# Recombinant Human Thrombopoietin

Catalog Number: 288E-GMP

## DESCRIPTION

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|---|---|
| **Source** | *E. coli*-derived human Thrombopoietin/Tpo protein<br>Ser22-Leu195<br>Accession # NP_000451.1<br>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

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|---|---|
| **SDS-PAGE** | 19 kDa, 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 $ED_{50}$ for this effect is 0.05-0.5 ng/mL.<br><br>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 for Mycoplasma. |
| **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

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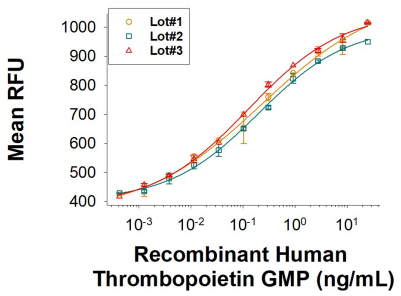
| **Reconstitution** | Reconstitute at 100-200 µg/mL in sterile, deionized water. |
| **Shipping** | The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below. |
| **Stability & Storage** | **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**<br>• A minimum of 12 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date.<br>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.<br>• 3 months, ≤ -20 °C under sterile conditions after reconstitution. |

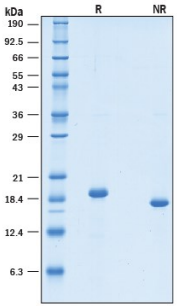
## DATA

**Bioactivity**




**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 $ED_{50}$ 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.

**SDS-PAGE**




**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.



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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:**

1. Deutsch, V.R. and A. Tomer (2006) Br. J. Haematol. **134**:453.
2. Kaushansky, K. (2005) J. Clin. Invest. **115**:3339.
3. Li, J. *et al.* (1999) Br. J. Haematol. **106**:345.
4. Bartley, T.D. *et al.* (1994) Cell **77**:1117.
5. de Sauvage, F.J. *et al.* (1994) Nature **369**:533.
6. Marcucci, R. and M. Romano (2008) Biochim. Biophys. Acta **1782**:427.
7. Kaushansky, K. *et al.* (1994) Nature **369**:568.
8. Kaushansky, K. *et al.* (1995) Proc. Natl. Acad. Sci. **92**:3234.
9. Broudy, V.C. *et al.* (1995) Blood **85**:1719.
10. Lok, S.I. *et al.* (1994) Nature **369**:565.
11. Chen, J. *et al.* (1995) Blood **86**:4054.
12. Oda, A. *et al.* (1996) Blood **87**:4664.
13. Van Os, E. *et al.* (2003) Br. J. Haematol. **121**:482.
14. Kato, T. *et al.* (1997) Proc. Natl. Acad. Sci. **94**:4669.
15. Foster, D. & Hunt, P. (1997) Thrombopoiesis and Thrombopoietins **13**:203.
16. Ehrenreich, H. *et al.* (2005) Proc. Natl. Acad. Sci. **102**:862.
17. Samoylenko, A. *et al.* (2008) Cell. Signal. **20**:154.



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- Mycoplasma testing by ribosomal RNA hybridization assay

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