

**APA133Mu01 100µg**  
**Active Tumor Necrosis Factor Alpha (TNFa)**  
**Organism Species: Mus musculus (Mouse)**  
***Instruction manual***

FOR IN VITRO USE AND RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1th Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Leu80~Leu235

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

**Purity:** >95%

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

**Predicted isoelectric point:** 5.0

**Predicted Molecular Mass:** 20.5kDa

**Accurate Molecular Mass:** 21kDa as determined by SDS-PAGE reducing conditions.

**Applications:** Cell culture; Activity Assays; In vivo assays.

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

## **[ 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

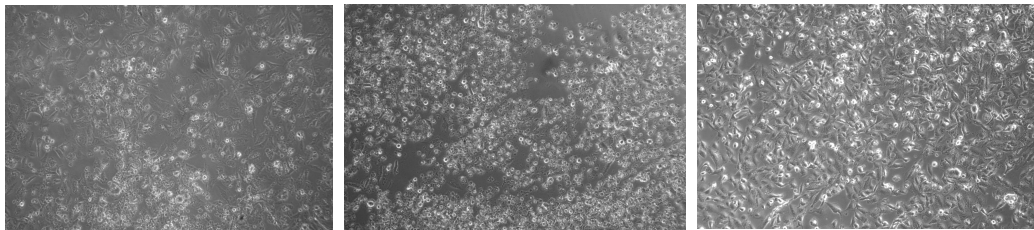
protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

### **[ SEQUENCE ]**

```
L RSSSQNSSDK PVAHVVANHQ  
VEEQLEWLSQ RANALLANGM DLKDNQLVVP ADGLYLVYSQ VLFKGGQCPD  
YVLLTHTVSR FAISYQEKVN LLSAVKSPCP KDTPEGAELK PWYEPIYLG  
VFQLEKGDQL SAEVNLPKYL DFAESGQVYF GVIAL
```

### **[ ACTIVITY ]**

TNF $\alpha$ , being an endogenous pyrogen, is able to induce fever, apoptotic cell death, inflammation and inhibit tumorigenesis. As reported, TNF $\alpha$  could inhibit the proliferation and induce apoptosis of A549 cells, and the concentration of IL-1 $\beta$  in cell supernatant will increase after stimulation. A549 cells were incubated in DMEM with TNF $\alpha$  (1ng/mL, 10ng/mL) for 2h, 4h, 8h, 24h, 48h, then cells were observed by inverted microscope and IL-1 $\beta$  in cell supernatant was detected by ELISA. Cell apoptosis of A549 after incubation of 48h was shown in Figure 1.



A

B

C

**Figure 1. Cell apoptosis of A549 cells after stimulated by TNF $\alpha$ .**

- (A) A549 cells cultured in DMEM, stimulated with 1ng/mL TNF $\alpha$  for 48h;**
- (B) A549 cells cultured in DMEM, stimulated with 10ng/mL TNF $\alpha$  for 48h;**
- (C) A549 cells cultured in DMEM for 48h.**

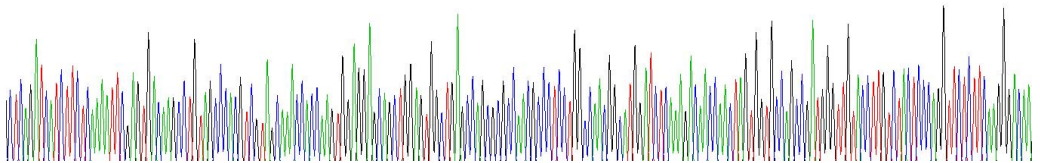
Effect of TNF $\alpha$  on the expression of IL-1 $\beta$  is shown in Table 1.

**Table 1. ELISA detection of IL-1 $\beta$  expression from A549 cells by TNFa.**

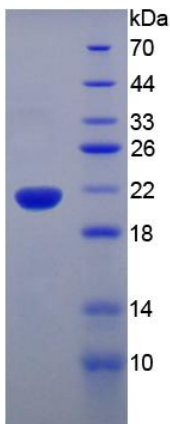
Sample (cell supernatant of A549 cells)	Concentration of IL-1 $\beta$ (ng/mL)
Stimulated with TNFa (1ng/mL)	9.304
Stimulated with TNFa (10ng/mL)	29.064
Unstimulated	1.344

## [ IDENTIFICATION ]

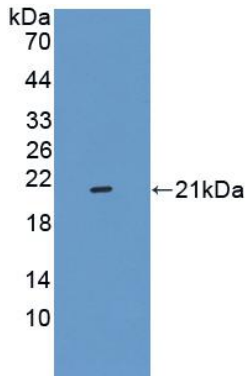
CTCGAGTCACTCTTCGAAATTCGAGTCAGAGCCCTGAGGCGAGTGTGACAAAGCCGAGTGGAGAGCCAGTGGAGTGGCTGAGCCAGGCGGCGCAAGCGCCCTCTGGCGAGGGCAATGGATCTCAAGAGCAAGCCATGAGTGGCGAGCGATGGAGTGGTCTCTCTCCAGGATCTCTTCAGGGAGCA  
L R S S Q W S S P P V A R H Y A R H Q V E E L E L S Q R A V A L L A N V R D S F R D L F P A D G L T L Y C Q Y L E F K Q I



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



**Figure 3. SDS-PAGE, Sample: Active recombinant TNFa, Mouse**



**Figure 4. Western Blot, Sample: Recombinant TNF $\alpha$ , Mouse;  
Antibody: Rabbit Anti-Mouse TNF $\alpha$  Ab (PAA133Mu01)**

**[ 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.