

**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%

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

**Buffer Formulation:** 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 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.

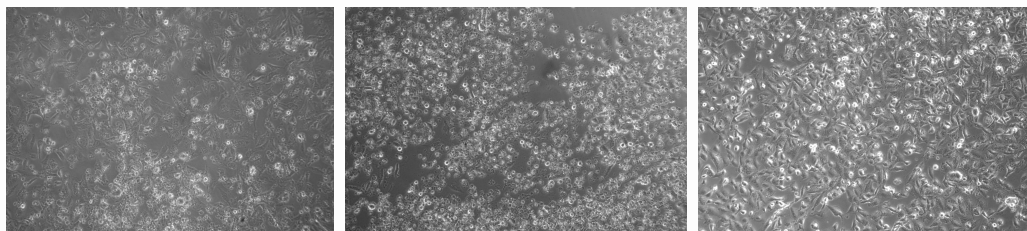
**Stability Test:** The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration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

## **[ SEQUENCE ]**

```
L RSSSQNSSDK PVAHVVAHQ  
VEEQLEWLSQ RANALLANGM DLKDNQLVVP ADGLYLVYSQ VLFKGGQCPD  
YVLLTHTVSR FAISYQEKVN LLSAVKSPCP KDTPEGAELK PWYEPYILGG  
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 stimulated by TNF $\alpha$ .

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

[ IDENTIFICATION ]

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

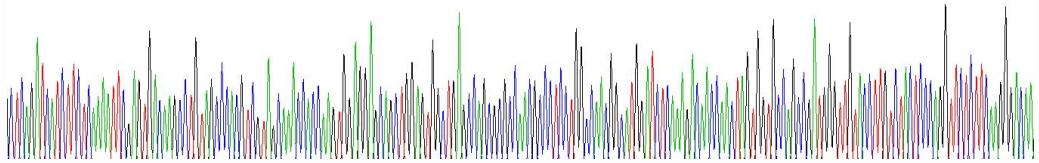


Figure 2. Gene Sequencing (extract)

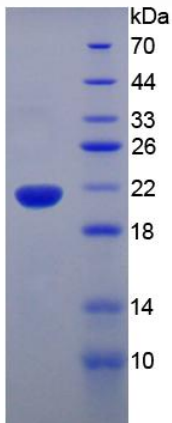
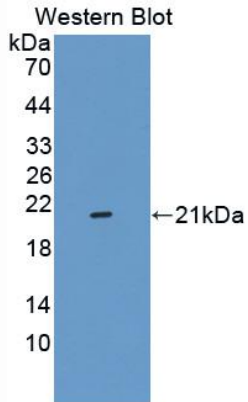


Figure 3. SDS-PAGE, Sample: Active recombinant TNF $\alpha$ , Mouse



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