

Product Information

Anti-phospho-PDGF R α [pTyr⁷⁶²]

Developed in Rabbit, Affinity Isolated Antibody

Product Number **P 7996**

Product Description

Anti-phospho-PDGF R α [pTyr⁷⁶²] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human PDGF receptor α that contains tyrosine 762 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 R α peptide.

The antibody detects mouse PDGF R α . Human, rat (100% homologous) and frog (83%) PDGF R α have not been tested, but are expected to react. PDGF R β (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 which 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 \rightarrow Raf \rightarrow ERK1&2 pathway. PDGF R α tyrosine 762 is autophosphorylated in the activated receptor, and serves as a binding site for Crk proteins.

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg²⁺ and Ca²⁺), 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 $\mu\text{g}/\text{mL}$ 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 $\mu\text{g}/\text{mL}$ anti-phospho-PDGF R α [pTyr⁷⁶²] 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.

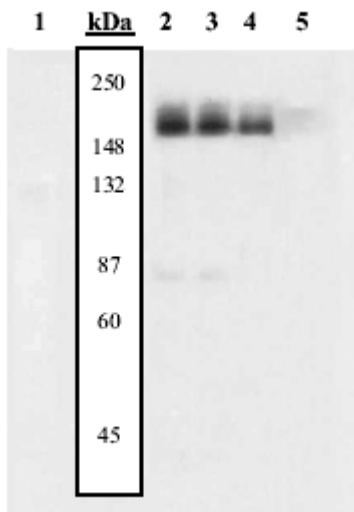


Figure 1 Peptide Competition

The data in Figure 1 show that only the peptide corresponding to PDGF R α [pTyr⁷⁶²] blocks the antibody signal, thereby demonstrating the specificity of the antibody.

References

1. Rolny, C., et al., Heparin amplifies platelet-derived growth factor (PDGF)- BB-induced PDGF α -receptor but not PDGF β -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. Matsumoto, T., et al., Differential interaction of CrkII adaptor protein with platelet-derived growth factor α - and β -receptors is determined by its internal tyrosine phosphorylation. *Biochem. Biophys. Res. Commun.*, **270**, 28-33 (2000).
4. Yokote, K., et al., Identification of Tyr-762 in the platelet-derived growth factor α -receptor as the binding site for Crk proteins. *Oncogene*, **16**, 1229-1239 (1998).
5. Heldin, C.H., et al., Signal transduction via platelet-derived growth factor receptors. *Biochim. Biophys. Acta.*, **1378**, F79-F113 (1998).

AH/PHC 08/04

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.