

APA133Ca01 100µg
Active Tumor Necrosis Factor Alpha (TNFα)
Organism Species: *Canis familiaris*; Canine (Dog)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Leu63~Leu233

Tags: N-terminal His-tag

Purity: >95%

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

Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 6.3

Predicted Molecular Mass: 17.7kDa

Accurate Molecular Mass: 20kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) 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]

LPNGLQLI SPLAQTVKSS SRTPSDKPVA HVVANPEAEG QLQWLSRRAN ALLANGVELT
DNQLIVPSDG LYLIYSQVLF KGQGCPSTHV LLTHTISRFA VSYQTKVNULL SAIKSPCQRE
TPEGTEAKPW YEPIYLGGVF QLEKGDRLSA EINLPNYLDF AESGQVYFGI IAL

[ACTIVITY]

Tumor necrosis factor (TNF, tumor necrosis factor alpha, TNF α , cachexin, or cachectin) is a cell signaling protein (cytokine) involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction. The primary role of TNF is in the regulation of immune cells. TNF, being an endogenous pyrogen, is able to induce fever, apoptotic cell death, cachexia, inflammation and to inhibit tumorigenesis and viral replication and respond to sepsis via IL1 & IL6 producing cells. To test the effect of TNF α on cell apoptosis, Hepa1-6 cells were seeded into 96-well plates at a density of 4,000 cells/well with 5% serum standard DMEM including various concentrations of recombinant dog TNF α . After incubated for 72h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 μ l of CCK-8 solution was added to each well of the plate, then the absorbance at 450nm was measured using a microplate reader after incubating the plate for 1h at 37 °C . Apoptosis of Hepa1-6 cells after incubation with TNF α for 72h observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant dog TNF α for 72h. The result was shown in Figure 2. It was obvious that TNF α significantly inhibit cell viability of Hepa1-6 cells. The ED50 is 11.9 μ g/mL.

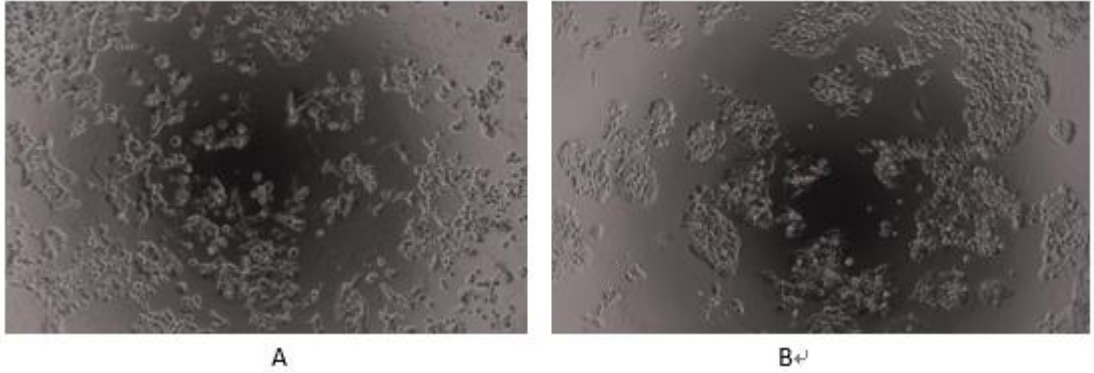


Figure 1. Inhibition of Hepa1-6 cells proliferation after stimulated with TNF α
(A) Hepa1-6 cells cultured in DMEM, stimulated with 10 μ g/ml TNF α for 72h;
(B) Unstimulated Hepa1-6 cells cultured in DMEM for 72h.

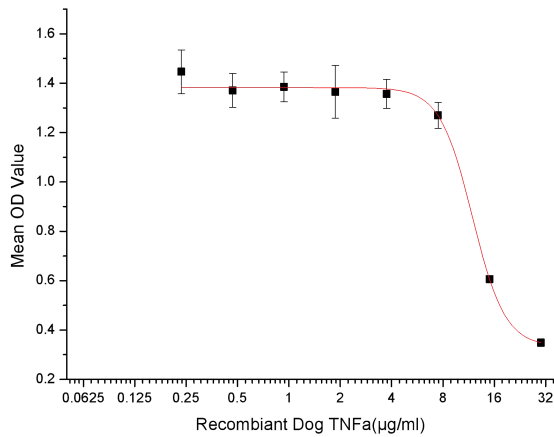


Figure 2. Inhibition of Hepa1-6 cells proliferation after stimulated with TNF α .
[IDENTIFICATION]

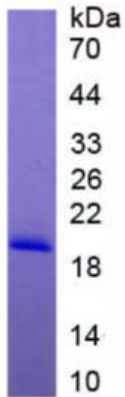


Figure 3. SDS-PAGE

Sample: Active recombinant TNFa, Dog

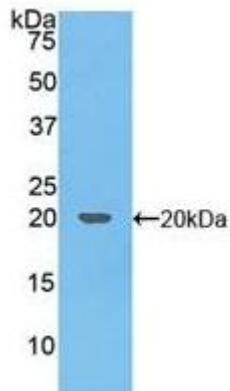


Figure 4. Western Blot

Sample: Recombinant TNFa, Dog;

Antibody: Rabbit Anti- Dog TNFa Ab (PAA133Ca01)

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.