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ProductInformation

Anti-phospho-PDGF Rα [pTyr^{742}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **P 8246**

Product Description

Anti-phospho-PDGF Rα [pTyr^{742}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human PDGF receptor α that contains tyrosine 742 as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated PDGF Rα peptide.

The antibody detects mouse PFGF Rα. Human, rat (100% homologous) and frog (83%) PDGF Rα have not been tested, but are expected to react. PDGF Rβ (50%) has not been tested, but is not expected to react. The antibody has been used in immunoblotting applications.

Platelet-derived growth factor receptor (PDGF R) is a transmembrane glycoprotein of 170-185 kDa that undergoes homo- or heterodimerization into complexes of α and β subunits upon ligand binding, depending on the isoform of PDGF (PDGF-AA, -BB or -AB) that binds.

The phosphorylation of tyrosine residues in the ligand-activated receptor can control multiple signaling events such as actin reorganization, transcription, cell growth, migration and differentiation, and also lead to activation of the Ras → Raf → ERK1&2 pathway. Tyrosine 742 in PDGFRα is a binding site for PI3-kinase.

Reagent

Anti-phospho- PDGF Rα [pTyr^{742}] 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.

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 –70 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

A recommended working concentration of 0.35 to 1.0 µg/mL is determined by immunoblotting using NIH3T3 cells +/- PDGF.

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

Results

Peptide Competition

1. Extracts prepared from NIH3T3 cells left unstimulated (Lane 1) and stimulated with PDGF (Lanes 2-5) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:
   - Lane 1,2 no peptide
   - Lane 3 non-phosphorylated peptide corresponding to the immunogen
   - Lane 4 a generic phosphotyrosine containing peptide
   - Lane 5 immunogen
4. After preincubation membranes were incubated with 0.50 µg/mL PDGF Rα [pTyr^{742}] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase and signals were detected.

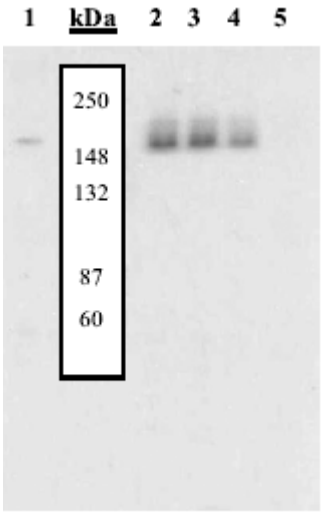

Figure 1 Peptide Competition

The data in Figure 1 show that only the peptide corresponding to PDGF Rα [pTyr^{742}] blocks the antibody signal, demonstrating the specificity of the antibody.

References

1. Rolny, C., et al., Heparin amplifies platelet-derived growth factor (PDGF)- BB-induced PDGF alpha -receptor but not PDGF beta -receptor tyrosine phosphorylation in heparan sulfate deficient cells. Effects on signal transduction and biological responses. J. Biol. Chem., **277**, 19315-19321 (2002).
2. Carloni, V., et al. Cell adhesion regulates platelet-derived growth factor-induced MAP kinase and PI-3 kinase activation in stellate cells. Hepatology, **36**, 582-591 (2002).
3. Heldin, C.H., et al., Signal transduction via platelet-derived growth factor receptors. Biochim. Biophys. Acta., **1378**, F79-F113 (1998).
4. Matsumoto, T., et al. Differential interaction of CrkII adaptor protein with platelet-derived growth factor alpha- and beta-receptors is determined by its internal tyrosine phosphorylation. Biochem. Biophys. Res. Commun., **270**, 28-33 (2000).

AH/JK 5/12/2004