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Product Information

Anti-Platelet-Derived Growth Factor Receptor α
produced in goat, affinity isolated antibody

Catalog Number **P2110**

Product Description

Anti-Platelet-Derived Growth Factor Receptor α (PDGF Rα) is produced in goat using a purified recombinant soluble human platelet-derived growth factor receptor α, expressed in the insect cell line *Sf* 21, as immunogen. Affinity isolated antibody is obtained from goat anti-PDGF Rα antiserum by immuno-specific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide. Recombinant human PDGF sRα is a 501 amino acid (~56 kDa) transmembrane protein, expressed in a mouse myeloma NSO cell line.[1].

Anti-Platelet-Derived Growth Factor Receptor α recognizes recombinant human PDGF Rα by various immunochemical techniques including immunoblotting, ELISA, and neutralization.

Platelet-Derived Growth Factor Receptor α (PDGF Rα) is a member of the class III subfamily of receptor tyrosine kinases (RTK) that includes PDGF Rβ, and also receptors for M-CSF, SCF, and Flt 3 ligand. Characteristic of the class III RTKs is the presence of five immunoglobulin-like regions in their extracellular domain, and a split kinase region in their intracellular domain. PDGF Rα and PDGF Rβ share 44% sequence identity. Within the extracellular domain, 30% of the amino acid residues are identical. [1]

Platelet derived growth factor (PDGF), first identified in serum, is a major mitogen for cells of mesenchymal origin and is released from platelets during clot formation.[2] PDGF elicits multifunctional actions with a variety of cells, including mitogenesis of mesoderm-derived cells, increased extracellular matrix synthesis, and chemotaxis and activation of neutrophils, monocytes, and fibroblasts. PDGF is mitogenic for dermal and tendon fibroblasts, vascular smooth muscle cells, glial cells, and chondrocytes. PDGF appears to interact with TGF-1 in accelerating wound healing [3] and may also be pathogenic in arteriosclerosis and neoplasia.[4]

PDGF exists as a homodimeric or heterodimeric protein consisting of disulfide-linked PDGF-A and PDGF-B chains. PDGF exerts its actions via specific receptors on the cell surface. Two distinct human PDGF receptors have been identified, PDGF α and PDGF β, which are structurally related, consist of an extracellular region, a single transmembrane region, and an intracellular region. The three different isoforms of PDGF (PDGF-AA, PDGF-AB, and PDGF-BB) bind with different affinities to two both receptors. [5] Ligand binding induces receptor dimerization. The A-subunit of PDGF binds to α-receptors, whereas the B-subunit binds to both α- and β-receptors. Binding of PDGF to its receptor activates the tyrosine kinase domain and leads to enhanced phosphorylation of intracellular substrates as well as autophosphorylation of the receptor itself. Autophosphorylation is induced by allowing binding and activation of the cytoplasmic SH2-domain, which contains signal transduction molecules. Thereby, a number of different signaling pathways are initiated leading to cell growth, actin reorganization, migration and differentiation. Recent observations suggest that extensive cross talk occurs between the different signaling pathways and that stimulatory signals are modulated by inhibitory signals arising in parallel. [6]

PDGF Rα is expressed in oligodendrocyte progenitor cells, mesothelial cells, and liver endothelial cells. It has also been detected in cell conditioned medium and human plasma.

Reagent

Supplied as a lyophilized powder from a 0.2 μm filtered solution in phosphate buffered saline with 5% trehalose.

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of sterile phosphate buffered saline (PBS) to produce a 0.1 mg/ml stock solution of antibody.

Storage/Stability

Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2-8 °C for at least one month. For prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing.

Product Profile
Neutralization: Anti-Platelet Growth Factor Receptor α is measured by its ability to block cell surface human or mouse PDGF Rα mediated bioactivity induced by PDGF AA. [7]

To measure this biological activity, concentrations of the antibody from 0.01 to 100 μg/ml are added to quiescent confluent cultures of mouse NR6R-3T3 or human WS1 cells in MEM with 2% bovine plasma-derived serum in a 96 well plate The cell-antibody mixture is incubated for 60 minutes at room temperature. Following this preincubation, recombinant human PDGF AA (10 ng/mL) is added to the wells. The assay mixture, in a total volume of 100 μL, containing antibody at the concentrations indicated, and recombinant human PDGF AA at 10 ng/ml and NR6R-3T3 cells is incubated at 37 °C in a humidified CO_2 chamber for 18 to 20 hours. ^{3}H-thymidine is added during the last 2 hours of the incubation. The cells are detached and harvested onto glass fiber filters and the ^{3}H-thymidine incorporated into the DNA is measured.

The Neutralization Dose$_{50}$ (ND$_{50}$) of this antibody is defined as that concentration required to yield one-half maximal inhibition of the cell surface PDGF Rα mediated PDGF AA response on a responsive cell line, at a specific PDGF AA concentration.

The exact concentration of antibody required to neutralize the human cell surface PDGF Rα mediated bioactivity is dependent on the PDGF AA concentration as well as on the number of PDGF receptors present on the cell surface (a function of the cell type and culture conditions).

Immunoblotting: a working antibody concentration of 0.1-0.2 μg/mL is recommended. The detection limit for recombinant human PDGF Rα is ~5 ng/lane under non-reducing and reducing conditions.

ELISA: a working antibody concentration of 0.5-1.0 μg/mL is recommended. The detection limit for recombinant human PDGF Rα is ~0.5 ng/well.

Immunohistochemistry: a working antibody concentration of 15 μg/mL is recommended using paraffin-embedded human tissue sections.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

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