

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Ala279-Ser390 Accession # P01137 Manufactured and tested under cGMP guidelines.
N-terminal Sequence Analysis	Ala-Leu-Asp-Thr-Asn-Tyr-(Cys)-Phe-Ser-Ser
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. <i>et al.</i> (1995) Cytokine 7:389. The ED ₅₀ for this effect is 0.04-0.2 ng/mL. The specific activity of recombinant human TGF-β1 GMP is approximately 2.5 x 10 ⁴ U/μg, which is calibrated against human TGF-β1 Standard (NIBSC code: 89/514).
Endotoxin Level	<0.10 EU per 1 μg of the protein by the LAL method.
Purity	>97%, by SDS-PAGE with silver staining.
Host Cell Protein	<0.5 ng per μg of protein when tested by ELISA.
Mycoplasma	Negative when tested in a ribosomal RNA hybridization assay.
Formulation	Lyophilized from a 0.2 μm filtered solution in Acetonitrile and TFA. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 μg/mL in 4 mM HCl.
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. <ul style="list-style-type: none"> ● A minimum of 6 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 3 months, ≤ -20 °C under sterile conditions after reconstitution.

DATA

<p>Bioactivity</p> <p>GMP-grade Recombinant Human TGF-β1 (Catalog # 240-GMP) inhibits Recombinant Mouse IL-4 (Catalog # 404-ML) induced proliferation in the HT-2 mouse T cell line. The ED₅₀ for this effect is 0.04-0.2 ng/mL.</p>	<p>SDS-PAGE</p> <p>1 μg/lane of GMP-grade Recombinant Human TGF-β1 (Catalog # 240-GMP) was resolved with SDS-PAGE under reducing (R) and nonreducing (NR) conditions and visualized by silver staining, showing single bands at 12 kDa and 24 kDa, respectively.</p>
--	---

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 metalloproteinases, 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:

1. Derynck, R. and K. Miyazono (2008) Cold Spring Harbor Laboratory Press, 29.
2. Dunker, N. and K. Kriegstein (2000) Eur. J. Biochem. **267**:6982.
3. Wahl, S.M. (2006) Immunol. Rev. **213**:213.
4. Chang, H. et al. (2002) Endocr. Rev. **23**:787.
5. Lin, J.S. et al. (2006) Reproduction **132**:179.
6. Hinck, A.P. et al. (1996) Biochemistry **35**:8517.
7. Mittl, P.R.E. et al. (1996) Protein Sci. **5**:1261.
8. Derynck, R. et al. (1985) Nature **316**:701.
9. Miyazono, K. et al. (1988) J. Biol. Chem. **263**:6407.
10. Oklu, R. and R. Hesketh (2000) Biochem. J. **352**:601.
11. de Caestecker, M. et al. (2004) Cytokine Growth Factor Rev. **15**:1.
12. Zuniga, J.E. et al. (2005) J. Mol. Biol. **354**:1052.

MANUFACTURING SPECIFICATIONS

GMP Proteins

R&D Systems, a Bio-Techne Brand's GMP proteins are produced according to relevant sections of the following documents: WHO TRS, No. 822, 1992 Annex 1, Good Manufacturing Practices for Biological Products; USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and USP Chapter 92, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.

R&D Systems' quality focus includes:

- Manufactured and tested under an ISO 9001:2015 and ISO 13485:2016 certified quality system
- Documented processes and QA control of documentation and process changes
- Personnel training programs
- Raw material testing and vendor qualification/monitoring
- Fully validated equipment, processes and test methods
- Equipment calibration schedules using a computerized calibration program
- Facility maintenance, safety programs and pest control
- Material review process for variances
- Monitoring of stability over product shelf-life

R&D Systems strives to provide our customers with the analytical characteristics of each product so that customers may determine whether our products are appropriate for their research. The Certificate of Analysis provided contains the following lot specific information:

- N-terminal amino acid analysis, SDS-PAGE analysis, and endotoxin level (as determined by LAL assay) performed on each bulk QC lot, not on individual bottlings of each QC lot
- Post-bottling lot-specific bioassay results (compliance with an established range) and results of microbial bioburden testing (using broth culture, Sabourand's dextrose and blood agar plates with results reported at 3 days and at 7 days)
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

Additional testing and documentation requested by the customer can be arranged at an additional cost. Testing may include, but is not limited to, USP <61> bioburden testing, positive identity testing, testing for adventitious agents and testing for residual host cell content.

