**APA133Mu61 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

1th Edition (Apr, 2016)

## [ PROPERTIES ]

**Source:** Eukaryotic expression.
**Host:** 293F cell
**Residues:** Gly57~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:** 21.4kDa
**Accurate Molecular Mass:** 24kDa 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$^{o}$C for one month.
Aliquot and store at -80$^{o}$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.

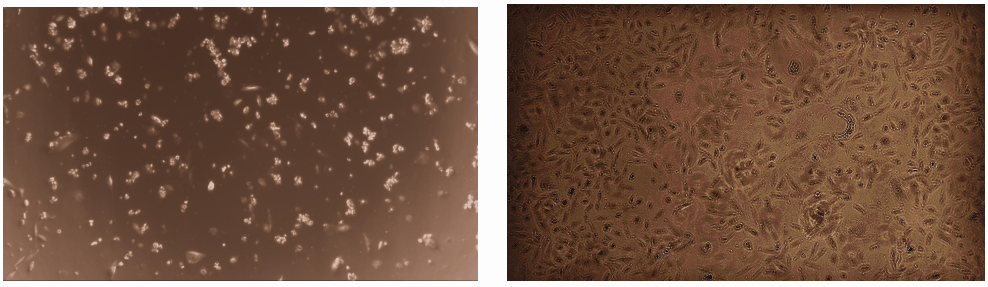
## [ SEQUENCE ]

GPQR DEKFPNGLPL ISSMAQTLTL RSSSQNSSDK PVAHVVANHQ
VEEQLEWLSQ RANALLANGM DLKDNQLVVP ADGLYLVYSQ VLFKGQGCPD
YVLLTHTVSR FAISYQEKVN LLSAVKSPCP KDTPEGAELK PWYEPIYLGG
VFQLEKGDQL SAEVNLPKYL DFAESGQVYF GVIAL

## [ ACTIVITY ]

TNF-a, being an endogenous pyrogen, is able to induce fever, necrosis, inflammation and to inhibit tumorigenesis. As reported, TNF-a could inhibit the proliferation and induce necrosis of A549 cells, and the concentration of IL-1β in cell supernatant will increase after stimulation. Therefore, A549 cells were incubated in DMEM with TNFa (1ng/mL, 10ng/mL) for 2h, 4h, 8h, 24h, 48h, then cells were observed by inverted microscope and IL-1β was detected in the cell supernatant by ELISA .

Cell necrosis after incubation with TNF-a (10ng/mL) for 72h was shown in Figure1.



A B

**Figure 1. Effect of TNF-a on A549 cells.**

**(A) A549 cells cultured in DMEM, stimulated with 10ng/mL TNF-a for 72h;**

**(B) Unstimulated A549 cells cultured in DMEM for 72h.**

The production of IL-1β after incubation with TNF-a (10ng/mL) for 8h is shown in Table 1.

**Table 1. Effect of TNF-β on A549 cells by ELISA.**

| Sample (cell supernatant of A549 cells) | Concentration of IL-1β (ng/mL) |
|---|---|
| Stimulated with TNF-β (10ng/mL) | 52.0 |
| Unstimulated | 4.9 |

# [ IDENTIFICATION ]

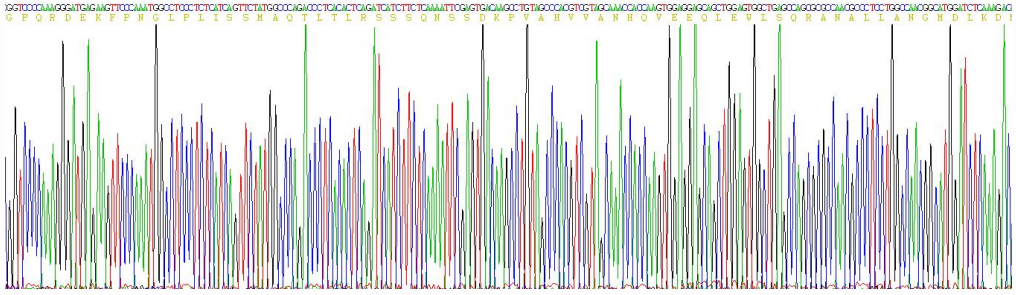


**Figure 2. Gene Sequencing (extract)**

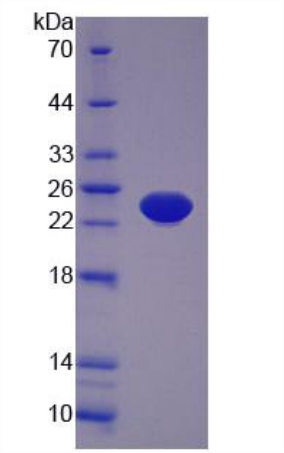



**Figure 3. SDS-PAGE**

**Sample: Active recombinant TNFa, Mouse**

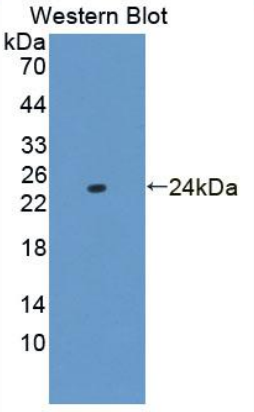



**Figure 4. Western Blot**

**Sample: Recombinant TNFa, Mouse;**

**Antibody: Rabbit Anti-Mouse TNFa Ab (PAA133Mu06)**