

TGF-B1 Protein: Product Information Sheet

DOCUMENT NUMBER: PROT-DAT-008

CELLARIA

Recombinant TGFB1

Transforming Growth Factor Beta-1 Proprotein, TGF-beta 1,
Latency-associated peptide (LAP)



V1.1 07/27/21 LD

GENERAL INFORMATION

CATALOG NO: CP-TGF Beta 1

Recombinant human TGFB1 is a glycosylated protein containing 112 amino acids (Ala278-Ser390), with a molecular weight of approximately 12.79 kDa. It does not contain additional tags.

Expression system/Source: Protein, purified, expressed in CHO, animal-free product.

Target formulation: Lyophilized from a 0.2 μ M filtered solution in 30 mM Sodium Citrate, 50 mM NaCl, 10mM Glycine pH 4.0

Sterility: Sterile

Purity: >95%, by SDS-PAGE with Coomassie staining and HPLC

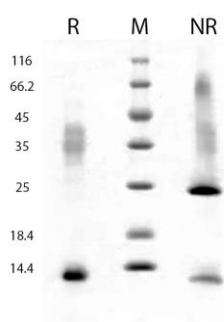
Endotoxin Level: <0.01 EU per 1 μ g of the protein by the LAL method.

UniProt Accession #: P01137

Structure / Isoform: Dimer

Predicted Molecular Mass/ Molecular weight: 12793.58 Da, determined by high-resolution TOF-MS to confirm correct protein sequence.

SDS-PAGE:



~13 kDa, reducing condition, ~25 kDa, non-reducing conditions.

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UniProt Description of TGF:

Multifunctional protein that regulates the growth and differentiation of various cell types and is involved in various processes, such as normal development, immune function, microglia function and responses to neurodegeneration (By similarity). Activation into mature form follows different steps: following cleavage of the proprotein in the Golgi apparatus, Latency-associated peptide (LAP) and Transforming growth factor beta-1 (TGF-beta-1) chains remain non-covalently linked rendering TGF-beta-1 inactive during storage in extracellular matrix (PubMed:29109152). At the same time, LAP chain interacts with 'milieu molecules', such as LTBP1, LRRC32/GARP and LRRC33/NRROS that control activation of TGF-beta-1 and maintain it in a latent state during storage in extracellular milieus (PubMed:2022183, PubMed:8617200, PubMed:8939931, PubMed:19750484, PubMed:22278742, PubMed:19651619). TGF-beta-1 is released from LAP by integrins (ITGAV: ITGB6 or ITGAV: ITGB8): integrin-binding to LAP stabilizes an alternative conformation of the LAP bowtie tail and results in distortion of the LAP chain and subsequent release of the active TGF-beta-1 (PubMed:22278742, PubMed:28117447). Once activated following release of LAP, TGF-beta-1 acts by binding to TGF-beta receptors (TGFBR1 and TGFBR2), which transduce signal (PubMed:20207738).

Sequence:

ALDTNYCFSS TEKNCCVRQL YIDFRKDLGW KWIHEPKGYH ANFCLGPCPY IWSLDTQYSK VLALYNQHNP GASAAPCCVP
QALEPLPIVY YVGRKPKVEQ LSNMIVRSCKCS

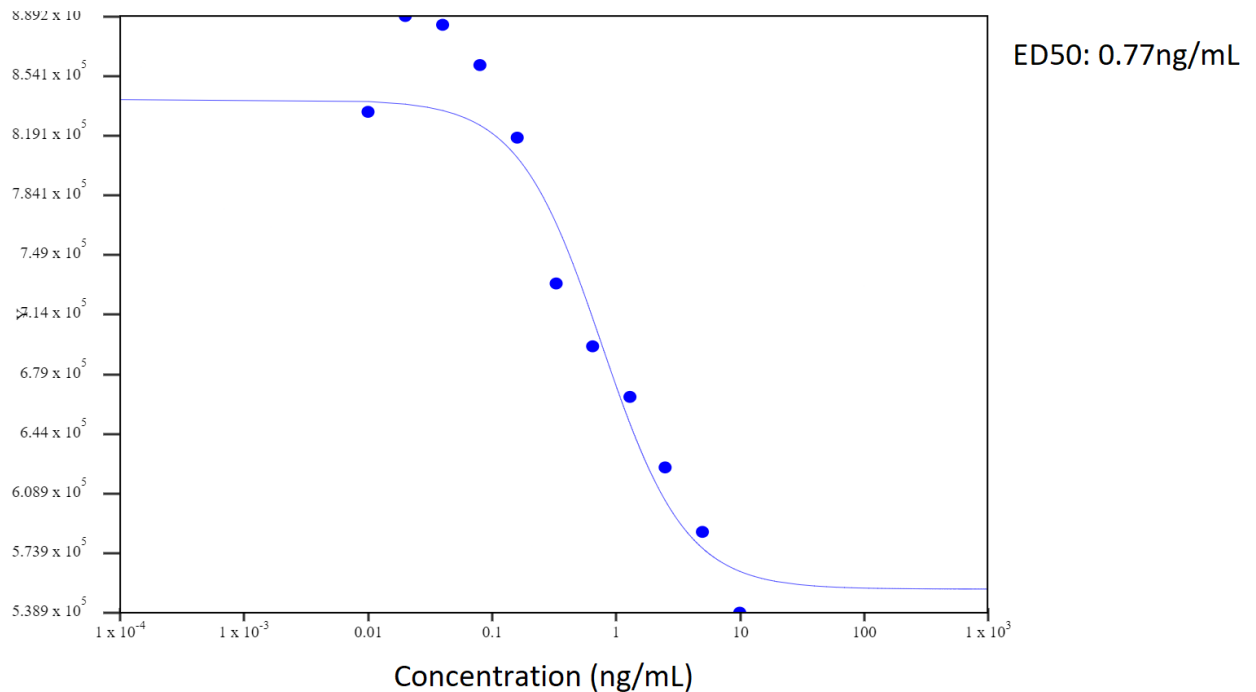
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ED50 ANALYSIS



BIOACTIVITY ASSAY SUMMARY PROTOCOL

The bioactivity (ED50) of TGFβ1 is determined in a 48-hour inhibition of proliferation assay using TF-1 erythroleukemia suspension cells (ATCC CRL-2003) in the presence of 2 ng/mL granulocyte-macrophage colony stimulating factor (GM-CSF), serum free media and CellTiter-Glo® Luminescent Cell Viability Assay (Promega G7570).