Production records and facilities are available for examination by appropriate personnel on-site at R&D Systems in Minneapolis, Minnesota USA.

R&D Systems sells its GMP grade recombinant protein products for research use or further manufacturing use in *ex vivo* cell therapy applications. They are not for *in vivo* use or for use as therapeutic or other drugs, biologic products or devices. Please read the following End User Terms prior to using this product.

PRODUCT SPECIFIC NOTICES

END USER TERMS OF USE OF PRODUCT

The following terms are offered to you upon your acceptance of these End User Terms of Use of Product. By using this product, you indicate your acknowledgment and agreement to these End User Terms of Use of Product. If you do not agree to be bound by and comply with all of the provisions of these End User Terms of Use of Product, you should contact your supplier of the product and make arrangements to return the product.

We suggest you print and retain a copy of these End User Terms of Use of Product for your records.

The End User is aware that R&D Systems, Inc. sells its GMP products for research use only or further manufacturing and not for *in vivo* use, the production of therapeutics or other drugs or for biologic products or devices. The End User further agrees, as a condition of the sale of R&D Systems' GMP products that: a) the End User will not use this GMP Product in any procedure wherein the product may be directly or indirectly administered to humans, unless the End User has obtained, or prior to their use will have obtained, an Investigational New Drug (IND) exemption from the FDA and will use the product only in accordance with the protocols of such IND and of the Institutional Review Board overseeing the proposed research, or b) the End User will use the products outside of the United States in accordance with the protocols of research approved by the Institutional Review Board or authorized ethics committee and regulatory agencies to which the End User is subject to in their territory.

R&D Systems, Inc. has the right, at its sole discretion, to modify, add or remove any terms or conditions of these End User Terms of Use without notice or liability to you. Any changes to these End User Terms of Use are effective immediately following the printing of such changes on this product insert. The most recent version of these End User Terms of Use of Product may be found at: RnDSystems.com/Legal.

You agree to review these End User Terms of Use of Product to ensure any subsequent use by you of R&D Systems' GMP Products following changes to these End User Terms of Use of Product constitutes your acceptance of all such changes.

TERMS AND CONDITIONS

The following limitation applies to R&D Systems' warranty and liability for damages: All products are warranted to meet R&D Systems' published specifications when used under normal laboratory conditions.

R&D SYSTEMS DOES NOT MAKE ANY OTHER WARRANTY OR REPRESENTATION WHATSOEVER, WHETHER EXPRESS OR IMPLIED, WITH RESPECT TO ITS PRODUCTS. IN PARTICULAR, R&D SYSTEMS DOES NOT MAKE ANY WARRANTY OF SUITABILITY, NONINFRINGEMENT, MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

NOTWITHSTANDING ANY OTHER PROVISIONS OF THESE TERMS AND/OR ANY OTHER AGREEMENT BETWEEN R&D SYSTEMS AND PURCHASER FOR THE PURCHASE OF THE PRODUCTS, R&D SYSTEMS' TOTAL LIABILITY TO PURCHASER ARISING FROM OR IN RELATION TO THESE TERMS, AN AGREEMENT BETWEEN THE PARTIES OR THE PRODUCTS, WHETHER ARISING IN CONTRACT, TORT OR OTHERWISE SHALL BE LIMITED TO THE TOTAL AMOUNT PAID BY PURCHASER TO R&D SYSTEMS FOR THE APPLICABLE PRODUCTS. IN NO EVENT WILL R&D SYSTEMS BE LIABLE FOR THE COST OF PROCUREMENT OF SUBSTITUTE GOODS.

Full details of R&D Systems' Terms and Conditions of Sale can be found online at: RnDSystems.com/Legal.