

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

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Leu92~Trp263

Tags: Two N-terminal Tags, His-tag and GST-tag

Purity: >92%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 0.01% sarcosyl, 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: 49.3kDa

Accurate Molecular Mass: 49kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) 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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                                LRLHENADL
QDSTLESED T LPDSCR RMKQ AFQGA VQKEL QHIVGPQRFS GAPAMMEGSW
LDVAQRGKPE AQPFAH LTIN AASIPSGSHK VTLSSWYHDR GWAKISNMTL
SNGKLRVNQD GFYYLYANIC FRHHETSGSV PTDY LQLMVY VVKTSIKIPS
SHNLMKGGST KNW
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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 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. RANKL also has a function in the immune system, where it is expressed by T helper cells and is thought to be involved in dendritic cell maturation. This protein was shown to be a dendritic cell survival factor and is involved in the regulation of T cell-dependent immune response. Besides, Peroxisome Proliferator Activated Receptor Gamma (PPAR γ) has been identified as an interactor of RANKL, thus a binding ELISA assay was conducted to detect the interaction of recombinant mouse RANKL and recombinant mouse PPAR γ . Briefly, RANKL were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then

transferred to PPAR γ -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 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37°C. Finally, add 50 μ L stop solution to the wells and read at 450nm immediately. The binding activity of RANKL and PPAR γ was shown in Figure 1, and this effect was in a dose dependent manner.

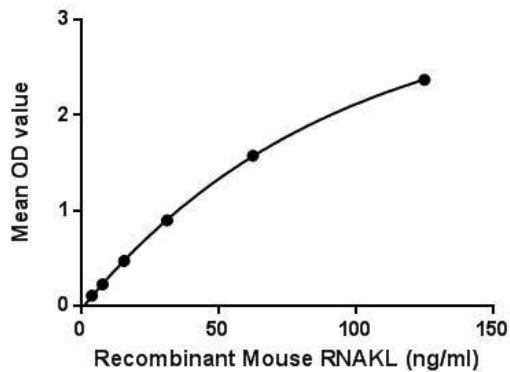


Figure 1. The binding activity of RANKL with PPAR γ .

[IDENTIFICATION]

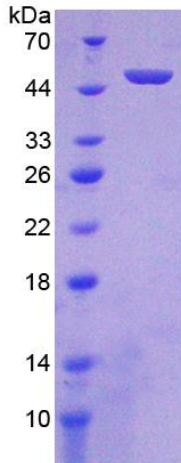


Figure 2. SDS-PAGE

Sample: Active recombinant RANKL, Mouse

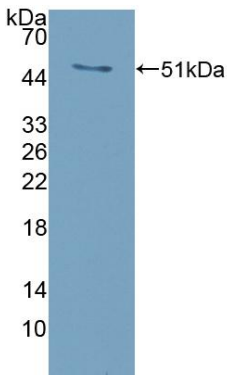


Figure 3. Western Blot

Sample: Recombinant RANKL, Mouse;

Antibody: Rabbit Anti-Mouse RANKL Ab (PAA855Mu01)

[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.