APA133Mu62 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: Gly57~Leu235
Tags: N-terminal His Tag and C-terminal Fc Region of Human IgG1
Purity: >80%
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: 4.8
Predicted Molecular Mass: 50.7kDa
Accurate Molecular Mass: 42&44kDa 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]

GPQRDEKFPNGLPLISSMAQTLTLRSSSQNSSDKPVAHVVANHQVEEQLEWLSQRANALLANGMDLKDN
QLVVPADGLYLVYSQVLFKGQGCPDYVLLTHTVSRFAISYQEKVNLLSAVKSPCPKDTPEGAELKPWYE
PIYLGGVFQLEKGDQLSAEVNLPKYLDFAESGQVYFGVIAL

[ACTIVITY]

Tumor Necrosis Factor Alpha (TNFa), also known as TNF-α, is a potent pro-inflammatory cytokine that plays a central role in the body's immune response, cell survival, and apoptosis. It is primarily produced by activated macrophages, but also by other cell types including lymphocytes, neutrophils, and endothelial cells. TNFa exists in two forms: a transmembrane precursor and a soluble cytokine, both of which are biologically active and mediate their effects by binding to its receptors, TNFR1 and TNFR2.Its functions are diverse and crucial. In its beneficial role, TNFa helps orchestrate the inflammatory response to combat infections and can induce the death of tumor cells, which is the origin of its name. It is essential for the proper functioning of the immune system. However, TNFa is a classic "double-edged sword." Dysregulated or excessive production of TNFa is a key driver of pathological inflammation and is heavily implicated in the pathogenesis of

a wide range of autoimmune and chronic inflammatory diseases, such as rheumatoid arthritis, inflammatory bowel disease (Crohn's disease), and psoriasis. Consequently, TNFa has become a major therapeutic target, and drugs that inhibit its action (e.g., infliximab, adalimumab) are widely used to treat these conditions, highlighting its profound clinical significance.To detect the activity of recombinant TNFa, a functional ELISA assay was performed to evaluate the interaction between recombinant mouse TNFa and recombinant human TNFR1.Briefly, biotin-linked TNFa were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100μl were then transferred to TNFR1-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 TNFa and TNFR1 was shown in Figure 1, the EC50 for this effect is 0.26852μg/mL.

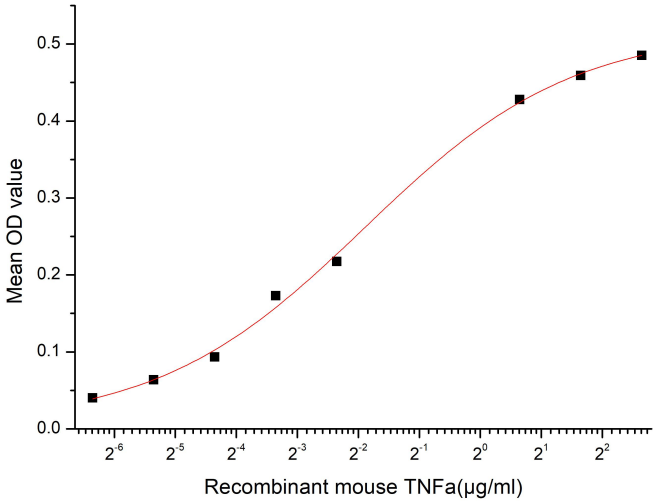


Figure 1. The binding activity of recombinant TNFa and TNFR1

[IDENTIFICATION]

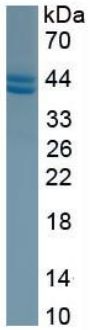


Figure 2. SDS-PAGE

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.