

**APC921Hu01 100µg**  
**Active Platelet Derived Growth Factor Subunit B (PDGFB)**  
**Organism Species: Homo sapiens (Human)**  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1st Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Glu21~Ala241

**Tags:** N-terminal His-tag

**Purity:** >95%

**Buffer Formulation:** 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

**Applications:** Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

**Predicted isoelectric point:** 9.5

**Predicted Molecular Mass:** 28.6kDa

**Accurate Molecular Mass:** 33kDa as determined by SDS-PAGE reducing conditions.

### **Phenomenon explanation:**

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

## **[ USAGE ]**

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

## **[ STORAGE AND STABILITY ]**

**Storage:** Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

**Stability Test:** The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

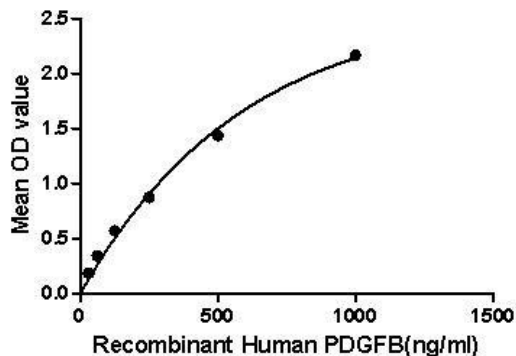
## **[ SEQUENCE ]**

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EGDPIPEELY EMLSDHSIRS FDDLQRLLLHG  
DPGEEDGAEL DLNMTRSHSG GELESLARGR RSLGSLTIAE PAMIAECKTR  
TEVFEISRRL IDRTNANFLV WPPCVEVQRC SGCCNNRNQV CRPTQVQLRP  
VQVRKIEIVR KKPIFKKATV TLEDHLACKC ETVAAARPVT RSPGGSQEQR  
AKTPQTRVTI RTVRVRRPPK GKHRKFKHTH DKTALKETLG A
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## **[ ACTIVITY ]**

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

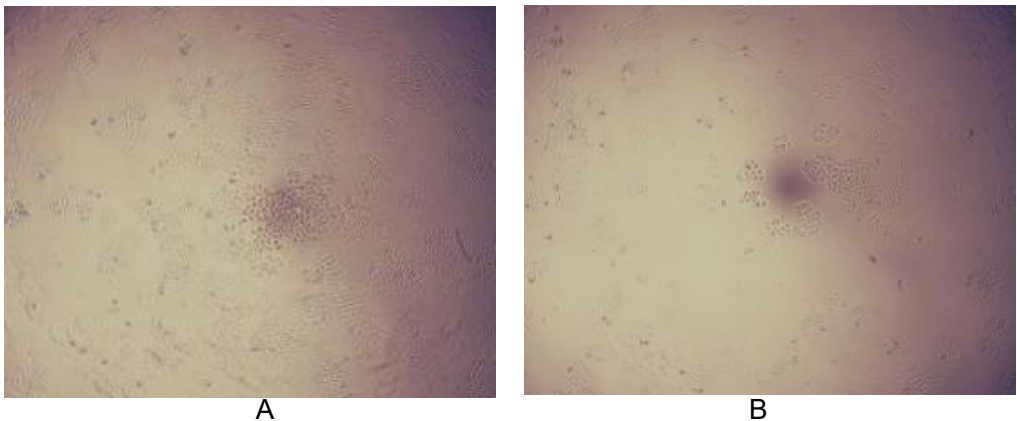
were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 $\mu$ L were then transferred to NRP-1-coated microtiter wells and incubated for 2h at 37 $^{\circ}$ C. Wells were washed with PBST and incubated for 1h with anti- PDGFB pAb, then aspirated and washed 3 times. After incubation 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 $^{\circ}$ C. Finally, add 50 $\mu$ L stop solution to the wells and read at 450nm immediately. The binding activity of PDGFB and NRP-1 was shown in Figure 1, and this effect was in a dose dependent manner.



**Figure 1. The binding activity of PDGFB with NRP-1.**

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 PDFGFB using MCF-7 cells. Briefly, MCF-7 cells were seeded

into triplicate wells of 96-well plates at a density of 5,000 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, 10 $\mu$ L 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 $^{\circ}$ C. Proliferation of MCF-7 cells after incubation with PDGFB for 96h observed by inverted microscope was shown in Figure 2. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant PDGFB for 96h. The result was shown in Figure 3. It was obvious that PDGFB significantly increased cell viability of MCF-7 cells.



**Figure 2. Cell proliferation of MCF-7 cells after stimulated with IGF2.**

**(A) MCF-7 cells cultured in DMEM, stimulated with 1ng/mL PDGFB for 96h;**

**(B) Unstimulated MCF7 cells cultured in DMEM for 96h.**

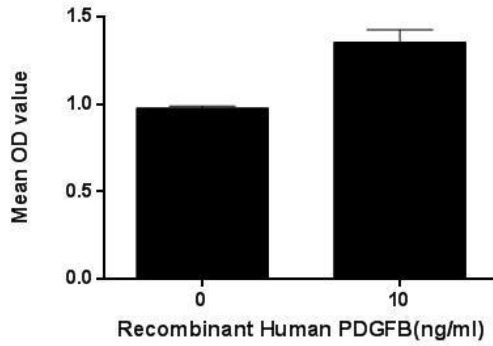


Figure 3. Cell proliferation of MCF-7 cells after stimulated with PDGFB.

### [ IDENTIFICATION ]

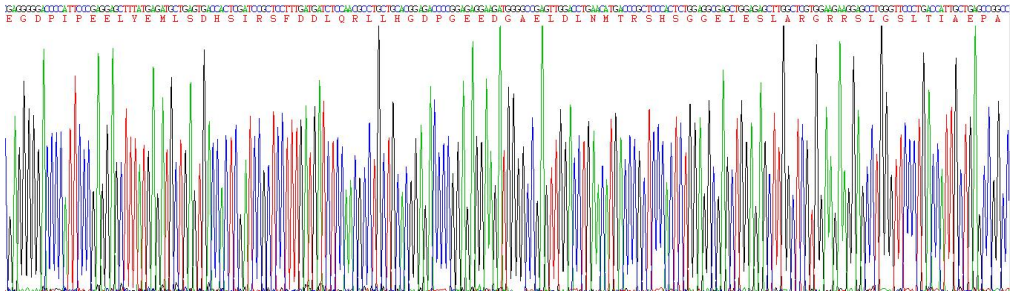


Figure 4. Gene Sequencing (extract)

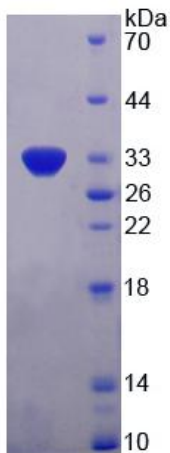
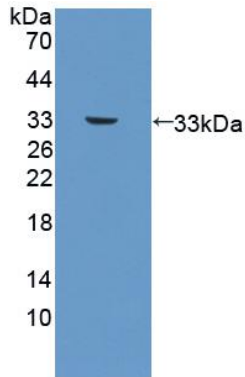


Figure 5. SDS-PAGE

Sample: Active recombinant PDGFB, Human



**Figure 6. Western Blot**

**Sample: Recombinant PDGFB, Human;**

**Antibody: Rabbit Anti-Human PDGFB Ab (PAC921Hu01)**

**[ IMPORTANT NOTE ]**

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.