APA855Mu01 10µg Active Receptor Activator Of Nuclear Factor Kappa B Ligand (RANkL) 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: Prokaryotic expression. Host: *E. coli* Residues: Leu92~Trp263 Tags: Two N-terminal Tags, His-tag and GST-tag Purity: >80% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 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: 7.1 Predicted Molecular Mass: 49.3kDa Accurate Molecular Mass: 498.34kDa 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.

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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.

[SEQUENCE]

LRLHENADL QDSTLESEDT LPDSCRRMKQ AFQGAVQKEL QHIVGPQRFS GAPAMMEGSW LDVAQRGKPE AQPFAHLTIN AASIPSGSHK VTLSSWYHDR GWAKISNMTL SNGKLRVNQD GFYYLYANIC FRHHETSGSV PTDYLQLMVY VVKTSIKIPS SHNLMKGGST KNW

[ACTIVITY]

Receptor activator of nuclear factor kappa-B ligand (RANKL), also known as tumor necrosis factor ligand superfamily member 11 (TNFSF11), TNF-related activation-induced cytokine (TRANCE), osteoprotegerin ligand (OPGL), and osteoclast differentiation factor (ODF), is a protein that in humans is encoded by the TNFSF11 gene. RANKL is a member of the tumor necrosis factor (TNF) cytokine family, it binds to RANK on cells of the myeloid lineage and functions as a key factor for osteoclast differentiation and activation. It may also bind to osteoprotegerin, a protein secreted mainly by cells of the osteoblast lineage which is a potent inhibitor of osteoclast formation by preventing binding of RANKL to RANK. V-Fos FBJ Murine Osteosarcoma Viral Oncogene Homolog (FOS) has been identified as an interactor of RANKL, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant mouse RANKL and recombinant human FOS. Briefly, RANKL were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 ul were then transferred to FOS-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-RANKL pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 5 times. With the addition of

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substrate solution , wells were incubated 15-25 minutes at 37 $^\circ$ C. Finally, add 50 μ L stop solution to the wells and read at 450 nm immediately. The binding activity of recombinant mouse RANKL and recombinant human FOS was shown in Figure 1, the EC50 for this effect is 0.08 ug/mL.

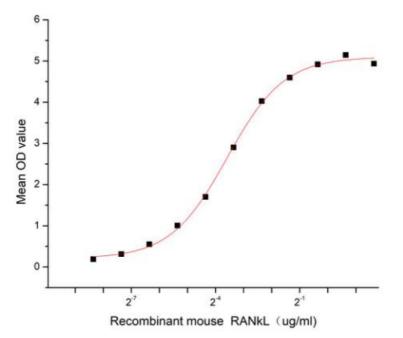
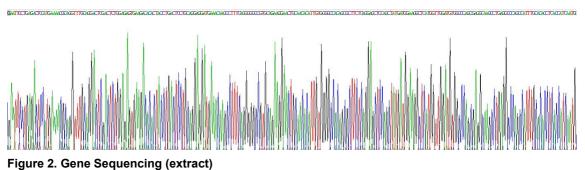


Figure 1. The binding activity of recombinant mouse RANKL and recombinant human

FOS

[IDENTIFICATION]



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-	kDa 70
-	44
-	33
	26
	22
	18
	14
	10

Figure 3. SDS-PAGE

Sample: Active recombinant RANkL, 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.