

**User's Manual**

Mouse TNF- α ELISA Development Kit

REF DEIA149 Σ 45 plates**RUO**

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Mouse TNF- α ELISA Development Kit contains the key components required for the quantitative measurement of natural and/or recombinant TNF- α in a sandwich ELISA format. Using the ELISA protocol described below, this kit provides sufficient reagents to assay TNF- α in approximately 1500 ELISA plate wells.

General Description

CD120 can refer to two members of the tumor necrosis factor receptor superfamily- CD120a (TNFR1) or CD120b (TNFR2). CD120a is a 55kD Type I transmembrane protein receptor that binds both TNF- α and TNF- β (LT- α). In association with TRADD and RIP, the receptor crosslinking induced by TNF- α or TNF- β trimers is vital for signal transduction, leading to apoptosis, NF- κ B activation, increased expression of proinflammatory genes, tumor necrosis, and cell differentiation depending on cell type and differentiation state. CD120b is a 75 kD type I transmembrane protein that binds both TNF- α and TNF- β . In conjunction with TRAF1 and TRAF2, the receptor crosslinking induced by TNF- α or TNF- β trimers is critical for signal transduction that may lead to apoptosis, NF- κ B activation, increased expression of proinflammatory genes, tumor necrosis, and cell differentiation depending on cell type and differentiation state. TNF- α is a 17.5 kD protein that mediates inflammation and immunity caused by the invasion of viruses, bacteria, and parasites by initiating a cascade of cytokines that increase vascular permeability, thus bringing macrophages and neutrophils to the site of infection. TNF- α secreted by the macrophage causes the blood to clot which provides containment of the infection. TNF- α binding to surface receptors brings about various biologic activities that include cytolysis and cytostasis of many tumor cell lines In vitro, hemorrhagic necrosis of tumors In vivo, increased fibroblast proliferation, and enhanced chemotaxis and phagocytosis in neutrophils. TNF- β (LT- α) is a 25 kD protein that has a significant impact on the maintenance of the immune system including the development of secondary lymphoid organs. TNF- β has dual functions. It may function to prevent growth of cancer cells or it may facilitate the development of tumors. TNF- β is involved in the regulation of cell survival, proliferation, differentiation, and apoptosis and, if unregulated, can result in a constantly active signaling pathway, resulting in uncontrolled cellular growth and creation of tumors. Additionally, TNF- β is involved in innate immune regulation and has been shown to prevent tumor growth and obliterate cancerous cell lines.

Reagents And Materials Provided

1. Capture Antibody
2. Detection Antibody
3. Standard
4. StreptAvidin-HRP
5. TMB Liquid Substrate "Ready to Use"

Materials Required But Not Supplied

Additional Required Materials

ELISA microplates

BSA

Dulbecco's PBS (DPBS) [10x]

Stop Solution: 450 nm Stop Reagent for TMB Microwell

Required Solutions

PBS: dilute 10xPBS to 1xPBS, pH 7.2, in sterile water

Wash Buffer: 0.05% Tween-20 in PBS

Reagent Diluent: 1.0% BSA in PBS*

Blocking Buffer 1.0% BSA in PBS

Note: Other acceptable blocking buffers such as CD's Ultra-FISH Block or Ultra-Synthetic Block may be used for assay optimization.

Storage

See "Reagent Preparation"

Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS without carrier protein and immediately add 100 μ L 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 300-400 μ L of wash buffer per well.

Note: We recommend using an autowasher, although a squirt bottle or manifold dispenser would suffice.

3. After the last wash, invert plate to remove residual buffer and blot on paper towel.
4. Add 300 μ L block buffer to each well and incubate for at least 1 hour at room temperature.
5. Aspirate and wash plate 4 times.

Note: Complete removal of liquid at each step is essential for good performance and sensitivity of assay.

Reagent Preparation

Goat Anti-Mouse TNF- α Capture Antibody: Centrifuge vial prior to opening. Reconstitute in 0.5 mL sterile PBS. Refer to the lot-specific datasheet for amount supplied and dilute in PBS without carrier protein to the working concentration indicated on the C of A. Following reconstitution the capture antibody 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. Avoid repeated freeze and thaw cycles.

