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APA135Mu61 100µg Active Thrombopoietin (TPO) Organism Species: *Mus musculus (Mouse) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Ser22~Thr356

Tags: N-terminal His Tag and C-terminal Fc Region of Human IgG1

Purity: >90%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 9.6

Predicted Molecular Mass: 66.6kDa

Accurate Molecular Mass: 90kDa as determined by SDS-PAGE reducing conditions. Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

- 1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
- 2. Relative charge: The composition of amino acids may affects the charge of the protein.
- 3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.

4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.

5. Polymerization of the target protein: Dimerization, multimerization etc.

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[<u>USAGE</u>]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[<u>SEQUENCE</u>]

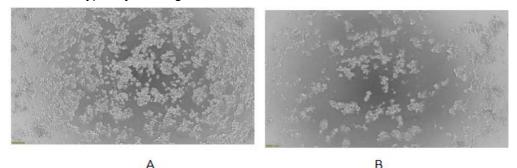
SPVAPACDP RLLNKLLRDS HLLHSRLSQC PDVDPLSIPV LLPAVDFSLG EWKTQTEQSK AQDILGAVSL LLEGVMAARG QLEPSCLSSL LGQLSGQVRL LLGALQGLLG TQLPLQGRTT AHKDPNALFL SLQQLLRGKV RFLLLVEGPT LCVRRTLPTT AVPSSTSQLL TLNKFPNRTS GLLETNFSVT ARTAGPGLLS RLQGFRVKIT PGQLNQTSRS PVQISGYLNR THGPVNGTHG LFAGTSLQTL EASDISPGAF NKGSLAFNLQ GGLPPSPSLA PDGHTPFPPS PALPTTHGSP PQLHPLFPDP STTMPNSTAP HPVTMYPHPR NLSQET

[<u>ACTIVITY</u>]

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. The activity of recombinant mouse TPO was measured in a cell proliferation assay using MO7e human megakaryocytic leukemic cells. MO7e cells were seeded into triplicate wells of 96-well plates at a density of 30,000 cells/well in RPMI-1640 with the addition of various concentrations of rmTPO. After incubated for 72h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 μ l of CCK-8 solution was added to each well of the plate, then

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the absorbance at 450 nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37 ° C. Cell proliferation of MO7e cells after incubation with rmTPO for 72h observed by inverted microscope was shown in Figure 1. The dose-effect curve of rmTPO was shown in Figure 2. It was obvious that rmTPO significantly promoted cell proliferation of MO7e cells .The EC50 for this effect is typically 0.26 ug/ml.





(A) MO7e cells cultured in RPMI-1640 , stimulated with 0.31 ug/ml rMTPO for 72h;(B) Unstimulated MO7e cells cultured in RPMI-1640 for 72h.

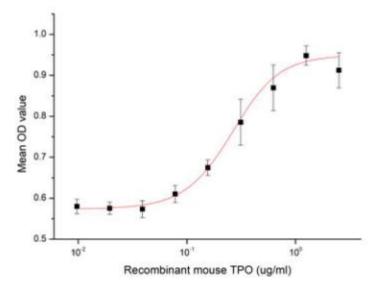


Figure 2. The dose-effect curve of rmTPO on MO7e cells

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[IDENTIFICATION]

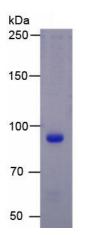


Figure 3. SDS-PAGE Sample: Active recombinant TPO, Mouse

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.