Human Tumor Necrosis Factor, TNF ELISA Kit

Cat. No.: DEIA143
Lot No.: (See product label)
Pkg. Size: 15 plates, 45 plates

INTENDED USE

Human TNF-α ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant TNF-α in a µg/mL format within the range of 16-2,000 pg/ml. Using the ELISA protocol described below, this kit provides sufficient reagents to assay TNF-α in approximately 1,500 ELISA plate wells.

DESCRIPTION

This gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. This cytokine is mainly secreted by macrophages. It can bind to, and thus functions through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFRB. This cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation.

RECONSTITUTION & STORAGE

Capture Antibody: 720 µg of antigen-affinity purified goat anti-TNF-α. Centrifuge vial prior to opening. Reconstitute in 1.0 ml PBS for a concentration of 720 µg/mL. Following reconstitution the Capture antibodies may be stored at 2-8°C for up to 6 months. For long term storage it is recommended to aliquot into working volumes and store at -70°C in a manual defrost freezer.

Detection Antibody: 63 µg of biotinylated antigen-affinity purified goat anti-TNF-α. Centrifuge vial prior to opening. Reconstitute in 1.0 ml sterile 1.0% BSA in PBS for a concentration of 63 µg/mL. Following reconstitution the Detection antibodies may be stored at 2-8°C for up to 6 months. For long term storage it is recommended to aliquot into working volumes and store at -70°C in a manual defrost freezer.

Human TNF-α Standard: 160 ng of recombinant TNF-α. Centrifuge vial prior to opening. Reconstitute in 0.5 ml sterile 1.0% BSA in PBS for a concentration of 320 ng/ml. The Standard may be stored at 2-8°C for one month or aliquoted and stored at -70°C for up to three months in a manual defrost freezer.

UltraAvidin-HRP Conjugate: 40 µl vial. Upon receipt, UltraAvidin-HRP conjugate should be stored at 2-8°C, DO NOT FREEZE.

TMB Liquid Substrate: Aspirate and wash plate 4 times. Add 100 µl of TMB HRP Microwell Substrate Standard Kinetic One Component "Ready Use" to each well. Incubate at room temperature and monitor color development with an ELISA plate reader at 450 nm with wavelength correction set at 540 nm or 570 nm. The reaction may be stopped after 15-20 minutes by adding 100 µl of 2 M sulfuric acid to each well.

ANALYTE GENE INFORMATION

Gene Name: TNF tumor necrosis factor (TNF superfamily, member 2) [Homo sapiens]
Official Symbol: TNF
Synonyms: TNF, tumor necrosis factor (TNF superfamily, member 2); DIF; TNFA; TNFSF2; TNF-alpha; tumor necrosis factor alpha; cachectin; APC1 protein; TNF, monocyte-derived; TNF, macrophage-derived; TNF superfamily, member 2; OTTHUMP00000029281; OTTHUMP00000037669; Tumor necrosis factor ligand superfamily member 2
GenelID: 7124
mRNA Refseq: NM_000594
Protein Refseq: NP_000585
MIM: 191160
UniProt ID: P01375
Chromosome Location: 6p21
Function: cytokine activity; identical protein binding; tumor necrosis factor receptor binding

RECOMMENDED MATERIALS

• ELISA microplates
• BSA
• Stop Solution 2 M Sulfuric Acid
• Dulbecco’s PBS [10x]

RECOMMENDED SOLUTIONS

PBS: Dilute 10xPBS to 1xPBS, pH 7.2 in sterile water.
Wash Buffer: 0.05% Tween-20 in PBS.
Block Buffer: 1.0% BSA in PBS.
Diluent: 1.0% BSA in PBS.

PLATE PREPARATION

1. Dilute to 2.0 µg/ml of capture antibody and immediately add 100 µl to each ELISA plate well. Seal the plate and incubate overnight at room temperature.
2. Aspirate the wells to remove liquid and wash the plate 4 times using 300µl of wash buffer per well.
3. After the last wash invert plate to remove residual buffer and blot on paper towel.
4. Add 300µl block buffer to each well. Incubate for at least 1 hour at room temperature.
5. Aspirate and wash plate 4 times.

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**ELISA PROTOCOL**

**Standard/Sample:** Dilute standard from 2,000 pg/ml to zero in diluent. Immediately add 100 μl of standard or sample to each well in duplicate and incubate at room temperature for at least 2 hours.

**Detection:** Aspirate and wash plate 4 times. Dilute a portion of detection antibody in diluent to a concentration of 50 ng/ml. Add 100 μl per well and incubate at room temperature for 2 hours.

**Avidin-HRP Conjugate:** Aspirate and wash plate 4 times. Dilute 3.0 μl of avidin-HRP conjugate 1:5,000 (this dilution factor may require some optimization) in diluent for a total volume of 15 ml. Add 100 μl per well and incubate at room temperature for 30 minutes.

**TMB Liquid Substrate:** Add 100 μl of TMB HRPO Microwell Substrate Standard Kinetic One Component "Ready Use" to each well. Incubate at room temperature for 20-30 minutes and monitor color development with an ELISA plate reader at 450 nm with wavelength correction set at 540 nm or 570 nm. Avoid placing plates in direct light.

**Stop Solution:** The reaction may be stopped after 15–20 minutes by adding 100 μl of 2 M sulfuric acid to each well. All eye, hand, face, and clothing protection should be used when working with sulfuric acid.

**REFERENCES**


**CROSS REACTIVITY**

When tested at 50 ng/ml the following antigen exhibited less than 5% cross reactivity: Human TNF-β, sTNF RI, sTNF RII and mouse TNF-α, sTNF RI & sTNF RII. Also rat and porcine TNF-α.

**TYPICAL STANDARD CURVE**

The standard curve is provided as an example only. A standard curve should be prepared with each assay.