

Human PDGF beta protein, His tag (active)

Cat. No. GTX00204-pro

Applications Functional Assay**Species** Human**Package**

10 µg

Applications

Application Note

Platelet-derived growth factor subunit B (PDGFB) is a member of the platelet-derived growth factor family in humans. The four members of this family are mitogenic factors for cells of mesenchymal origin and are characterized by a motif of eight cysteines. This gene product can exist either as a homodimer (PDGF-BB) or as a heterodimer with the platelet-derived growth factor alpha (PDGFA) polypeptide (PDGF-AB), where the dimers are connected by disulfide bonds. Besides, Neuropilin-1 (NRP-1) has been identified as an interactor of PDGFB, thus a binding ELISA assay was conducted to detect the interaction of recombinant human PDGFB and recombinant human NRP-1. Briefly, PDGFB were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to NRP-1-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti- PDGFB pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37°C. Finally, add 50 µl stop solution to the wells and read at 450nm immediately. The binding activity of PDGFB and NRP-1 was in a dose dependent manner.

PDGFs are mitogenic during early developmental stages, driving the proliferation of undifferentiated mesenchyme and some progenitor populations. During later maturation stages, PDGF signalling has been implicated in tissue remodelling and cellular differentiation, and in inductive events involved in patterning and morphogenesis. In addition to driving mesenchymal proliferation, PDGFs have been shown to direct the migration, differentiation and function of a variety of specialised mesenchymal and migratory cell types, both during development and in the adult animal. A proliferation assay was conducted to detect the bioactivity of recombinant human PDGFB using MCF-7 cells. Briefly, MCF-7 cells were seeded into triplicate wells of 96-well plates at a density of 5000 cells/well and allowed to attach, replaced with serum-free overnight, then the medium was replaced with 1% serum standard DMEM prior to the addition of various concentrations of PDGFB. After incubated for 96h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10µL of CCK-8 solution was added to each well of the plate, then the absorbance at 450nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37°C. Proliferation of MCF-7 cells after incubation with PDGFB for 96h observed by inverted microscope. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant PDGFB for 96h. And PDGFB significantly increased cell viability of MCF-7 cells.

Observed MW (kDa) 33 kDa.

Properties

Form Lyophilized powder**Buffer** Reconstitute with 20mM Tris and 150mM NaCl to 0.1-1.0mg/ml. Do not vortex. Lyophilized from 20mM Tris, 150mM NaCl, 1mM EDTA, 1mM DTT, 0.01% SKL, 5% Trehalose.**Preservative** ProClin 300**Storage** For short-term storage (1-2 weeks), store at 4°C. For long-term storage, store at -20°C or below. After reconstitution, keep as concentrated solution. Avoid freeze-thaw cycles.**Region/Sequence** N-terminal His-Tag; Glu21~Ala241 (NP_002599.1)**Expression System** E. coli**Purity** > 95%**Endotoxin** < 1 EU/µg

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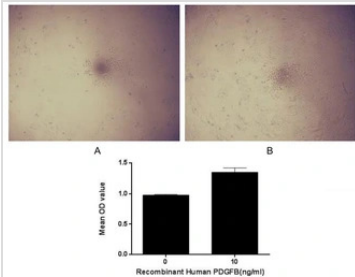
Date 2025 / 12 / 31 Page 1 of 2

Conjugation

Unconjugated

Note

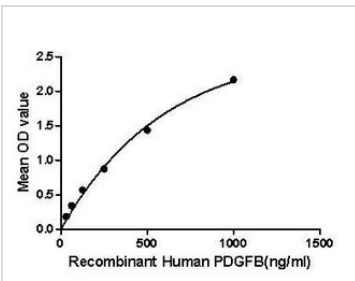
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DATA IMAGES

GTX00204-pro Functional Assay Image

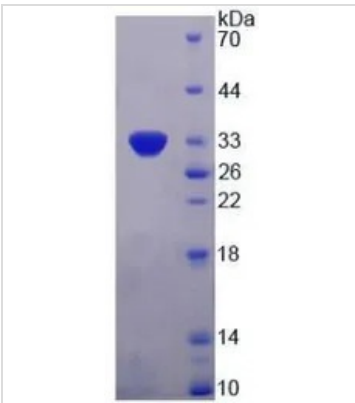
Cell proliferation effect of GTX00204-pro Human PDGF beta protein (active). Cell viability was measured by Cell Counting Kit-8 (CCK-8).

(A) Unstimulated MCF-7 cells cultured in DMEM for 96hrs.

(B) MCF-7 cells cultured in DMEM, stimulated with 1 ng/ml PDGFB for 96hrs.


GTX00204-pro Functional Assay Image

Functional ELISA analysis of GTX00204-pro Human PDGF beta protein (active) which can bind immobilized NRP-1 protein.


GTX00204-pro Image

SDS-PAGE analysis of GTX00204-pro Human PDGF beta protein (active).


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