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# ProductInformation

**Anti-phospho-Pyk2 (pTyr$^{402}$)**
Developed in Rabbit, Affinity Isolated Antibody

Product Number **P 6614**

## Product Description

Anti-phospho-Pyk2 (pTyr$^{402}$) was developed in rabbit using a synthetic phosphorylated peptide derived from the region of human Pyk2 that contains tyrosine$^{402}$ 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$^{402}$) detects human, rat and chicken Pyk2 (pTyr$^{402}$) (approximately 116 kDa). It has been used in immunoblotting applications.[1-3]

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).[4,5] Proline-rich/$Ca^{2+}$-activated tyrosine kinase (Pyk2) is a member of the FAK family of non-receptor, proline-rich protein tyrosine kinases. 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 cell. Pyk2 is found as a short isoform Pyk2H in normal circulating monocytes, B, T and NK cells.[6-8] Pyk2 signaling is initiated by a variety of extracellular stimuli including integrin ligation, CD surface marker ligation (e.g., CD3, CD28, TCR, VCAM), bioactive peptides, growth factors, cytokines, chemokines, and certain stress stimuli (reactive oxygen species and $Ca^{++}$ flux). Pyk2 is involved in the regulation of vesicular transport, osteoclastic bone resorption, modulation of ion channels, T- and B-cell receptor signaling and cell death. 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).[9-11]

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$^{402}$) is supplied as a solution in Dulbecco's phosphate buffered saline (without $Mg^{2+}$ and $Ca^{2+}$), pH 7.3, containing 50% glycerol, 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide. One vial is sufficient for 10 immunoblots.

## 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

A recommended 1:1000 dilution of the antibody is determined by immunoblotting using chick embryo fibroblasts (CEF) cells expressing human Pyk2. The data demonstrate that only the phosphopeptide corresponding to the region containing tyrosine 402 blocks the antibody signal, which confirms the specificity of Anti-phospho-Pyk2 (pTyr$^{402}$) 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.

## Results

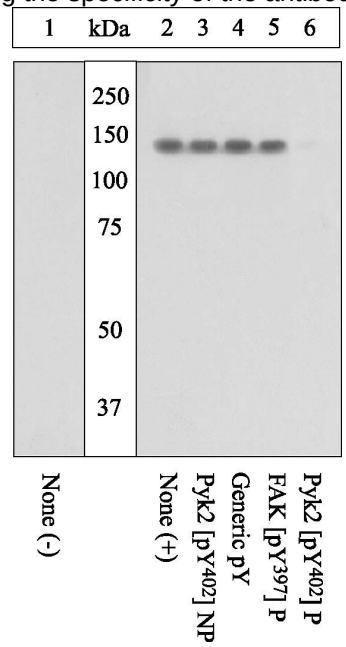
Peptide Competition

The specificity of this a phosphorylation site-specific

antibody, was demonstrated by peptide competition experiment.

- Extracts prepared from CEF cells (1) or CEF cells expressing Pyk2 (2-6) plated on fibronectin were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
- Membranes were blocked with a 5% BSA-TBST buffer overnight at 4°C
- Membranes were pre-incubated with:
  - 1,2 no peptide
  - 3 a generic peptide containing phosphorylated tyrosine
  - 4 the non-phosphorylated peptide corresponding to the phosphorylated immunogen
  - 5 the phosphopeptide to the corresponding tyrosine on FAK
  - 6 the immunogen phosphopeptide
- Subsequently all four membranes were incubated with 0.35 µg/mL Rb (pSer$^{807}$) antibody,
- After washing, membranes were incubated with a goat F(ab')$_2$ anti-rabbit IgG and alkaline phosphatase conjugate and the bands were visualized.

The data show that only the peptide corresponding to Rb (p Ser$^{807}$) blocks the antibody signal, thereby demonstrating the specificity of the antibody

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