

Human Transforming Growth Factor β 1 (hTGF- β 1)

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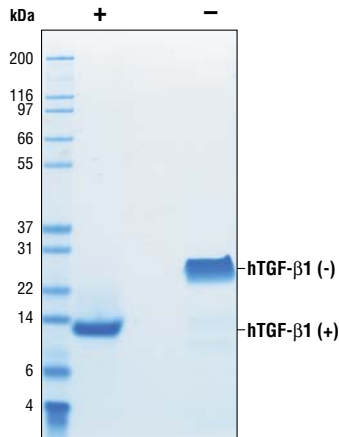
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Source: Recombinant human TGF- β 1 (hTGF- β 1) Ala279-Ser390 (Accession #P01137) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant hTGF- β 1 contains no "tags" and the nonglycosylated protein has a calculated MW of 12,794. DTT-reduced protein migrates as a 13 kDa polypeptide and the non-reduced cystine-linked homodimer migrates as a 25 kDa protein. The expected amino-terminal ALDTN of recombinant hTGF- β 1 was verified by amino acid sequencing.

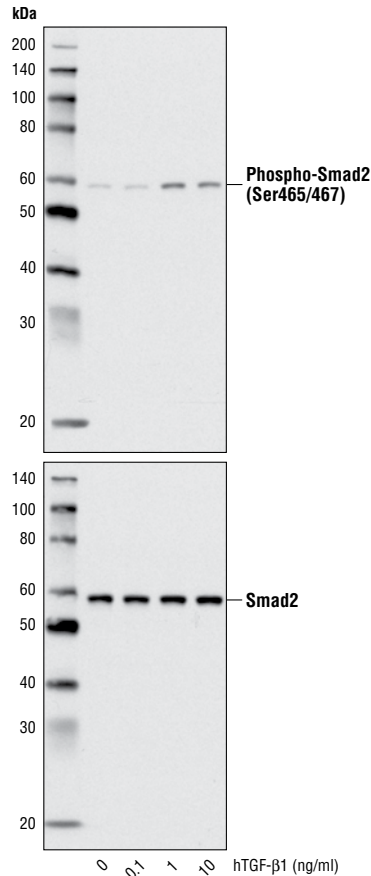
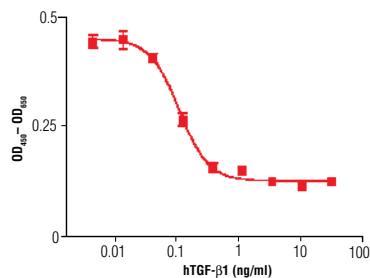
Endotoxin: Less than 0.01 ng endotoxin/1 μ g hTGF- β 1.

Purity: >98% as determined by SDS-PAGE of 6 μ g reduced (+) and non-reduced (-) recombinant hTGF- β 1. Less than 1% migrates as monomer hTGF- β 1 under non-reduced (-) conditions. All lots are greater than 98% pure.



The purity of recombinant hTGF- β 1 was determined by SDS-PAGE of 6 μ g reduced (+) and non-reduced (-) recombinant hTGF- β 1 and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hTGF- β 1 was determined by assessing inhibition of IL-4 induced HT-2 cell proliferation. The ED₅₀ of each lot is between 40–800 pg/ml.



Western blot analysis of extracts from HeLa cells, untreated or treated with hTGF- β 1 for 25 minutes, using Phospho-Smad2 (Ser465/467) (138D4) Rabbit mAb #3108 (upper) and Smad2 (86F7) Rabbit mAb #3122 (lower).

◀ The inhibition of IL-4 induced proliferation in HT-2 cells treated with increasing concentrations of hTGF- β 1 was assessed. After 48 hour treatment with hTGF- β 1, cells were incubated with a tetrazolium salt and the OD₄₅₀ - OD₆₅₀ was determined.

Formulation: With carrier: Lyophilized from a 0.22 μ m filtered solution of 20 mM citrate, pH 3.0 containing 100 mM NaCl and 20 μ g BSA per 1 μ g hTGF- β 1.

Carrier free: Lyophilized from a 0.22 μ m 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 hTGF- β 1 concentration of greater than 50 μ g/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 hTGF- β 1 to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hTGF- β 1 should be greater than 50 μ g/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: TGF- β 1 activities include proliferation, angiogenesis and promotion or inhibition of many immune events (1-3). TGF- β 1 is produced by a number of cell types including regulatory T cells, fibroblasts, epithelial cells, and endothelial cells (3). TGF- β 1 binds to T β RII homodimer, which then complexes with T β RI homodimer (1,4). The oligomeric receptor complex phosphorylates subsets of the SMAD proteins that then act to induce and repress a number of target genes (1,3,4). TGF- β 1 binding can also activate the Erk2, p38, and JNK pathways via TAK1 (4). TGF- β 1 appears to promote late stage progression and metastasis in some cancers (1,2).

Background References:

- (1) Siegel, P.M. and Massagué, J. (2003) *Nat Rev Cancer* 3, 807-21.
- (2) Bierie, B. and Moses, H.L. (2006) *Nat Rev Cancer* 6, 506-20.
- (3) Tian, M. and Schiemann, W.P. (2009) *Future Oncol* 5, 259-71.
- (4) Moustakas, A. and Heldin, C.H. (2009) *Development* 136, 3699-714.