

**APA855Ra61 100µg**

**Active Receptor Activator Of Nuclear Factor Kappa B Ligand (RANKL)**

**Organism Species: Rattus norvegicus (Rat)**

***Instruction manual***

FOR IN VITRO USE AND RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1st Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Eukaryotic expression.

**Host:** 293F cell

**Residues:** Arg72~Asp316

**Tags:** N-terminal His-tag

**Purity:** >95%

**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:** 7.9

**Predicted Molecular Mass:** 28.2kDa

**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 ]**

```
RAQMDPNRI SEDSTRCFYR ILRLRENTGL
QDSTLESED T EALPDSCRRM KQAFQGA VQR ELQHIVGPQR FSGVPAMMEG
SWLDVARRGK PEAQPFALT INAADIPSGS HKVSLSSWYH DRGWAKISNM
TLSNGKLRVN QDGFYYLYAN ICFRHHETSG SVPADYLQLM VYVVKTSIKI
PSSHNL MKGG STKNWSGNSE FHFYSINVGG FFKLRAGEEI SVQVSNPSLL
DPDQDATYFG AFKVQD
```

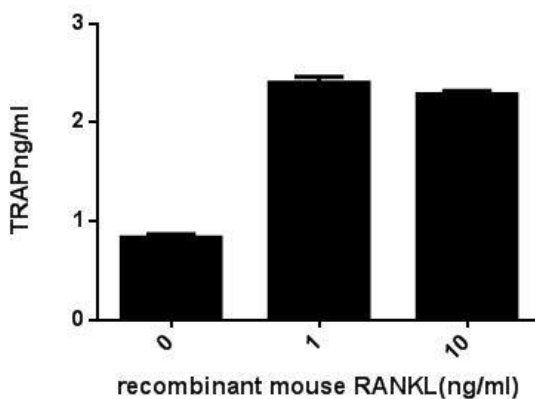
## **[ ACTIVITY ]**

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  $2 \times 10^5$  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 ELASA.

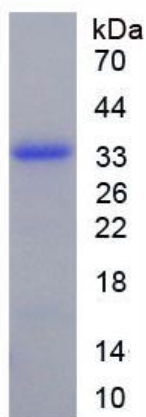
Result: TRAP levels in the cell supernatant of RAW264.7 cells increased significantly after stimulated with RANK, the data was shown in Table 1and Figure 1.

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



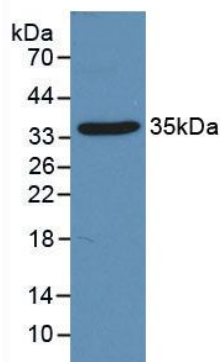
**Figure1.** TRAP levels in the cell of RAW264.7 cells induced by RANKL.

## [ IDENTIFICATION ]



**Figure 2. SDS-PAGE**

**Sample: Active recombinant RANKL, Rat**



**Figure 3. Western Blot**

**Sample: Recombinant RANKL, Rat;**

**Antibody: Rabbit Anti-Rat RANKL Ab (PAA855Ra06)**