 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety 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

For continuous use, store at 2 °C to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A minimum working dilution of 1:2,000 is determined by immunoblotting using a whole extract of LPS-stimulated P388 mouse monocyte-macrophage cells.

Pyk2 is immunoprecipitated from 150 to 200 μ g of PC-12 rat pheochromocytoma RIPA lysate using 3 to 5 μ g of the antibody.

A minimum working dilution of 1:100 is determined by indirect immunoperoxidase staining of protease-digested, formalin-fixed, paraffin-embedded tissue sections of human cerebellum.

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

References

- 1. Lev, S., et al., Nature., 376, 737-745 (1995).
- 2. Sasaki, H., et al., J. Biol. Chem., **270**, 21206-21219 (1995).
- 3. Avraham, S., et al., J. Biol. Chem., **270**, 27742-27751 (1995).
- Hu, H., et al., J. Biol. Chem., 271, 29993-29998 (1996).
- 5. Li, Y., et al., Blood, 88, 417-428 (1996).
- Dikic, I., et al., J. Biol. Chem., 273, 14301-14308 (1998).
- 7. Li, X., et al., J. Biol. Chem., **273**, 9361-9364 (1998).
- 8. Brinsson, A. E., et al., J. Biol. Chem., **273**, 1711-1718 (1998).
- 9. Girault, Y., A., et al., Trends Neurosci., **22**, 257-263 (1999).
- 10. Avraham, H., et al., Cell Signal., **12**, 123-133 (2000).

lpg 2/01