

Thrombopoietin Active Protein | Thpo active protein

mouse Thrombopoietin Recombinant

CATALOG #	HOST
AAA76396	E coli (Note: Host protein expression may be lot-specific and can be subject to change during production, including bulk orders.)

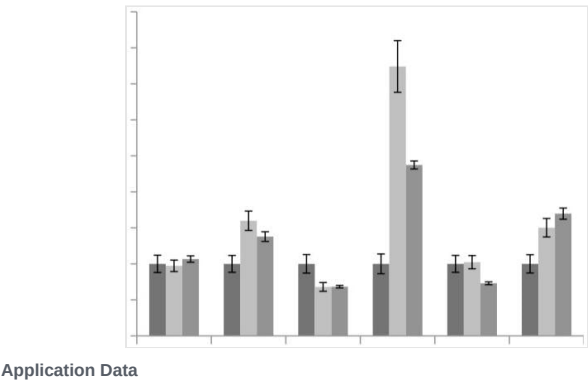
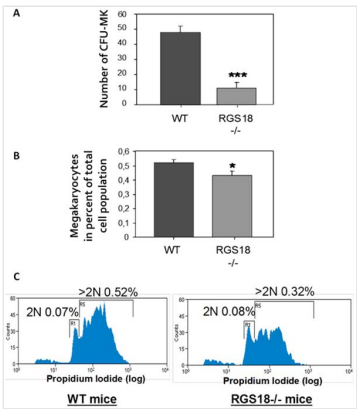
PRODUCT SYNONYMS

Thrombopoietin; mouse Thrombopoietin Recombinant; Thpo active protein

PRODUCT SPECIFICATIONS

Host	E coli (Note: Host protein expression may be lot-specific and can be subject to change during production, including bulk orders.)
Purity/Purification	>95%, as determined by SDS-PAGE and HPLC.
Form/Format	Recombinant mouse TPO was lyophilized from a 0.2 um filtered PBS solution.
Sequence	MELTDLLAA MLLAVARLTL SSPVAPACDP RLLNKLLRDS HLLHSRLSQC PDVDPLSIPV LLPAVDFSLG EWKTQTEQSK AQDILGAVSL LLEGVMAARG QLEPSCSSSL LGQLSGQVRL LLGALQGLLG TQLPLQGRTT AHKDPNALFL SLQQLLRGKV RFLLLVEGPT LCVRRTLPTT AVPSSTSQLL TLNKFNRSTS GLETNFSVT ARTAGPGLLS RLQGFRVKIT PGQLNQTSRS PVQISGYLNR THGPVNGTHG LFACTSLQTL EASDISPGAF NKGSLAFNLQ GGLPPSPSLA PDGHTPFPPS PALPTTHGSP PQLHPLFPDP STTMPNSTAP HPVTMYPHPR NLSQET
Source	Optimized DNA sequence encoding mouse TPO erythropoietin like domain was expressed in Escherichia Coli.
Endotoxin	Endotoxin content was assayed using a LAL gel clot method. Endotoxin level was found to be less than 0.1 ng/ug (1EU/ug).
Biological Activity	The ED(50) was determined by the dose-dependent proliferation of human MO7e cells was found to be less than 0 ng/ml.

