APA855Mu61 100µg 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: Eukaryotic expression. Host: 293F cell Residues: Arg72~Asp316 Tags: N-terminal His-tag **Purity: >95% Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method). **Buffer Formulation:** PBS, pH7.4, containing 5% Trehalose. Original Concentration: 50µg/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: 29.2kDa Accurate Molecular Mass: 35kDa 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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Reconstitute in ddH_2O to a concentration of 0.1-0.5 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>]

RAQMDPNRI SEDSTHCFYR ILRLHENADL QDSTLESEDT LPDSCRRMKQ AFQGAVQKEL QHIVGPQRFS GAPAMMEGSW LDVAQRGKPE AQPFAHLTIN AASIPSGSHK VTLSSWYHDR GWAKISNMTL SNGKLRVNQD GFYYLYANIC FRHHETSGSV PTDYLQLMVY VVKTSIKIPS SHNLMKGGST KNWSGNSEFH FYSINVGGFF KLRAGEEISI QVSNPSLLDP DQDATYFGAF KVQDID

[ACTIVITY]

Receptor activator of nuclear factor kappa B ligand (RANkL) is a member of the TNF superfamily of proteins. RANkL is expressed by macrophages and osteoblasts, and functions as an activator of RANK and is an important factor in osteoclast differentiation, activation and survival. Cathepsin K (CTSK) plays an essential role in osteoclastic bone resorption and CTSK levels increase with osteoclast differentiation and activation, a process that is controlled by RANkL. Thus a functional binding ELISA assay was conducted to detect the interaction of recombinant mouse RANkL and recombinant human CTSK. Briefly, CTSK was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 $\,\mu$ I were then transferred to RANkL-coated microtiter wells and incubated for 1h at 37 $^\circ$ C. Wells were washed with PBST and incubated for 1h with anti-CTSK pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 $^\circ$ C, wells were aspirated and

Washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at $37 \,^{\circ}$ C. Finally, add 50 µL stop solution to the wells and read at 450/630 nm immediately. The result was shown in Figure 1. When recombinant mouse RANkL is Immobilized at 2 ug/mL (100 ul/well), the concentration of recombinant human CTSK that produces 50% optimal binding response is found to be approximately 2.17 ug/mL.

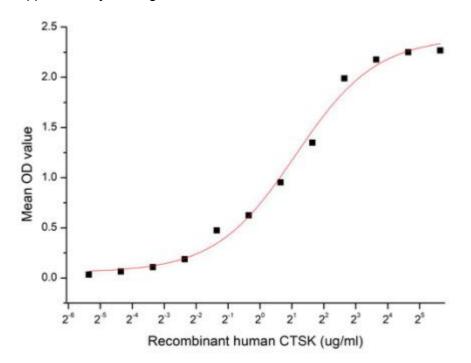


Figure 1. The binding activity of recombinant mouse $\ensuremath{\mathsf{RANkL}}$ and recombinant human

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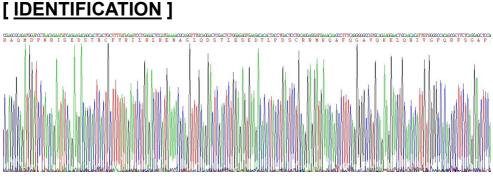


Figure 2. Gene Sequencing (extract)

KDa 70 44 33 26 22 18 14 10 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.