

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

1st Edition (Apr, 2016)

[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: 10mM PBS, pH7.6, containing 5% trehalose.

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.

[USAGE]

Reconstitute in 10mM PBS (pH7.6) 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.

[SEQUENCE]

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RAQMDPNRI SEDSTHCFYR ILRLHENADL
QDSTLESED T LPDSCRRMKQ AFQGA VQKEL QHIVGPQRFS GAPAMMEGSW
LDVAQRGKPE AQPFAHLTIN AASIPSGSHK VTLSSWYHDR GWAKISNMTL
SNGKLRVNQD GFYYLYANIC FRHHETSGSV PTDYLQLMVY VVKTSIKIPS
SHNLMKGGST KNWSGNSEFH FYSINVGGFF KLRAGEEISI QVSNPSLLDP
DQDATYFGAF KVQDID
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[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 member of the tumor necrosis factor (TNF) superfamily. RANKL has been identified to affect the immune system and control bone regeneration and remodeling. RANKL is an apoptosis regulator gene, a binding partner of osteoprotegerin (OPG), a ligand for the receptor RANK and controls cell proliferation by modifying protein levels of Id4, Id2 and cyclin D1. The protein can bind to RANK on cells of the myeloid lineage and functions as a key

factor for osteoclast differentiation and activation. Thus, we use recombinant RANKL to induce RAW264.7 mouse monocyte/macrophage cells to differentiate to osteoclast cell. RAW264.7 mouse monocyte/macrophage cells were seeded into 24-well plates at a density of 2×10^5 and allowed to attach overnight, then treated with RANKL (1ng/mL, 10ng/mL) and incubated for 72h. Then we assay TRAP (a kind of enzyme expressed by osteoclast cell) by ELISA.

Result: TRAP levels in the cell supernatant of RAW264.7 cells increased significantly after stimulated with RANK, the data was shown in Table 1 and Figure1.

Sample (cell supernatant of RAW264.7 cells)	O.D. value	Corrected	Concentration of TRAP (ng/mL)
stimulated with RANKL(1ng/mL)	0.665	0.609	2.36
stimulated with RANKL(10ng/mL)	0.631	0.574	2.21
unstimulated	0.209	0.152	0.86

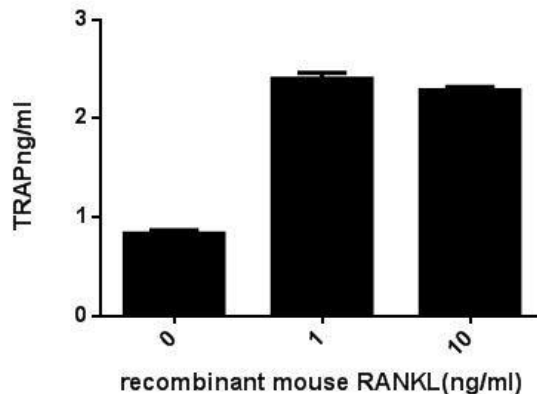


Figure1. TRAP levels in the cell of RAW264.7 cells induced by RANKL.

[IDENTIFICATION]

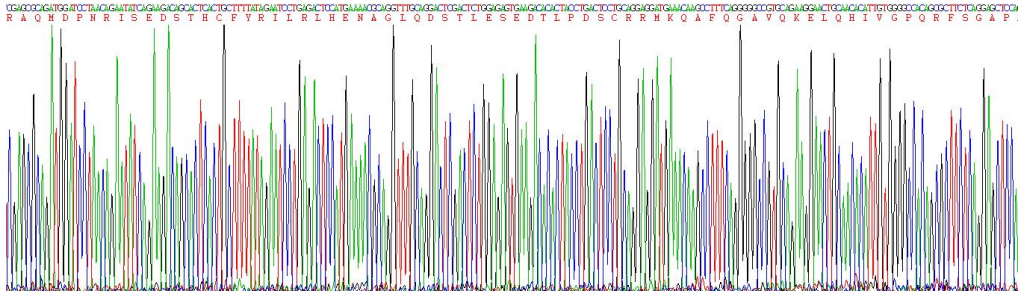


Figure 2. Gene Sequencing (extract)

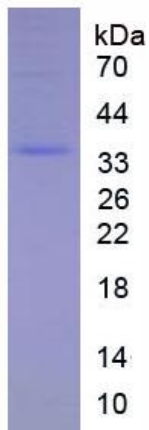


Figure 3. SDS-PAGE

Sample: Active recombinant RANKL, mouse

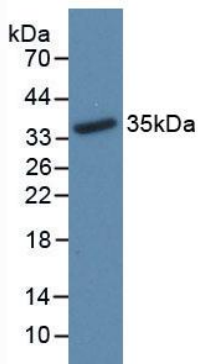


Figure 4. Western Blot

Sample: Recombinant RANKL, mouse;

Antibody: Rabbit Anti-mouse RANKL Ab (PAA855Mu06)

[IMPORTANT NOTE]

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