

**APC057Hu01 100µg**  
**Active Receptor Activator Of Nuclear Factor Kappa B (RANK)**  
**Organism Species: *Homo sapiens* (Human)**  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1st Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Val330~Lys615

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

**Purity:** >98%

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

**Buffer Formulation:** 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 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:** 4.8

**Predicted Molecular Mass:** 33.9kDa

**Accurate Molecular Mass:** 44kDa 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 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.

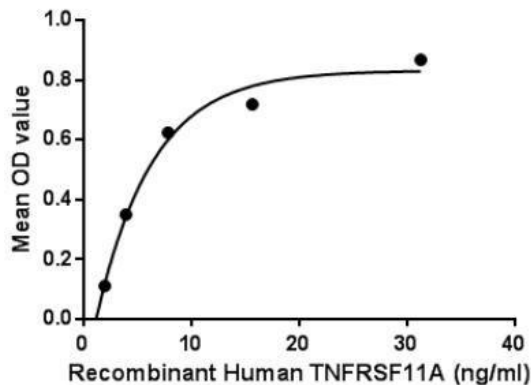
## **[ SEQUENCE ]**

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DRPSQPTDQL LFLTEPGSKS TPPFSEPLEV GENDSLSQCF TGTQSTVGSE
SCNCTEPLCR TDWTPMSEN YLQKEVDSGH CPHWAASPSP NWADVCTGCR
NPPGEDCEPL VGSPKRGPLP QCAYGMGLPP EEEASRTEAR DQPEDGADGR
LPSSARAGAG SGSSPGGQSP ASGNVTGNSN STFISSGQVM NFKGDIIVVY
VSQTSQEGAA AAAEPMGRPV QEETLARRDS FAGNGPRFPD PCGGPEGLRE
PEKASRPVQE QGGAK
```

## **[ ACTIVITY ]**

RANK tumor necrosis factor receptor superfamily member 11A protein (TNFRSF11A) also known as receptor Activator of Nuclear Factor  $\kappa$  B (RANK) or TRANCE Receptor is a member of the tumor necrosis factor receptor (TNFR) molecular sub-family. TNFRSF11A is the receptor for RANK-Ligand (RANKL) and part of the RANK/RANKL/OPG signaling pathway that regulates osteoclast differentiation and activation. It is associated with bone remodeling and repair, immune cell function, lymph node development, thermal regulation, and mammary

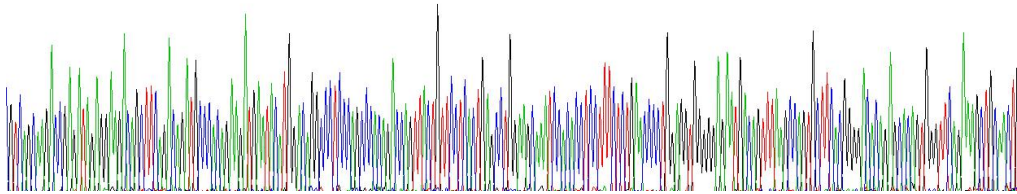
gland development. Besides, TNF Receptor Associated Factor 5 (TRAF5) has been identified as an interactor of TNFRSF11A, thus a binding ELISA assay was conducted to detect the interaction of recombinant human TNFRSF11A and recombinant human TRAF5. Briefly, TNFRSF11A were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 $\mu$ L were then transferred to TRAF5-coated microtiter wells and incubated for 2h at 37 $^{\circ}$ C. Wells were washed with PBST and incubated for 1h with anti-TNFRSF11A 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 $^{\circ}$ C. Finally, add 50 $\mu$ L stop solution to the wells and read at 450nm immediately. The binding activity of TNFRSF11A and TRAF5 was shown in Figure 1, and this effect was in a dose dependent manner.



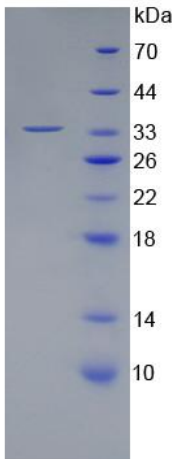
**Figure 1. The binding activity of TNFRSF11A with TRAF5.**

## [ IDENTIFICATION ]

ATGACGAGCGGATGAGGAGCGCTTGGCGGCTGGCGAGGATGATGAGAGGCGCTGGCGCGGACGTTCCTCCAGCGCTGGAGGATCCGCTCTTCGAGACCGGAGGAGGATGCGATTTAGCGCGCTTCCGGGCGGAGCGAGCGTGGAGATCGACT  
VSKTEIEEDSFRQHPTEDEYMDRPSQPTDQLLFLTEPGSKSTPPFSEPLEVGENDSLSCQFTGTQSTVGSESCNC

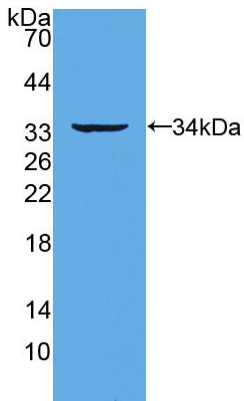


**Figure 2. Gene Sequencing (Extract)**



**Figure 3. SDS-PAGE**

**Sample: Active recombinant RANK, Human**



**Figure 4. Western Blot****Sample: Recombinant RANK, Human;****Antibody: Rabbit Anti-Human RANK Ab (PAC057Hu01)****[ 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.