Briefly,

1. A stock solution of TGFβ1 is prepared with sterile DPBS and the concentration is confirmed using a NanoDrop spectrophotometer or equivalent piece of equipment.
2. TF-1 suspension cells are seeded at 10,000 cells per well in a 96 well plate in serum free DMEM media (DMEM with 0.1% Bovine Serum Albumin) on **Day 0**.
3. A titration of TGFβ1 is prepared in serum free DMEM at **2X** concentration by serial dilution and added at equal volume to the cell suspension and incubated for 48 hours in a 37°C 5% CO₂ incubator.
4. On **Day 2**, CellTiter-Glo® is added to the plate and luminescence is measured on a plate reader.
5. ED50 analysis is performed and recorded.

PREPARATION AND STORAGE

1. Reconstitute at 1 mg/mL in distilled water (final buffer composition 30 mM Sodium citrate, 50 mM NaCl, 10mM Glycine, pH 4.0)
 - a. *It is recommended to filter the solution after reconstitution.*
2. The product is shipped with cold packs. Upon receipt, store it immediately at the temperature

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recommended below.

3. Avoid repeated freeze-thaw cycles. A minimum of 6 months when stored at $\leq -20\text{ }^{\circ}\text{C}$ as supplied. Refer to lot specific COA for the Use by Date. 1 month, $2\text{ to }4\text{ }^{\circ}\text{C}$ under sterile conditions after reconstitution. 3 months, $\leq -20\text{ }^{\circ}\text{C}$ under sterile conditions after reconstitution.

GRADE:	STORAGE CONDITIONS:	SAFETY PRECAUTIONS:
<input type="checkbox"/> USP	<input type="checkbox"/> Ambient	<input type="checkbox"/> Corrosive
<input type="checkbox"/> NF	<input checked="" type="checkbox"/> $2^{\circ}\text{C to }8^{\circ}\text{C}$	<input type="checkbox"/> Flammable
<input type="checkbox"/> EP	<input checked="" type="checkbox"/> $-25^{\circ}\text{C to }-15^{\circ}\text{C}$	<input checked="" type="checkbox"/> Carcinogen
<input type="checkbox"/> JP	<input type="checkbox"/> $\leq -70^{\circ}\text{C}$	<input checked="" type="checkbox"/> Avoid Inhalation
<input type="checkbox"/> multi-compendial	<input type="checkbox"/> Store desiccated	<input checked="" type="checkbox"/> Avoid Contact with Skin
<input checked="" type="checkbox"/> Other: RUO	<input type="checkbox"/> Store protected from light	<input checked="" type="checkbox"/> Avoid Contact with Eyes
<input type="checkbox"/> N/A	<input type="checkbox"/> Other: _____	<input type="checkbox"/> Other: _____
		<input type="checkbox"/> N/A

REFERENCES

1. Dong X, Zhao B, Iacob RE, et al. Force interacts with macromolecular structure in activation of TGF- β . Nature. 2017;542(7639):55-9.
2. Gleizes PE, Beavis RC, Mazziere R, Shen B, Rifkin DB. Identification and characterization of an eight-cysteine repeat of the latent transforming growth factor-beta binding protein-1 that mediates bonding to the latent transforming growth factor-beta1. J Biol Chem. 1996;271(47):29891-6.
3. Miyazono K, Olofsson A, Colosetti P, Heldin CH. A role of the latent TGF-beta 1-binding protein in the assembly and secretion of TGF-beta 1. EMBO J. 1991;10(5):1091-101.
4. Radaev S, Zou Z, Huang T, Lafer EM, Hinck AP, Sun PD. Ternary complex of transforming growth factor-beta1 reveals isoform-specific ligand recognition and receptor recruitment in the superfamily. J Biol Chem. 2010;285(19):14806-14.
5. Saharinen J, Taipale J, Keski-Oja J. Association of the small latent transforming growth factor-beta with an eight cysteine repeat of its binding protein LTBP-1. EMBO J. 1996;15(2):245-53.
6. Stockis J, Colau D, Coulie PG, Lucas S. Membrane protein GARP is a receptor for latent TGF-beta on the surface of activated human Treg. Eur J Immunol. 2009;39(12):3315-22.
7. Tran DQ, Andersson J, Wang R, Ramsey H, Unutmaz D, Shevach EM. GARP (LRRC32) is essential for the surface expression of latent TGF-beta on platelets and activated FOXP3+ regulatory T cells. Proc Natl Acad Sci U S A. 2009;106(32):13445-50.
8. Wang R, Zhu J, Dong X, Shi M, Lu C, Springer TA. GARP regulates the bioavailability and activation of TGF β . Mol Biol Cell. 2012;23(6):1129-39.
9. Zhao B, Xu S, Dong X, Lu C, Springer TA. Prodomain-growth factor swapping in the structure of pro-TGF- β 1. J Biol Chem. 2018;293(5):1579-89.