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# ProductInformation

**Anti-phospho-Pyk2 (pTyr$^{580}$)**
Developed in Rabbit, Affinity Isolated Antibody

Product Number **P 6739**

## Product Description

Anti-phospho-Pyk2 (pTyr$^{580}$) (proline-rich/Ca-activated tyrosine kinase) was developed in rabbit using a synthetic phosphorylated peptide derived from the region of human Pyk2 that contains tyrosine$^{580}$ as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward the non-tyrosine phosphorylated Pyk2 protein.

Anti-phospho-Pyk2 (pTyr$^{580}$) detects human and rat Pyk2 (pTyr$^{580}$) (approx. 116 kDa). It has been used in immunoblotting applications.[1-4]

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).[5-7]

Fak and Pyk2 share about 45% overall sequence identity and 60 % identity in the centrally located catalytic domain. 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 co-expressed 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.[8]

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. 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. 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 and bone resorption.[5-12]

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).[2,11,12] 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$^{580}$) is supplied as a solution in Dulbecco's phosphate buffered saline (without $Mg^{2+}$ and $Ca^{2+}$), 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 hazards 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. To ensure accurate dilutions mix gently, remove excess solution from pipette tip with clean absorbent paper, pipette slowly. 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**

One vial is sufficient for 10 immunoblots.

A recommended working 1:1000 dilution of the antibody is determined by immunoblotting using chicken embryo fibroblasts (CEFs) expressing human Pyk2 protein and plated on fibronectin.

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

**Results**

Peptide competition

1. Extracts from CEFs were left untreated or treated by plating on fibronectin and expressing Pyk2.
2. The extracts were preincubated with different peptides, as follows:
   Lane 1 – Untreated CEFs, no peptide
   Lane 2 – Treated CEFs no peptide
   Lane 3 – Treated CEFs plus the non-phosphopeptide corresponding to the immunogen
   Lane 4 – Treated CEF's plus phosphopeptide corresponding to Pyk2 ($pY^{579}$)
   Lane 5 - Treated CEFs plus a phosphopeptide derived from the corresponding region of FAK,
   Lane 6 - Treated CEFs plus a generic phosphotyrosine-containing peptide
   Lane 7 - Treated CEFs plus the immunogen
3. The extracts were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF.
4. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C
5. All lanes were incubated with Pyk2 ($pTyr^{580}$) antibody for two hours at room temperature in a 1% BSA-TBST buffer.
6. After washing, membranes were incubated with goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase.
7. Bands were detected.
8. The data show (Figure 1) that only the phosphopeptide corresponding Pyk2 ($pTyr^{580}$) completely blocks the antibody signal, which confirms the specificity of the antibody.

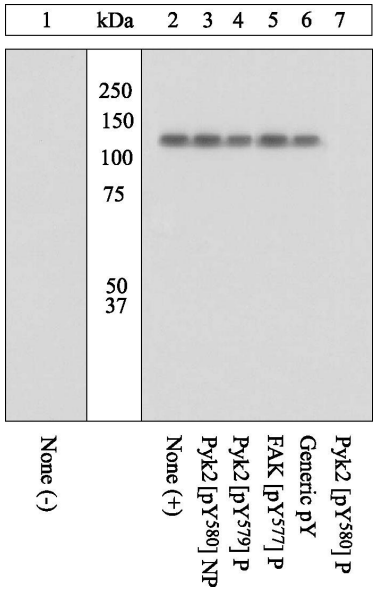


**Figure 1 Peptide competition with Anti-phospho-Pyk2 ($pTyr^{580}$)**

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