

**APA133Ra01 5µg**  
**Active Tumor Necrosis Factor Alpha (TNFa)**  
**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:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Leu80~Leu235

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

**Purity:** >92%

**Buffer Formulation:** 20mM Tris, 150mM NaCl, pH8.0, containing 0.01% sarcosyl, 5% trehalose, and Proclin300.

**Applications:** Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

**Predicted isoelectric point:** 6.0

**Predicted Molecular Mass:** 21.0kDa

**Accurate Molecular Mass:** 21kDa 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.

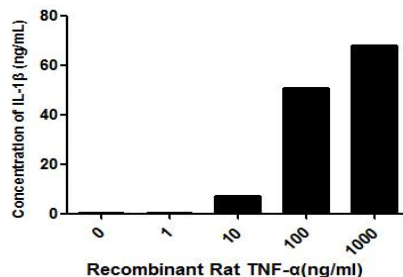
## [ **SEQUENCE** ]

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L  RSSSQNSSDK  PVAHVVANHQ  
AEEQLEWLSQ  RANALLANGM  DLKDNQLVVP  ADGLYLIYSQ  VLFKGQGPCD  
YVLLTHTVSR  FAISYQEKVS  LLSAIKSPCP  KDTPEGAELK  PWYEPMYLGG  
VFQLEKGDLL  SAEVNLPKYL  DITESGQVYF  GVIAL
```

## [ **ACTIVITY** ]

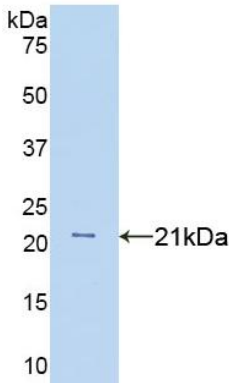
Mechanism: TNF $\alpha$ , being an endogenous pyrogen, is able to induce fever, apoptotic cell death, inflammation and to inhibit tumorigenesis. As reported, TNF $\alpha$  could inhibit the proliferation and induce apoptosis of A549 cells, and the concentration of IL-1 $\beta$  and IL-8 in cell supernatant will increase after stimulation. Therefore, A549 cells were incubated in DMEM with TNF $\alpha$  (1ng/mL, 10ng/mL, 100ng/mL, 1000ng/mL) for 8h, IL-1 $\beta$  and IL-8 were detected in the cell supernatant by ELISA.

Results: After incubation with TNF- $\alpha$  for 8h, IL-1 $\beta$  and IL-8 significantly increased in the cell supernatant. The concentration of IL-1 $\beta$  and IL-8 detected in the cell supernatant was shown in Figure 1 and Figure 2 respectively.



**Figure 1. The concentration of IL-1 $\beta$  in the cell supernatant of A549 cells stimulated by TNF- $\alpha$ .**





**Figure 5. Western Blot**

**Sample: Recombinant TNFa, Rat;**

**Antibody: Rabbit Anti-Rat TNFa Ab (PAA133Ra01)**

**[ 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.