

Product Information

Anti-Platelet-Derived Growth Factor

produced in goat, IgG Fraction of Antiserum

Catalog Number **P 6101**

Synonym: Anti-PDGF

Product Description

Anti-Human Platelet-Derived Growth Factor (PDGF) is produced in goat using as immunogen natural, human PDGF. The antibody is purified using protein G affinity chromatography.

Anti-Human Platelet-Derived Growth Factor (PDGF) will neutralize the biological activity of natural human PDGF. It will also neutralize the activity of recombinant human PDGF-AB, recombinant human PDGF-AA, recombinant human PDGF-BB, and natural porcine PDGF-BB. The antibody may be used in immunoblotting and ELISA. It shows cross-reactivity with recombinant human PDGF-AB, recombinant human PDGF-AA, recombinant human PDGF-BB, and natural porcine PDGF-BB. In ELISA and immunoblotting, the antibody will recognize all the PDGF isoforms mentioned above.

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 - 10.4) having a molecular weight of ~30 kDa and composed of two covalently linked subunits, designated as chains, A (16 kDa) and B (14 kDa).⁵⁻⁸ In platelets, ~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 27-31 kDa, 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- β 1 (TGF- β 1), 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 sequence²³ that is virtually identical to the PDGF-B that is processed into a PDGF-BB-like homodimer.²⁴ This 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 on 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 autocrinally growth stimulated³⁴ 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.

Reagent

The antibody is supplied lyophilized from phosphate buffered saline, pH 7.4, with 5% trehalose.

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.

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of 0.2 μ m filtered phosphate buffered saline to produce a 1 mg/ml stock solution of antibody. 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 $2-8^{\circ}\text{C}$ for up to one month. For prolonged storage, freeze in working aliquots at -20°C . Avoid repeated freezing and thawing. Do not store in "frost-free" freezers.

Procedure

Anti-Human PDGF is tested for its ability to neutralize the bioactivity of PDGF in a cell proliferation assay using PDGF-responsive NR6R-3T3 fibroblasts.³⁶ In this bioassay, natural, human PDGF is preincubated with various dilutions of the antibody in a 96-well plate. Quiescent confluent cultures of NR6R-3T3 cells are added to each well. The total volume of 100 μ L, containing antibody, PDGF at 10 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 ^3H -thymidine. Cells are harvested onto glass filters and the ^3H -thymidine incorporation into DNA is measured.

The ND_{50} of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of bioactivity of PDGF that is present at a concentration just high enough to elicit a maximum response.

Product Profile

Indirect ELISA: 0.25 μ g/mL antibody detects < 5 ng/well of natural human PDGF.

Indirect Immunoblotting: 1 μ g/mL antibody detects natural human PDGF at 1 ng/lane under non-reducing and reducing conditions.

References

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