

Human Platelet-Derived Growth Factor BB (hPDGF-BB)



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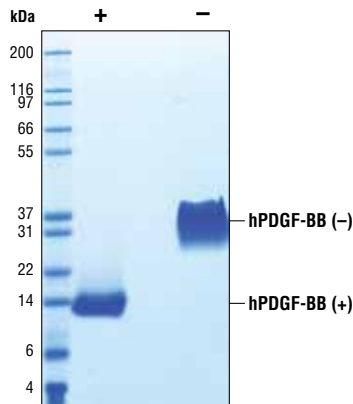
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Source: Recombinant human PDGF-BB (hPDGF-BB) Ser82-Thr190 (Accession #NP_002599) was produced in *E. coli* at Cell Signaling Technology.

Molecular Characterization: Recombinant hPDGF-BB does not have a Met on the amino terminus and has a calculated MW of 12,294. DTT-reduced protein migrates as a 14 kDa polypeptide and the non-reduced cystine-linked homodimer migrates as a 32 kDa protein. The expected amino-terminal SLGSL of recombinant hPDGF-BB was verified by amino acid sequencing.

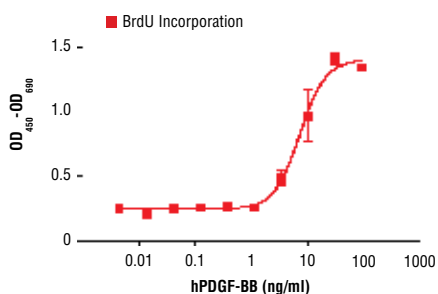
Endotoxin: Less than 0.01 ng endotoxin/1 µg hPDGF-BB.

Purity: >98% as determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hPDGF-BB. All lots are greater than 98% pure.

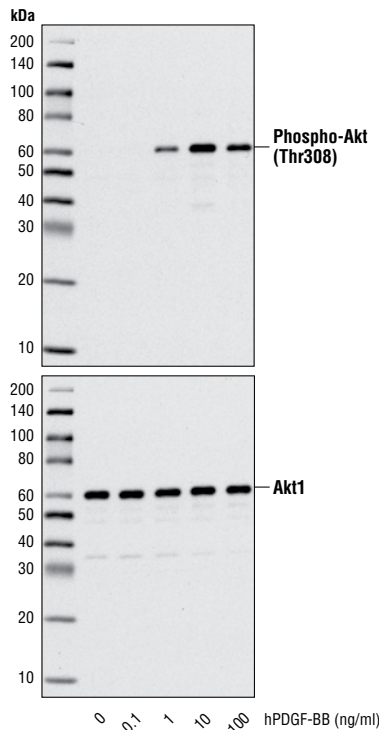


The purity of recombinant hPDGF-BB was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hPDGF-BB and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hPDGF-BB was determined in a NIH/3T3 cell proliferation assay. The ED₅₀ of each lot is between 4.0 - 15 ng/ml.



The proliferation of NIH/3T3 cells treated with increasing concentrations of hPDGF-BB basic was assessed. After 24 hr treatment, cells were labeled with BrdU for 4 hrs. BrdU incorporation was determined by ELISA and the OD₄₅₀-OD₆₉₀ was determined.



Western blot analysis of extracts from NIH/3T3 cells, untreated or treated with hPDGF-BB for 10 minutes, using Phospho-Akt (Thr308) (C31E5E) Rabbit mAb #2965 (upper) and Akt1 (C73H10) Rabbit mAb #2938 (lower).

Formulation: With carrier: Lyophilized from a 0.22 µm filtered solution of 20 mM citrate, pH 3.0 containing 100 mM NaCl and 20 µg BSA per 1 µg hPDGF-BB.

Carrier free: Lyophilized from a 0.22 µm filtered solution of 20 mM citrate, pH 3.0 containing 100 mM NaCl.

Reconstitution:

With carrier: Add sterile 20 mM citrate, pH 3.0 to a final hPDGF-BB concentration of greater than 50 µg/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile 20 mM citrate, pH 3.0 or 20 mM citrate, pH 3.0 containing protein to minimize absorption of hPDGF-BB to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hPDGF-BB should be greater than 50 µg/ml.

Storage: Stable in lyophilized state at 4°C for 1 year after receipt. Sterile stock solutions reconstituted with carrier protein are stable at 4°C for 2 months and at -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Maintain sterility. Storage at -20°C should be in a manual defrost freezer.

Applications: Optimal concentration for the desired application should be determined by the user.

Background: PDGF-BB is a cystine-linked homodimer PDGF family member with key roles in development, cell proliferation, cell survival, and angiogenesis (1,2). PDGF-BB is expressed by vascular endothelium, megakaryocytes and Leydig cells (2). PDGF-BB targets pericytes, fibroblasts, monocytes and other cell types (1-3). PDGF induces fibroblast growth and migration (3) and is a chemoattractant for monocytes and granulocytes. Precursor PDGF-BB is cleaved intracellularly to generate a form that contains a carboxy-terminal stretch that serves to retain PDGF-BB in the extracellular matrix. In a second cleavage event, the carboxy-terminal stretch is removed extracellularly to generate mature PDGF-BB (1,2). PDGF-BB binds to PDGFRβ and induces receptor dimerization. Signaling is through the PI3K/Akt, JNK, and PLCγ pathways (1, 2). PDGF-BB may have a role in some cancer types (2).

Background References:

- (1) Hoch, R.V. and Soriano, P. (2003) *Development* 130, 4769-84.
- (2) Andrae, J. et al. (2008) *Genes Dev* 22, 1276-312.
- (3) Siegbahn, A. et al. (1990) *J Clin Invest* 85, 916-20.