ProductInformation

ANTI-HUMAN PLATELET-DERIVED GROWTH FACTOR-AA (PDGF-AA)
Developed in Goat, Affinity Isolated Antibody

Product Number **P5851**

Product Description

Anti-Human PDGF-AA is developed in goat using recombinant, human PDGF-AA (rhPDGF-AA) expressed in *E. coli* as immunogen. The antibody is purified using human PDGF-AA affinity chromatography.

Anti-Human PDGF-AA will neutralize the biological activity of rhPDGF-AA. It will also neutralize the activity of rhPDGF-AB and partially neutralized the activity natural human PDGF. It will not neutralize the activity of porcine PDGF-BB or rhPDGF-BB. The antibody may also be used in immunoblotting and ELISA. By immunoblotting, the antibody shows 100% cross-reactivity with rhPDGF-AB and < 5% cross-reactivity with rhPDGF-BB and rrPDGF-BB. In addition, by ELISA, the antibody shows no cross-reactivity with other cytokines tested.*

Platelet-Derived Growth Factor (PDGF), first identified by Ross et al.,[1] in serum is the principal mitogen present for cells of mesenchymal origin.[2,3] PDGF is localized in α-granules of platelets and released during clot formation.[4] PDGF from human platelets has been purified and described as a cationic glycoprotein (pI 9.5 to 10.4) having a molecular weight of approximately 30 kD and composed of two covalently linked subunits, designated as chains A (16 kD) and B (14 kD).[5-8] In platelets, approximately 70% of the PDGF is present as the AB dimer, with most of the remainder as BB.[9] Purified human PDGF shows substantial size heterogeneity, ranging from 27 to 31 kD, probably due to the presence of isoforms, glycosylation processing, aging of the platelets, and partial proteolysis during purification. The A and B chains are 40% homologous in sequence and are encoded by distinctly different genes.[10] Each chain contains 8 cysteine residues, which are involved in intra- and inter-chain disulfide bonds.[11,12] Cleavage of these bonds by reduction causes irreversible loss of biological activity.[8] PDGF elicits multifunctional actions with a variety of cells.[13-15] It is mitogenic to mesoderm-derived cells, such as dermal and tendon fibroblasts, vascular smooth muscle cells, glial cells and chondrocytes. PDGF is a potent chemoattractant and activator of neutrophils, monocytes and fibroblasts. PDGF increases the synthesis of phospholipids, cholesterol esters, glycogen and prostaglandins, and modulates LDL receptor binding. Other actions of PDGF include its ability to regulate the synthesis and degradation of extracellular matrix protein and to stimulate the synthesis of additional growth factors. PDGF may increase erythropoiesis and stimulate vaso-constriction. PDGF is believed to play an essential role in the cellular response to tissue injury, both as a stimulant of mesodermal cell growth and activity and as a chemoattractant to other cells involved in the repair process[16]. In this role, PDGF appears to interact with Transforming Growth Factor-β1 (TGF-β1), which is also released by degranulating platelets at the source of the damaged tissue.[17] The sources of PDGF during wound repair include platelets (predominantly PDGF-AB), smooth muscles (PDGF-A)[18], monocyte-derived macrophages (PDGF-B),[19] and endothelial cells (PDGF-B).[20] PDGF may play a role during normal embryonic development[14]. Pathologically, PDGF appears to be an initial mediator and a contributing sustaining factor in the development of atherosclerosis.[18-21] Abnormal cellular expression of PDGF is associated with certain malignant transformations.[13] In fact, a transforming protein ($p28^{sis}$) encoded by the simian sarcoma virus oncogene (*v-cis*) contains an amino acid sequence,[23] that is virtually identical to the PDGF-B and is processed into a PDGF-BB-like homodimer,[24]. That exhibits biological actions identical to PDGF.[25] Detection of *v-cis*-related mRNA (*c-sis* RNA) has been reported in certain malignancies of mesenchymal cell origin, including fibrosarcoma, glioblastoma and osteosarcoma.[26,27] Certain other tumor cell lines express PDGF-A chain or both A and B chains.[10,28]. Other pathological conditions in which PDGF has been implicated include scleroderma, inflammatory joint disease, myelofibrosis and pulmonary fibrosis.[9,14] Purified PDGF activates two distinct PDGF receptors encoded by separate genes.[29,30] PDGF-AA binds only to α-PDGF receptor

but PDGF-AB and PDGF-BB bind to both α and β receptors; i.e., the α receptor binds either A or B chain and the β receptor binds only the B chain.[29,31] Perhaps the independent expression of specific receptor types and the availability of the different isoforms of PDGF may explain the diverse range of observed cellular PDGF responses.[30] For example, the PDGF-B gene has a much greater transforming potential than the PDGF-A gene when transfected into NIH 3T3 cells, but the PDGF-A gene product is more efficiently secreted into the medium.[32] The sequence domains on each chain responsible for the greater receptor activation and secretory ability have been recently mapped.[33] Furthermore, certain tumors have been found to express the β-PDGF receptor with or without the coexpression of the PDGF-B chain, indicating that a tumor may be autocrinically growth stimulated[34] or it may be stimulated by exogenous PDGF.[35] Binding of either PDGF receptor to its substrate induces receptor autophosphorylation at a tyrosine residue,[31] which then becomes detectable by immunoreaction with monoclonal anti-phosphotyrosine.

