Sterile

#8913

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

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

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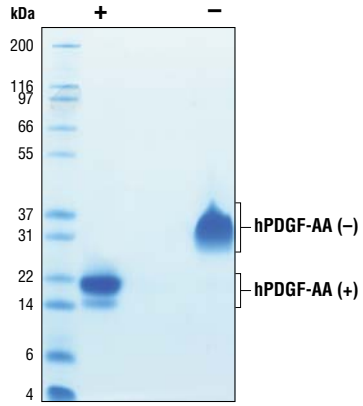
For Research Use Only. Not For Use In Diagnostic Procedures.

Source: Recombinant human PDGF-AA (hPDGF-AA) Ser87-Thr211 (Accession #NP_002598) was produced in *E. coli* at Cell Signaling Technology.

Molecular Characterization: Recombinant hPDGF-AA does not have a Met on the amino terminus and has a calculated MW of 14,305. DTT-reduced protein migrates as an 18 kDa polypeptide and the non-reduced cystine-linked homodimer migrates as a 34 kDa protein. The expected amino-terminal SIEEA of recombinant hPDGF-AA was verified by amino acid sequencing.

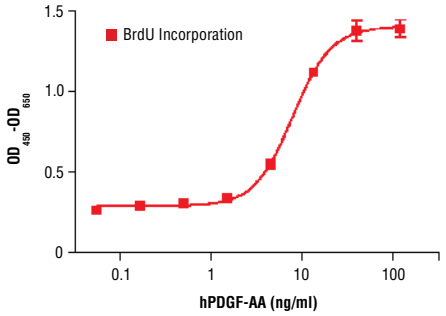
Endotoxin: Less than 0.01 ng endotoxin/1 μg hPDGF-AA.

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

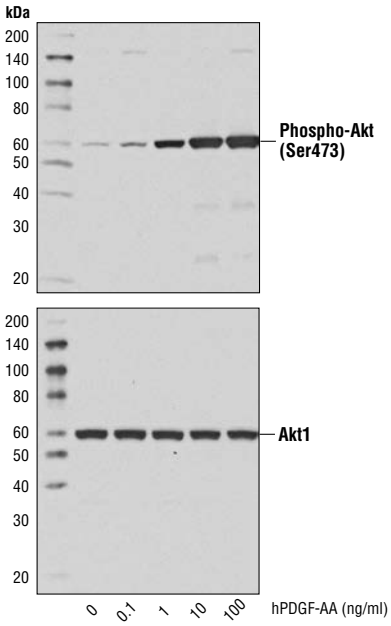


The purity of recombinant hPDGF-AA was determined by SDS-PAGE of 6 μg reduced (+) and non-reduced (-) recombinant hPDGF-AA and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hPDGF-AA was determined in a NIH/3T3 proliferation assay. The ED_{50} of each lot is between 4-12 ng/ml.



The proliferation of NIH/3T3 cells treated with increasing concentrations of hPDGF-AA was assessed. After 24 hr treatment, cells were labeled with BrdU for 4 hrs. BrdU incorporation was determined by ELISA and the OD_{450}-OD_{650} was determined.



Western blot analysis of extracts from NIH/3T3 cells untreated or treated with hPDGF-AA for 10 minutes, using Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb #4060 (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-AA.

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-AA 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-AA to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hPDGF-AA should be greater than 50 μg/ml.

Storage: Stable in lyophilized state at -20°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-AA is integrally involved in embryonic development, angiogenesis and organogenesis and induces fibroblast proliferation and migration (1,2). PDGF-AA is produced by epithelial, muscle, osteosarcoma and neuronal progenitor cells (1,3). Active PDGF-AA is formed through intracellular proteolytic cleavage of a large precursor. PDGF-AA is also concentrated in the extracellular matrix through alternative splicing that generates an extended carboxy-terminal that binds components of the extracellular matrix. The carboxy-terminal stretch is removed extracellularly to generate mature PDGF-AA (1,2). PDGF-AA binding to the PDGFR-α activates the receptor tyrosine kinase (1). PDGF-AA-induced signaling is through the Ras-MAPK, PI3K/AKT and PLCγ pathways (1). Dysregulation of PDGF-AA expression and/or signaling is often associated with cancer and fibrotic disorders (1).

Background References:

(1) Andrae, J. et al. (2008) *Genes Dev* 22, 1276-312.

(2) Hoch, R.V. and Soriano, P. (2003) *Development* 130, 4769-84.

(3) Siegbahn, A. et al. (1990) *J Clin Invest* 85, 916-20.

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