

# Human Transforming Growth Factor $\beta$ 3 (hTGF- $\beta$ 3)

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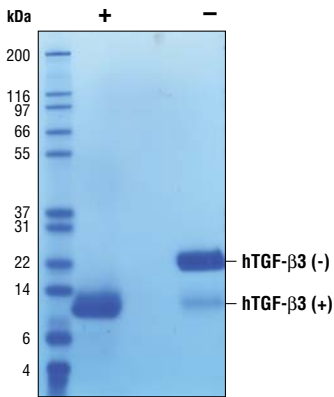
**For Research Use Only. Not For Use In Diagnostic Procedures.**

**Source:** Recombinant human TGF- $\beta$ 3 (hTGF- $\beta$ 3) Ala301-Ser412 (Accession #NP\_003230) was expressed in human 293 cells at Cell Signaling Technology.

**Molecular Characterization:** Recombinant hTGF- $\beta$ 3 contains no "tags" and the nonglycosylated protein has a calculated MW of 12,722. DTT-reduced protein migrates as a 12 kDa polypeptide and the non-reduced cysteine-linked homodimer migrates as a 22 kDa protein. The expected amino-terminal ALDTN of recombinant hTGF- $\beta$ 3 was verified by amino acid sequencing.

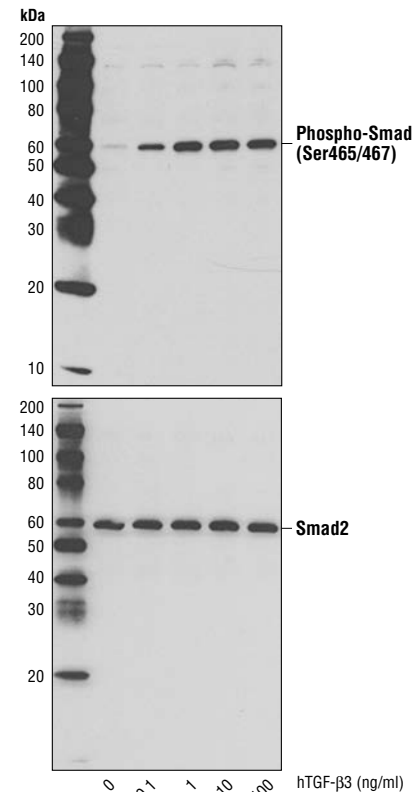
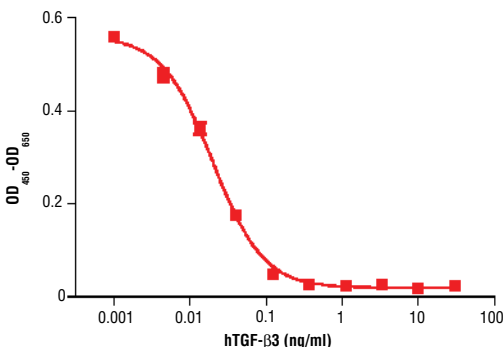
**Endotoxin:** Less than 0.01 ng endotoxin/1  $\mu$ g hTGF- $\beta$ 3.

**Purity:** >98% as determined by SDS-PAGE of 6  $\mu$ g reduced (+) and non-reduced (-) recombinant hTGF- $\beta$ 3. Less than 5% migrates as monomer hTGF- $\beta$ 3 under non-reduced (-) conditions. All lots are greater than 98% pure.



The purity of recombinant hTGF- $\beta$ 3 was determined by SDS-PAGE of 6  $\mu$ g reduced (+) and non-reduced (-) recombinant hTGF- $\beta$ 3 and staining overnight with Coomassie Blue.

**Bioactivity:** The bioactivity of recombinant hTGF- $\beta$ 3 was determined by assessing inhibition of IL-4 induced HT-2 cell proliferation. The ED<sub>50</sub> of each lot is between 20-250 pg/ml.



Western blot analysis of extracts from HeLa cells, untreated or treated with hTGF- $\beta$ 3 for 30 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- $\beta$ 3 was assessed. After 48 hour treatment with hTGF- $\beta$ 3, cells were incubated with a tetrazolium salt and the OD<sub>450</sub>-OD<sub>650</sub> was determined.

**Formulation:** With carrier: Lyophilized from a 0.22  $\mu$ m filtered solution of 20 mM Citrate, pH 3.0 containing 100 mM NaCl and 20  $\mu$ g BSA per 1  $\mu$ g hTGF- $\beta$ 3.

Carrier free: Lyophilized from a 0.22  $\mu$ 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- $\beta$ 3 concentration of greater than 50  $\mu$ 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- $\beta$ 3 to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hTGF- $\beta$ 3 should be greater than 50  $\mu$ g/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:** TGF- $\beta$ 3 can induce cell proliferation and angiogenesis, and can promote some immune events while inhibiting others (1-3). TGF- $\beta$ 3 is produced by chondrocytes, glial cells, astrocytes and some cancer cells (3,4). TGF- $\beta$ 3 binds to T $\beta$ RII homodimer, which then complexes with T $\beta$ RI homodimer (1,5). The oligomeric receptor complex phosphorylates subsets of SMAD proteins that then act to induce or suppress target genes (1,5). Based on knock-out studies in mice, TGF- $\beta$ 3 is critical for lung and palate development (6).

**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) Damstrup, L. et al. (1993) *Br J Cancer* 67, 1015-21.
- (4) Constam, D.B. et al. (1992) *J Immunol* 148, 1404-10.
- (5) Moustakas, A. and Heldin, C.H. (2009) *Development* 136, 3699-714.
- (6) Dünker, N. and Kriegelstein, K. (2000) *Eur J Biochem* 267, 6982-8.