Reagents

Mass/vial:	0.1 mg
Immunogen:	Recombinant Human PDGF-AA
Host animal:	Goat
Form:	Affinity Isolated Antibody
Formulation:	Lyophilized from PBS without additives.
Endotoxin:	≤10 ng/mg by LAL method
Sterility:	0.2 µm-filtered, aseptic fill

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of 0.2 µm-filtered PBS to produce a 0.1 mg/ml stock solution of Anti-Human PDGF-AA. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Storage/Stability

Prior to reconstitution, 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.

Procedure

Anti-Human PDGF-AA is tested for its ability to neutralize the bioactivity of rhPDGF-AA in a cell proliferation assay using PDGF-responsive NR6R-3T3 fibroblasts.[36] The ND_{50} of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of bioactivity of rhPDGF-AA that is present at a concentration just high enough to elicit a maximum response. In this bioassay, rhPDGF-AA is preincubated with various dilutions of the antibody in a 96-well microtiter plate. Quiescent confluent cultures of NR6R-3T3 cells are added to each well. The total volume of 100 ul, containing antibody, rhPDGF-AA at 25 ng/ml, is incubated for 20 hours at 37°C in a 5% CO_2 humidified incubator and then pulsed for the last 2 hours with ^{3}H-thymidine. Cells are harvested onto glass filters and the ^{3}H-thymidine incorporation into DNA is measured.

Results

Bioactivity:	ND_{50} = 0.1 – 0.3 µg/ml
Indirect ELISA:	0.5 – 1 µg/ml antibody detects 0.25 ng/well of rhPDGF-AA and rhPDGF-AB.
Indirect Immunoblotting:	0.1 – 0.2 µg/ml antibody detects rhPDGF-AA and rhPDGF-AB at 2 ng/lane under non-reducing and reducing conditions.

References

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- rhActivin β A, rhActivin RII, rhANG, rhAnnexin V, rhAR, rhB7-1, rhB7-2, rmB7-2, rhBTC, rhβ-NGF, rrβ-NGF, rhBDNF, rmC10, rhCD8, rhCD28, rrCINC-1, rhCNTF, rrCNTF, rhCNTF sRα, rrCNTF sRα, rhCPP 32, rhCTLA-4, rmCRG-2, rhEGF, rhENA-78, rhEotaxin, rmEotaxin, rhEpo, rh EPO R, rhFas, rhFGF acidic, rhFGF basic, rhFGF-4, rhFGF-5, rhFGF-6, rhFGF-7, rhFGF-8b, rhFGF-9,
- rhFlk2/Flt3 ligand, rhFlt-1 R, rhG-CSF, rmG-CSF, rhG-CSF sRα, rmGDF-9, rhGDNF, rrGDNF,
- rhGM-CSF, rmGM-CSF, rhGM-CSF Rα, rhGROα, rhGROβ, rhGROγ, rhHB-EGF, rhHCC-1, rhHRG-α, rhHRG-β, rhHGF, rhI-309, rhI-Linck, rhIAP-1, rhIFN-γ, rmIFN-γ, rrIFN-γ, rhIGF-I, rhIGF-I bp, rhIGF-I R, rhIGF-II, rhIGIF, rhIL-1α, rmIL-1α,
- rhIL-1 sRI, rhIL-1 sRII, rhIL-1β, rmIL-1β, rrIL-1β, rhIL-1ra, rmIL-1ra, rhIL-2, rmIL-2, rrIL-2, rhIL-2 sRα, rhIL-2 sRβ, rhIL-2sRγ, rhIL-3, rmIL-3, rhIL-3 sRα, rhIL-4, rmIL-4, rrIL-4, rhIL-4 sR, rhIL-5, rmIL-5, rhIL-5 sRα, rhIL-6, rmIL-6, rhIL-6 sR,
- rhIL-7, rmIL-7, rhIL-7 R, rhIL-8, rhIL-9, rmIL-9,
- rhIL-9 sR, rhIL-10, rmIL-10, rhIL-10 sR, rmIL-10 sR, rhIL-11, rmIL-11, rhIL-12, rmIL-12, rmIL-12 R β, rhIL-13, rmIL-13, rhIL-15, rhIL-17, rmIL-17, rhIP-10, rmJE, rmKC, rhLIF, rmLIF, rhLIF R, rmLymphotactin, rmMARC, rhM-CSF, rmM-CSF, rhMCP-1, rhMCP-1 R, rhMCP-2, rhMCP-3, rhMidkine, rhMIF, rhMIG, rmMIG, rhMIP-1α, rmMIP-1α, rhMIP-1β(rhACT II), rmMIP-1β,
- rmMIP-2, rhMIP-3α, rhMIP-3β, rhMSP, rhNT-3, rhNT-4, rhOB, rmOB, rhOSM, rmOSM,
- rhPD-ECGF, rhPDGF Rα, rhPlGF, rhPTN, rhRANTES, rmRANTES, rhSCF, rmSCF, rhSCF R, rhsgp130, rhSLPI, hTfR, rhTGF-α, rhTGF-β1, rhTGF-β2, rhTGF-β3, raTGF-β5, rhLAP (TGF-β1), rhLatent TGF-β1, rhTGF-β sRII, rhTGF-β sRIII, rhTNF-α, rmTNF-α, rrTNF-α, rhTNF-β, rhsTNF RI, rmsTNF RI, rhsTNF RII, rmsTNF RII, rhTpo, rmTpo, rhTyk-2, rhVEGF, rmVEGF, rhVEGF/PlGF

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