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

1st 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.
Applications: Cell culture; Activity Assays; In vivo assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 5.0
Predicted Molecular Mass: 21.4kDa
Accurate Molecular Mass: 24kDa 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 37ºC 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.

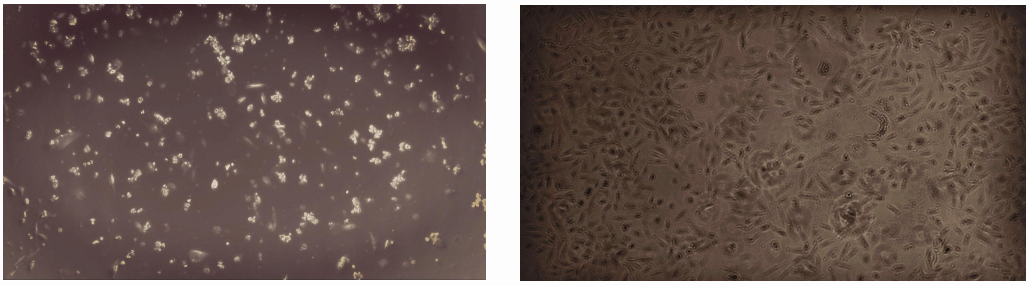
[SEQUENCE]

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

[ACTIVITY]

Mechanism: TNF-a, being an endogenous pyrogen, is able to induce fever, apoptotic cell death, inflammation and to inhibit tumorigenesis. As reported, TNF-a could inhibit the proliferation and induce apoptosis 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 .

Result 1: Cell apoptosis was observed after incubation with TNF-a (10ng/mL) for 72h.



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.

Results 2: After incubation with TNF-a (10ng/mL) for 8h, IL-1β significantly increased in the cell supernatant.

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

Sample (cell supernatant of A549 cells)	O.D. value	Corrected	Concentration of IL-1β (ng/mL)
Stimulated with TNF-β (10ng/mL)	1.210	1.046	52.0
Unstimulated	0.187	0.023	4.9

[IDENTIFICATION]

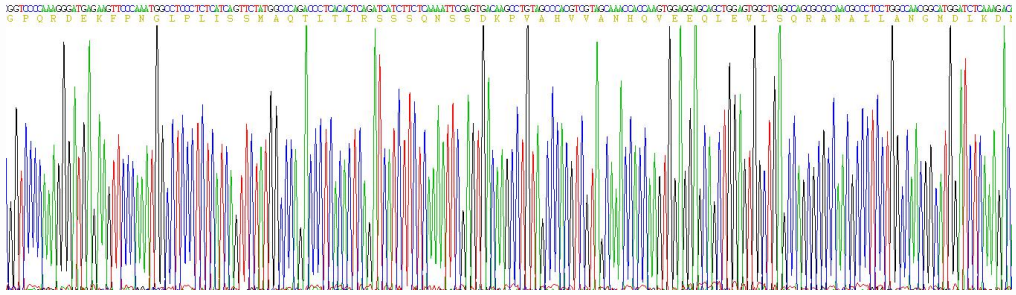


Figure 2. Gene Sequencing (extract)

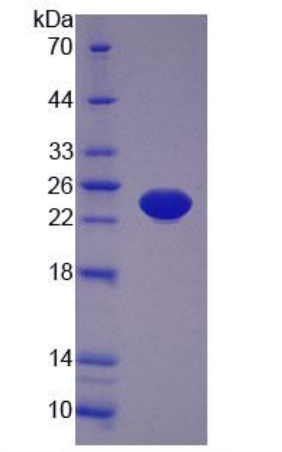


Figure 3. SDS-PAGE

Sample: Active recombinant TNFa, Mouse

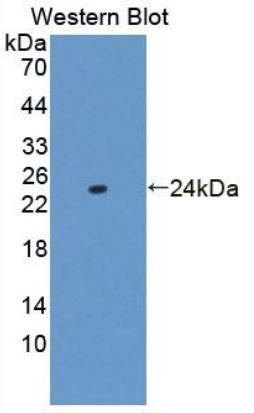


Figure 4. Western Blot

Sample: Recombinant TNFa, Mouse;

Antibody: Rabbit Anti-Mouse TNFa Ab (PAA133Mu06)