

**APA134Ra02 100µg**

**Active Tumor Necrosis Factor Beta (TNFb)**

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

***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:** Leu34~Leu202

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

**Purity:** >94%

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

**Buffer Formulation:** PBS, pH7.4, containing 0.01% SKL, 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:** 10.2

**Predicted Molecular Mass:** 22.4kDa

**Accurate Molecular Mass:** 22kDa as determined by SDS-PAGE reducing conditions.

## **[ USAGE ]**

Reconstitute in 10mM PBS (pH7.4) 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.

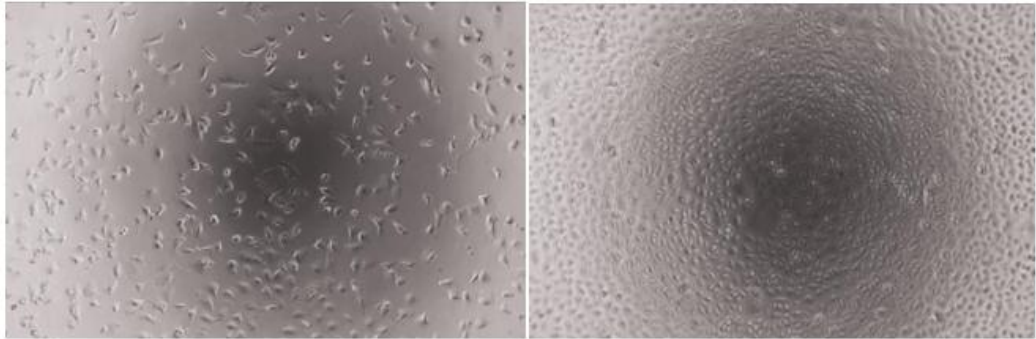
**Stability Test:** The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

## **[ SEQUENCE ]**

LSGVRFASRTAHQPPQ  
KHLTHGLLKPAAHLVGYPKQNSLLWRANTDRAFLRHGFSLNNNSLLIPT  
SGLYFVYSQVVFSGESCSPRAIPTIYLAHEVQLFSSQYPFHVPLLSAQK  
SVYPGLQGPWVRSMYQGAVFLLSKGDQLSTHTDGISHLHFSPSTVFFGAFAL

## **[ ACTIVITY ]**

TNF- $\beta$ , a member of the tumor necrosis factor family, is a potent lymphoid factor that exerts cytotoxic effects on a wide range of tumor cells. The biological effects of TNF- $\beta$  are very similar to TNF- $\alpha$ , due to the similarity of molecular structure and the receptors. As reported, TNF- $\alpha$  could inhibit the proliferation and induce apoptosis of A549 cells, therefore, A549 cells were seeded into triplicate wells of 96-well plates at a density of 4,000 cells/well with 5% serum standard DMEM including various concentrations of recombinant rat TNF- $\beta$ . After incubated for 48h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10  $\mu$ L of CCK-8 solution was added to each well of the plate, then the absorbance at 450 nm was measured using a microplate reader after incubating the plate for 2 hours at 37 °C. Proliferation of A549 cells after incubation with TNF- $\beta$  for 48h observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant mouse TNF- $\beta$  for 48h. The result was shown in Figure 2. It was obvious that TNF- $\beta$  significantly inhibit cell viability of A549 cells. The ED50 is 2.5 $\mu$ g/ml.



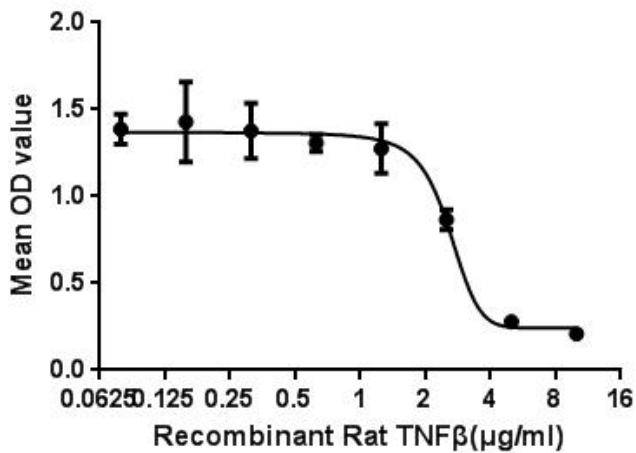
**A**

**B**

**Figure 1. Inhibition of A549 cells proliferation after stimulated with TNF-β**

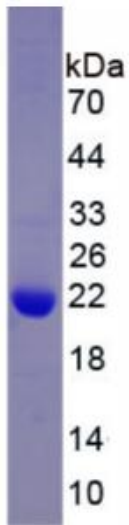
(A) A549 cells cultured in DMEM, stimulated with 2.5µg/ml TNF-β for 48h;

(B) Unstimulated A549 cells cultured in DMEM for 48h.



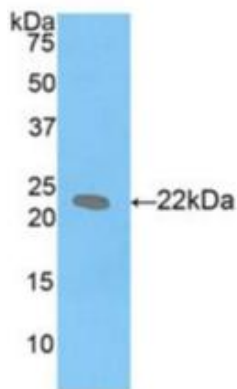
**Figure 2. Inhibition of A549 cells proliferation after stimulated with TNF-β**

**[ IDENTIFICATION ]**



**Figure 3. SDS-PAGE**

Sample: Active recombinant TNFb, Rat



**Figure 4. Western Blot**

Sample: Recombinant TNFb, Rat;

Antibody: Rabbit Anti- Rat TNFb Ab (PAA134Ra02)

**[ 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.