APA133Eq01 100μg
Active Tumor Necrosis Factor Alpha (TNFa)
Organism Species: *Equus caballus; Equine (Horse)*
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: Leu78~Leu234
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: 6.8
Predicted Molecular Mass: 20.8kDa
Accurate Molecular Mass: 21kDa 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 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]

LRS SSRTPSDKPV AHVVANPQAE GQLQWLSGRA NALLANGVKL TDNQLVVPLD GLYLIYSQVL FKGQGCPSTH VLLTHTISRL AVSYPSKVNL LSAIKSPCHT ESPEQAEAKP WYEPIYLGGV FQLEKGDQLS AEINQPNYLD FAESGQVYFG IIAL

[ACTIVITY]

Tumor necrosis factor (TNF, tumor necrosis factor alpha, TNFα, 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 TNFα on cell apoptosis, A549 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 horse TNFα. After incubated for 48h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10μl 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℃. Proliferation of A549 cells after incubation with TNFα for 48h 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 horse TNFα for 48h. The result was shown in Figure 2. It was obvious that TNFα significantly inhibit cell viability of A549 cells. The ED50 is 6.2μg/mL.

A B

Figure 1. Inhibition of A549 cells proliferation after stimulated with TNFα

(A) A549 cells cultured in DMEM, stimulated with 6.2μg/ml TNFα for 48h;

(B) Unstimulated A549 cells cultured in DMEM for 48h.

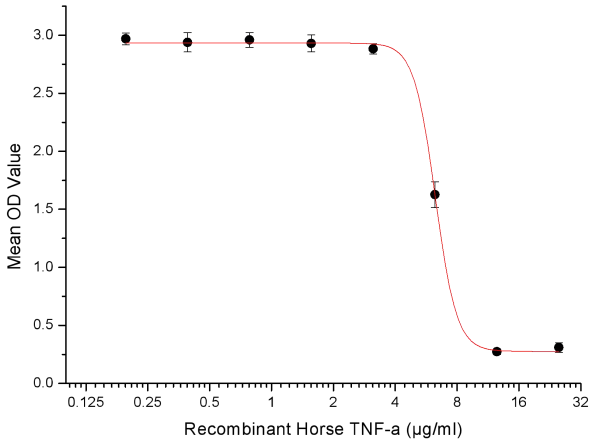

Figure 2. Inhibition of A549 cells proliferation after stimulated with TNFα.

[IDENTIFICATION]

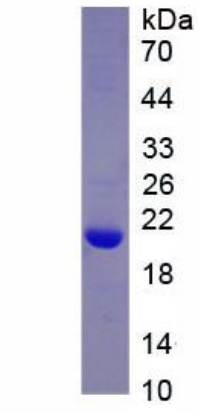

Figure 3. SDS-PAGE

Sample: Active recombinant TNFa, Horse

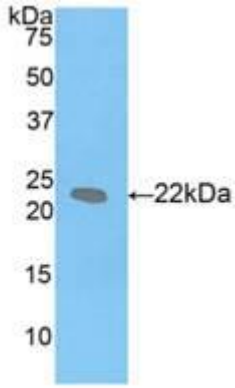

Figure 4. Western Blot

Sample: Recombinant TNFa, Horse;

Antibody: Rabbit Anti- Horse TNFa Ab (PAA133Eq01)

[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.