Recombinant Human TGF-β1

Catalog Number: 240-B

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human TGF-beta 1 protein Ala279-Ser390 Accession # P01137.2
N-terminal Sequence Analysis	Ala279
Structure / Form	Disulfide-linked homodimer
Predicted Molecular Mass	12.8 kDa (monomer)

SPECIFICATIONS

SDS-PAGE	12 kDa, reducing conditions 24 kDa, non-reducing conditions
Activity	Measured by its ability to inhibit the IL-4-dependent proliferation of HT-2 mouse T cells. Tsang, M. *et al.* (1995) Cytokine **7**:389. The ED_{50} for this effect is 0.0400-0.200 ng/mL.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>97%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Lyophilized from a 0.2 µm filtered solution in Acetonitrile and TFA with BSA as a carrier protein. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Purified recombinant human TGF-β1 is an extremely hydrophobic protein that adheres strongly to surfaces. To ensure recovery, reconstitute 2 µg vials at 20 µg/mL in sterile 4 mM HCl containing 1 mg/mL human or bovine serum albumin. Reconstitute 10 µg or larger vials at 100 µg/mL in sterile 4 mM HCl containing 1 mg/mL human or bovine serum albumin.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

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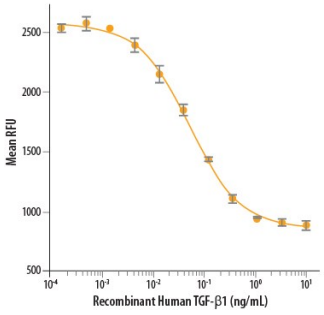
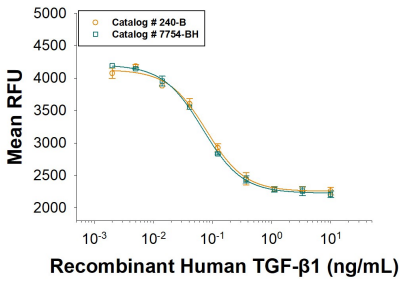
DATA

Bioactivity



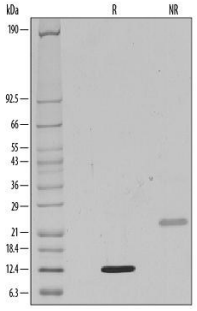
Recombinant Human TGF-beta 1 Protein Bioactivity
Recombinant Human TGF-beta 1 (Catalog # 240-B) inhibits Recombinant Mouse IL-4 (Catalog # Catalog # 404-ML) induced proliferation in the HT-2 mouse T cell line. The ED_{50} for this effect is 0.0400-0.200 ng/mL.

Bioactivity



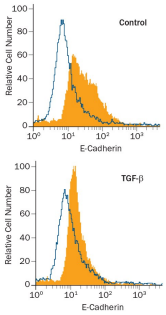
Equivalent bioactivity of CHO-derived and HEK293-derived Recombinant Human TGF-β1.
Equivalent bioactivity of CHO-derived (Catalog # 240-B) and HEK293-derived (Catalog # 7754-BH) Recombinant Human TGF-β1 as measured by its ability to inhibit the IL-4-dependent proliferation of HT-2 mouse T cell. (orange, green, respectively).

SDS-PAGE



Recombinant Human TGF-beta 1 Protein SDS-PAGE 1 µg/lane of Recombinant Human TGF-beta 1 was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by silver staining, showing single bands at 12 kDa and 24 kDa, respectively.

Bioactivity



Recombinant Human TGF-beta 1 Protein Bioactivity
Epithelial to Mesenchymal Transition (EMT) was induced in the A549 human lung carcinoma cell line with cell culture media supplemented with Recombinant Human (rh) TGF-beta 1 (Catalog # 240-B). Control cells were cultured without rhTGF-beta 1. EMT induction was confirmed at 48 h by flow cytometric staining for E-Cadherin (filled; Catalog # Catalog # FAB18381P), an epithelial cell marker, or an isotype control (Catalog # Catalog # IC0041P). TGF-beta 1 decreased the expression of E-Cadherin.

BACKGROUND

TGF-β1 (transforming growth factor beta 1) is one of three closely related mammalian members of the large TGF-β superfamily that share a characteristic cystine knot structure (1-7). TGF-β1, -2 and -3 are highly pleiotropic cytokines that are proposed to act as cellular switches that regulate processes such as immune function, proliferation and epithelial-mesenchymal transition (1-4). Each TGF-β isoform has some non-redundant functions; for TGF-β1, mice with targeted deletion show defects in hematopoiesis and endothelial differentiation, and die of overwhelming inflammation (2). Human TGF-β1 cDNA encodes a 390 amino acid (aa) precursor that contains a 29 aa signal peptide and a 361 aa proprotein (8). A furin-like convertase processes the proprotein to generate an N-terminal 249 aa latency-associated peptide (LAP) and a C-terminal 112 aa mature TGF-β1 (8, 9). Disulfide-linked homodimers of LAP and TGF-β1 remain non-covalently associated after secretion, forming the small latent TGF-β1 complex (8-10). Covalent linkage of LAP to one of three latent TGF-β binding proteins (LTBPs) creates a large latent complex that may interact with the extracellular matrix (9, 10). TGF-β is activated from latency by pathways that include actions of the protease plasmin, matrix metalloproteases, thrombospondin 1 and a subset of integrins (10). Mature human TGF-β1 shares 100% aa identity with pig, dog and cow TGF-β1, and 99% aa identity with mouse, rat and horse TGF-β1. It demonstrates cross-species activity (1). TGF-β1 signaling begins with high-affinity binding to a type II ser/thr kinase receptor termed TGF-β RII. This receptor then phosphorylates and activates a second ser/thr kinase receptor, TGF-β RI (also called activin receptor-like kinase (ALK) -5), or alternatively, ALK-1. This complex phosphorylates and activates Smad proteins that regulate transcription (3, 11, 12). Contributions of the accessory receptors betaglycan (also known as TGF-β RIII) and endoglin, or use of Smad-independent signaling pathways, allow for disparate actions observed in response to TGF-β in different contexts (11).

References:

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