

DESCRIPTION

Source Human embryonic kidney cell, HEK293-derived human Thrombopoietin/Tpo protein
Ser22-Leu195, with a C-terminal 6-His tag
Accession # P40225.1

N-terminal Sequence Analysis Ser22

Predicted Molecular Mass 19 kDa

SPECIFICATIONS

SDS-PAGE 26-30 kDa, under reducing conditions.

Activity Measured in a cell proliferation assay using MO7e human megakaryocytic leukemic cells. Avanzi, G. *et al.* (1988) Br. J. Haematol. **69**:359. The ED₅₀ for this effect is 0.0500-0.500 ng/mL.

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.

Formulation Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution Reconstitute the 10 µg size at 100 µg/mL in PBS. Reconstitute all other sizes at 500 µg/mL in PBS.

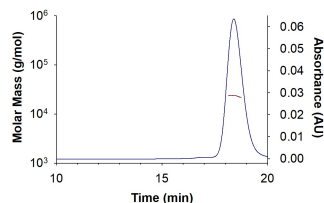
Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

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.

DATA

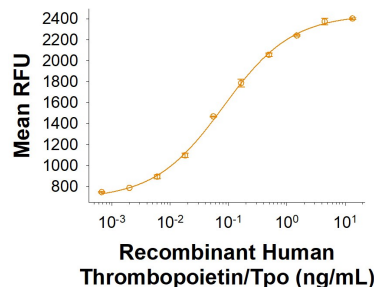
SEC-MALS



SEC-MALS Data	
Retention Time	18.2 - 18.8 min
MW - Predicted (Monomer)	19.0 kDa
MW - MALS	23.3 kDa
Polydispersity	1.001
System Suitability: BSA Monomer 66.4 ± 3.32 kDa	Pass

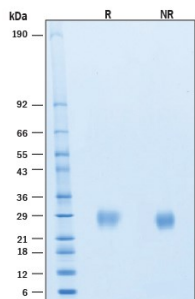
Recombinant Human Thrombopoietin/Tpo His-tag (HEK-293-expressed) Protein SEC-MALS. Recombinant Human Thrombopoietin (Catalog # 288-TPH) has a molecular weight (MW) of 23.3 kDa as analyzed by SEC-MALS, suggesting that this protein is a monomer. MW may differ from predicted MW due to post-translational modifications (PTMs) present (i.e. Glycosylation).

Bioactivity



Recombinant Human Thrombopoietin/Tpo His-tag (HEK-293-expressed) Protein Bioactivity. Recombinant Human Thrombopoietin/Tpo His-tag Protein (HEK-293-expressed) (Catalog # 288-TPH) stimulates proliferation in the MO7e human megakaryocytic leukemic cell line. The ED₅₀ for this effect is 0.0500-0.500 ng/mL.

SDS-PAGE



Recombinant Human Thrombopoietin/Tpo His-tag (HEK-293-expressed) Protein SDS-PAGE. 2 µg/lane of Recombinant Human Thrombopoietin/Tpo His-tag (HEK-293-expressed) Protein (Catalog # 288-TPH) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 26-30 kDa.

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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