APA135Mu01 10μg Active Thrombopoietin (TPO) Organism Species: *Mus musculus (Mouse) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition (Revised in Aug, 2016)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Ser22~Thr356 Tags: N-terminal His-tag Purity: >90% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose . Original Concentration: 600µg/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: 39.3kDa Accurate Molecular Mass: 40kDa as determined by SDS-PAGE reducing conditions.

[<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

[ACTIVITY]

Thrombopoietin (TPO) is a humoral growth factor that is necessary for megakaryocyte proliferation and maturation, as well as for thrombopoiesis.TPO can promote platelet production, aggregation, ECM adhesion, and activation. It is principally produced in the liver and is bound and internalized by the receptor Tpo R/c-mpl. This protein is the ligand for MLP/C MPL, the product of myeloproliferative leukemia virus oncogene. Mutations in this gene are the cause of thrombocythemia 1. Besides, GCSF has been identified as an interactor of TPO, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant mouse TPO and recombinant mouse GCSF. Briefly, biotin-linked TPO were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to GCSF-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30 min, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C. Finally, add 50 µl stop solution to the wells and read at 450 nm immediately. The binding activity of TPO and GCSF was shown in Figure 1, the EC50 for this effect is 0.68 ug/mL.



Figure 1. The binding activity of recombinant mouse TPO and recombinant mouse GCSF

[IDENTIFICATION]

	kDa 70
-	44
H	33
	26
	22
	18
	14
	10

Figure 2. 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.