Method	Expansion of CD34+ cells in serum free mediumIsolated CD34+ cells were seeded at a density of 5×10⁴cells per 500 ul of medium'>Stempro medium, in 24-well tissue culture plates. Growth factors used were IL-6, SCF, TPO, and Flt-3-L at a final concentration of 25 ng/ml with (test cells) and without (control cells) the addition of either zVADfmk -100 nM or zLLYfmk -10 uM (MP,,). After the 10th day of culture, the cells were collected from the suspension culture, and centrifuged (1000 rpm for 5 minutes). The cells were used for assessing the in vitro homing properties or were used for in vivo homing studies in NOD/SCID mice.Lentiviral transduction of bone marrow cellsFreshly isolated bone marrow (BM) cells were plated in Iscove's Modified Dulbecco's Medium (IMDM) (31980,,) containing 5% fetal bovine serum (FBS), 1% penicillin/streptomycin, 200 mM glutamine, 1% non-essential amino acids, 1% sodium pyruvate, 50 uM 2-mercaptoethanol, stem cell factor (SCF, 10 ng/ml,,), Flt3-L (10 ng/ml), IL-11 (10 ng/ml), thrombopoietin (TPO, 10 ng/ml), IL-6 (10 ng/ml), and IL-3 (10 ng/ml). Bone marrow cells were spin-infected with 8 ug/ml polybrene and lentivirus (MOI of 0.1-0.5), at 2,500 rpm for 90 min. BM cells were then incubated for 3 hours at 37 degree C, counted and injected into NOD.SCID mice.Generation and isolation of MLL-AF9 leukemia cellsMLL-AF9 mouse leukemias were generated in Dr. David Scadden's laboratory as described below. Actin-DsRED mice (JAX) were backcrossed for 10 generations onto C57BL/6J mice (JAX). These mice were sacrificed four days after injection with 150 mg/kg 5FU. BM cells were isolated from femurs and tibias, and red blood cells were lysed using ACK lysing buffer. Cells were incubated in RPMI supplemented with 20% FBS, 1% Pen-Strep, 6 ng/ml of IL-3, 10 ng/ml of TPO, 10 ng/ml of IL-6, at 37 degree C 5% CO₂ overnight. MLL-AF9 was introduced by spin-infection (1 000 g for 90 minutes) using a retroviral vector (MSCV-MLL-AF9-neo) 6 live cells per mouse were injected into lethally irradiated (9Gy) C57BL/6 recipient mice 12 hours after viral infection. The mice were sacrificed once they became moribund. BM cells were isolated as described above, and subjected to ACK lysis. 200 000 cells were injected into sublethally irradiated (4.5 Gy) C57BL/6 recipients. Once these mice were moribund, ACK-lysed live BM cells were isolated as described above and 1×10⁶ cells/mouse were used for intramuscular injections.Single Cell Hematopoietic Colony Forming in vitro Assaycelltype'>BM-derived celltype'>cells were isolated, stained as described above using combination of CD45-FITC, Lin-PE, Sca-1-PE-Cy5, CD105-PE-Cy7 or c-Kit-APC-Cy7 antibodies, and sorted with MoFlo XPD cell sorter. Only DAPI-negative cells with integral membrane were chosen, using gating strategy presented in 2), then wells with colonies were counted, the colonies were harvested, diluted with PBS to final volume of 250 ul, cytopspined (1,000 rpm, 10 minutes, room temperature), air-dried, and stained using's method with Hemacolor Kit.Colony-forming unit assaysMouse BM cells were harvested via flushing of the long bones with medium'>Dulbecco medium'>modified medium'>Eagle medium (DMEM;,,,) supplemented with 10% fetal bovine serum (FBS,,,), was followed by filtering through a 70-um nylon mesh cell strainer, to remove bone debris. BM mononuclear cells were cultured in medium'>MethoCult medium'>M3231 medium supplemented with 50 ng/ml Thrombopoietin (TPO,,), for 7 days according to the manufacturer's protocols. Colonies containing >3 MKs were counted as CFU-MKs. Duplicate assays were performed for each mouse. At least two mice were analyzed for each sample group.
Molecular Function	Cytokine, Hormone
Reconstitution	A quick spin of the vial followed by reconstitution in distilled water to a concentration not less than 0.1 mg/mL. This solution can then be diluted into other buffers.
Preparation and Storage	The lyophilized protein is stable for at least years from date of receipt at-20 degree C. Upon reconstitution, this cytokine can be stored in working aliquots at 2 degree -8 degree C for one month, or at-20 degree C for six months, with a carrier protein without detectable loss of activity. Avoid repeated freeze/thaw cycles.



Application Data
RGS18^{-/-} mice present a defective megakaryopoiesis. Lin⁻ bone marrow cells (5×10⁴ cells/chamber) from 9/10-week-old WT and RGS18^{-/-} mice (n=25 per group) were cultured in Mega-Cult-C media containing 50 ng/mL rhTPO, 10 ng/ml rmlL3, and 3 ng/ml rmlL6 in double-chamber slides at 37°C for 11 days.

ADDITIONAL INFORMATION

Product Categories/Family for Thpo active protein	Hematopoietins; Single chain family; Recombinant Mouse Cytokines
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NCBI AND UNIPROT INFORMATION

NCBI GI #	291045237
NCBI GeneID	21832
NCBI Accession #	NP_001166976.1
NCBI GenBank Nucleotide #	NM_001173505.1
UniProt Accession #	P40226
Molecular Weight	160,000
NCBI Official Full Name	thrombopoietin
NCBI Official Synonym Full Names	thrombopoietin
NCBI Official Symbol	Thpo
NCBI Official Synonym Symbols	MI; Tpo; Mgdf; Tpo1; Tpo2; Tpo3; Tpo4; Mpllg
NCBI Protein Information	thrombopoietin; C-mpl ligand; megakaryocyte colony-stimulating factor; megakaryocyte growth and development factor; myeloproliferative leukemia virus oncogene ligand
UniProt Protein Name	Thrombopoietin
UniProt Gene Name	Thpo
UniProt Synonym Gene Names	ML; MGDF
UniProt Entry Name	TPO_MOUSE

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