APA133Mu63 100μg
Active Tumor Necrosis Factor Alpha (TNFa)
Organism Species: *Mus musculus (Mouse)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.
Host: 293F cell
Residues: Leu80~Leu235
Tags: N-terminal His-tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .
Original Concentration: 200μg/mL
Applications: Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 4.7
Predicted Molecular Mass: 18.9kDa
Accurate Molecular Mass: 18&24kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:
1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[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]

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

[ACTIVITY]

Tumor Necrosis Factor Alpha (TNFa) is a pleiotropic pro-inflammatory cytokine primarily produced by macrophages and lymphocytes. As a key mediator of systemic inflammation, it binds to TNF receptors (TNFR1/R2) to activate NF-κB and MAPK pathways, regulating immune responses, cell survival, and apoptosis. Clinically, TNFa is implicated in autoimmune diseases and anti-tumor therapies, with its inhibitors widely used clinically. TNFa interacts directly with TNFaIP3, an ubiquitin-editing enzyme that terminates TNFa-induced NF-κB activation through deubiquitination, forming a critical negative feedback loop to control excessive inflammation.Thus a functional binding ELISA assay was conducted to detect the interaction of recombinant mouse TNFa and recombinant human TNFaIP3 .Briefly, biotin-linked TNFa were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100μl were then transferred to TNFaIP3-coated microtiter

wells and incubated for 1h at 37℃. Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50μl stop solution to the wells and read at 450nm immediately. The binding activity of recombinant mouse TNFa and recombinant human TNFaIP3 was shown in Figure 1, the EC50 for this effect is 0.67μg/mL.

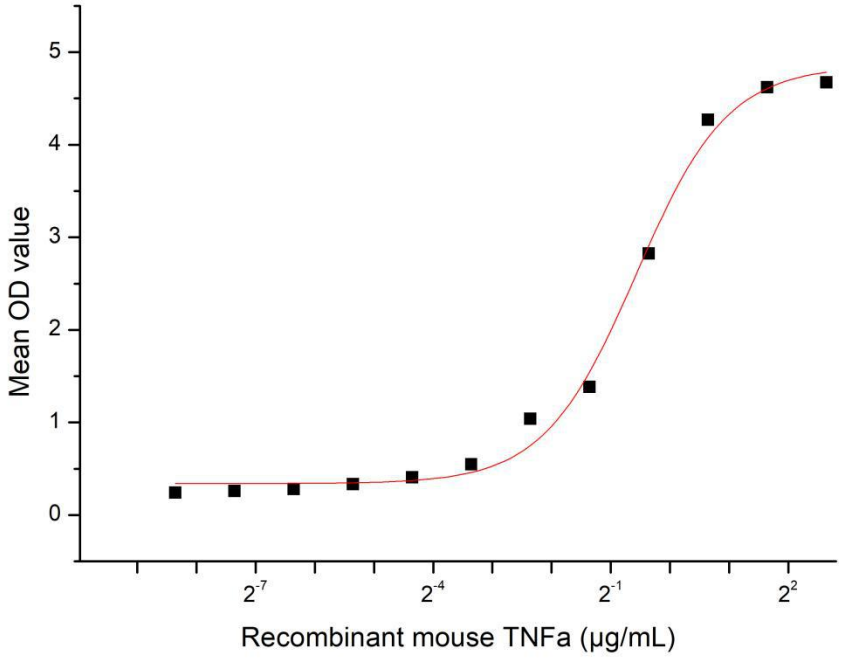

Figure 1. The binding activity of recombinant mouse TNFa and recombinant human TNFaIP3

[IDENTIFICATION]

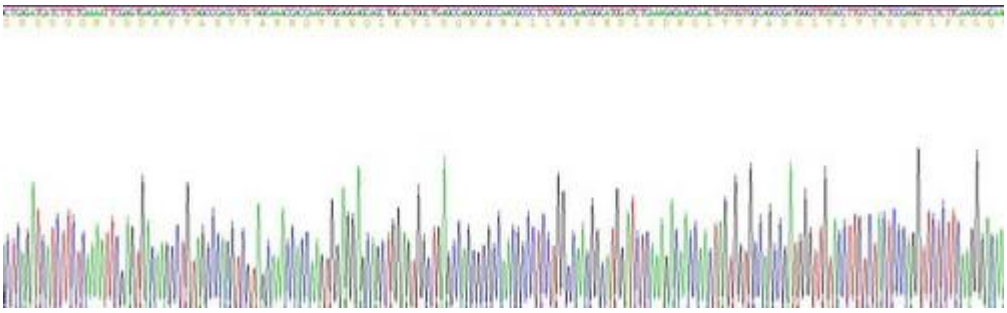

Figure 2. Gene Sequencing (extract)

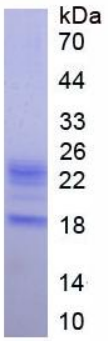

Figure 3. SDS-PAGE

Sample: Active recombinant TNFa, Mouse

[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.