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ProductInformation

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

Catalog Number **P2229**

Product Description

Anti-Platelet-Derived Growth Factor Soluble Receptor β (PDGF Rβ) is produced in goat using as immunogen a recombinant human soluble PDGF Rβ, expressed in NSO cells. The antibody is purified using human PDGF Rβ affinity chromatography.

Anti-Platelet-Derived Growth Factor Soluble Receptor β may be used to block the bioactivity of human soluble PDGF Rβ. By immunoblotting and ELISA, the antibody shows <1% cross-reactivity with recombinant human PDGF Rα. The antibody may also be used in immunohistochemistry (cells and tissues) and flow cytometry.

Platelet derived growth factor (PDGF), the major mitogen in serum for cultured connective tissue cells, exerts it actions via specific receptors on the cell surface. Two distinct human PDGF receptor transmembrane binding proteins have been identified, a 170 kDa, 1066 amino acid residue α-receptor (PDGF Rα)[1] and a 190 kDa, 1074 amino acid residue β-receptor (PDGF Rβ)[2]. The two receptor proteins are structurally related and consist of an extracellular portion containing five immuno-globulin-like domains, a single transmembrane region, and an intracellular portion with a protein-tyrosine kinase domain. Ligand binding induces receptor dimerization and autophosphorylation, allowing binding and activation of cytoplasmic SH2-domain containing 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 different signaling pathways, and that stimulatory signals are modulated by inhibitory signals arising in parallel.[3]

Between the two PDGF receptors, there is 44% overall sequence identity. Within the extracellular domain, 30% of the amino acid residues are identical.[4] The different isoforms of PDGF (PDGF-AA, PDGF-AB and PDGF-BB) bind with different affinities to two distinct 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 to autophosphorylation of the receptor itself. In addition, several other cellular responses are induced. Studies have indicated that PDGF β-receptors are not present on most cells of normal tissues, but are upregulated, in conjunction with inflammation,[3] excess cell proliferation,[6] malignancytic,[7] and fibrotic conditions.[8, 9]

Reagent

Supplied lyophilized 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 0.2 μm filtered phosphate buffered saline to produce a 0.1 mg/mL stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

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

Neutralization Procedure
For neutralization, the antibody is tested for its ability to block soluble PDGF Rβ mediated bioactivity in the presence of recombinant human PDGF-BB in a ^{3}H-thymidine incorporation assay using mouse NR6R-3T3 cells. To measure the ability of the antibody to block rhPDGF sRβ bioactivity on mouse NR6R-3T3 fibroblasts, rhPDGF sRβ was added to various concentratios of the antibody in a 96 well plate and incubated for 30 minutes at 37 °C. Following this incubation, rhPDGF-BB was added to the mixture and incubated for an additional 30 minutes at 37 °C. The antigen, receptor, and antibody mixture was then added to quiescent confluent cultures of NR6R-3T3 cells in DMEM with 2% bovine plasma-derived serum. The assay mixture in a total volume of 100 µL, containing antibody at the concentration of 0.01-10 µg/mL, rhPDGF sRb at 120 ng/mL, and rhPDGF-BB at 4 ng/mL, was incubated at 37 °C for 18-20 hours in a humidified CO_2 incubator. ^{3}H-thymidine was added during the final 2 hours of the incubation. The cells were subsequently detached and harvested onto glass fiber filters and the ^{3}H-thymidine incorporated into the DNA was determined.

The ND_{50} of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of the PDGF-BB activity on a responsive cell line.

Product Profile
Immunoblotting: a working concentration of 0.1-0.2 µg/mL is determined. The detection limit for recombinant human soluble PDGF Rβ is ~1 ng/lane under non-reducing and reducing conditions of

ELISA: a working concentration of 0.5-1 µg/mL is recommended for the detection of human PDGF sRβ. The detection limit is ~0.3 ng/well.

Immunohistochemistry: a working concentration of 15 µg/mL is determined for cells and tissues.

Flow cytometry: a working concentration of 50 µg/mL is recommended using Bud-8 cells. Dilute the anibody to 50 µg/mL, then add 10 µL of the diluted solution to 1-2.5 x 10^{5} cells in a total reaction volume not exceeding 200 µL. The binding of unlabeled antibodies may be visualized using a secondary antibody such as anti-goat IgG conjugated to a fluorochrome.

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

References

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KAA,PHC 03/07-1