

DESCRIPTION

Source	<i>E. coli</i> -derived human Thrombopoietin/Tpo protein Ser22-Leu195 Accession # NP_000451 Produced in an animal-free laboratory. Manufactured and tested under cGMP guidelines
N-terminal Sequence Analysis	Ala-Ser22-Pro-Ala-Pro-Pro-Ala-(Cys)-Asp-Leu
Predicted Molecular Mass	18.7 kDa

SPECIFICATIONS

SDS-PAGE	19 kDa, reducing conditions
Activity	Measured in a cell proliferation assay using MO7e human megakaryocytic leukemic cells. Avanzi, G. <i>et al.</i> (1988) Br. J. Haematol. 69 :359. The ED ₅₀ for this effect is 0.05-0.5 ng/mL. The specific activity of Recombinant Human Thrombopoietin is >1 x 10 ⁷ units/mg, which is calibrated against the human Thrombopoietin reference standard (NIBSC code: 03/124).
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Host Cell Protein	<5.00 ng per µg of protein when tested by ELISA.
Mycoplasma	Negative when tested in a ribosomal RNA hybridization assay.
Host Cell DNA	< 0.0010 ng per µg of protein when tested by PCR.
Formulation	Lyophilized from a 0.2 µm filtered solution in Sodium Acetate. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100-200 µg/mL in sterile, deionized water.
Shipping	The product is shipped with polar packs. 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>Recombinant Human Thrombopoietin GMP Protein Bioactivity GMP-grade Recombinant Human Thrombopoietin (Catalog # 288E-GMP) stimulates proliferation in the MO7e human megakaryocytic leukemic cell line. The ED₅₀ for this effect is 0.05-0.5 ng/mL. Three independent lots were tested for activity and plotted on the same graph to show lot-to-lot consistency of GMP Thrombopoietin.</p>	<p>SDS-PAGE</p> <p>Recombinant Human Thrombopoietin GMP Protein SDS-PAGE 2 µg/lane of GMP-grade Recombinant Human Thrombopoietin (Catalog # 288E-GMP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 19 kDa and 18 kDa, respectively.</p>
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BACKGROUND

Thrombopoietin (Tpo), is a key regulator of megakaryocytopoiesis and thrombopoiesis. It is principally produced in the liver and is bound and internalized by the receptor Tpo R/c-mpl. Defects in the Tpo-Tpo R signaling pathway are associated with a variety of platelet disorders (1-3). The 353 amino acid (aa) human Tpo precursor is cleaved to yield the 332 aa mature protein. Mature human Tpo shares approximately 70% aa sequence homology with mouse and rat Tpo. It is an 80-85 kDa protein that consists of an N-terminal domain with homology to Erythropoietin (Epo) and a C-terminal domain that contains multiple N-linked and O-linked glycosylation sites (4, 5). Tissue specific alternate splicing of human Tpo generates multiple isoforms with internal deletions, insertions, and/or C-terminal substitutions (6). Tpo promotes the differentiation, proliferation, and maturation of MK and their progenitors (4, 5, 7). Several other cytokines can promote these functions as well but only in cooperation with Tpo (8, 9). Notably, IL-3 independently induces MK development, although its effects are restricted to early in the MK lineage (8, 9). Tpo additionally promotes platelet production, aggregation, ECM adhesion, and activation (10-13). It is cleaved by platelet-derived thrombin following Arg191 within the C-terminal domain and subsequently at other sites upon extended digestion (14). Both full length Tpo and shorter forms circulate in the plasma, with the shorter, N-terminal EPO-like domain forms showing significantly increased specific activity (4, 5, 15). The C-terminal domain is not required for binding to Tpo R or inducing MK growth and differentiation (5). Aside from its hematopoietic effects, Tpo is expressed in the brain where it promotes the apoptosis of hypoxia-sensitized neurons and inhibits neuronal differentiation by blocking NGF induced signaling (16, 17).

References:

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MANUFACTURING SPECIFICATIONS

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.

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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 testing according to USP
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

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