Biotinylated Goat Anti-Mouse TNF- α Detection Antibody: Refer to the lot-specific datasheet for amount supplied. Centrifuge vial prior to opening. Reconstitute with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent to the working concentration indicated on the C of A. 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 the freezer. Avoid repeated freeze and thaw cycles.

E. coli - expressed Recombinant Mouse TNF- α Standard: Centrifuge vial prior to opening. Reconstitute each vial with 0.5 mL of Reagent Diluent. Refer to the lot-specific datasheet for amount supplied. The rProtein may be stored at 2-8°C for one (1) month or aliquoted and stored at -70°C for up to three months in a manual defrost freezer. Avoid repeated freeze and thaw cycles.

TMB Liquid Substrate "Ready to Use" (TMB Substrate should be at ambient temperature prior to use): 60.0 mL of TMB HRP Microwell Substrate Standard Kinetic One Component "Ready Use" is provided. The high quality of the substrate can be preserved by storing at temperatures between 2-8°C. When properly stored, TMB Microwell Substrate is stable for a minimum of 48 months from the manufactured date.

Assay Procedure

Standard/Sample: Add 100 μ L of the working dilution with reagent dilution standard or sample to each well (duplicate recommended). Cover plate with an adhesive plate cover and incubate at room temperature for at least 2 hours.

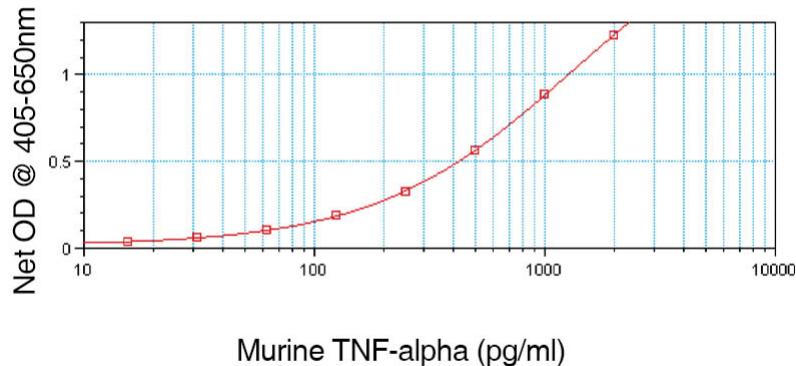
Detection: Aspirate and wash plate 4 times. Add 100 μ L of the detection antibody, diluted in Reagent Diluent to each well. Cover with a new adhesive plate cover and incubate at room temperature for 2 hours.

StreptAvidin-HRP Conjugate: Aspirate and wash plate 4 times. Add 100 μ L of the working dilution (the dilution factor may require optimization) to each well. Cover and incubate at room temperature for 20-30 minutes. Exposure to direct light should be avoided.

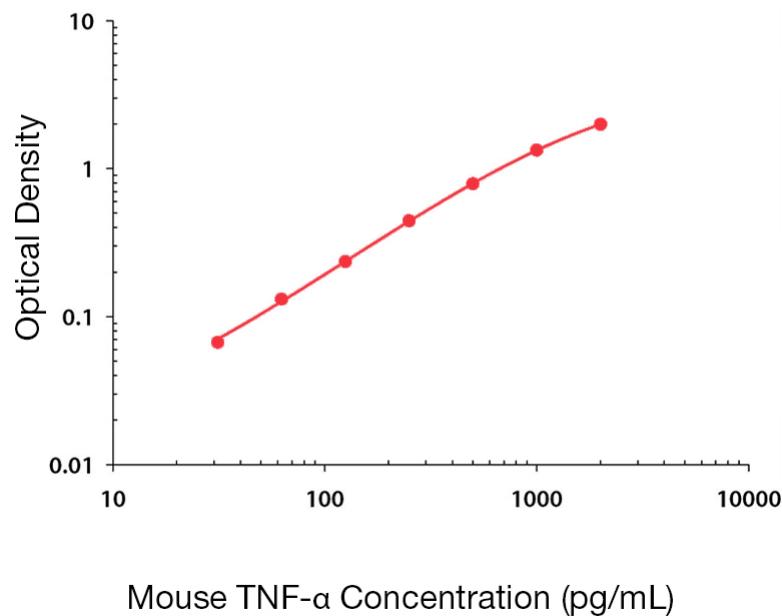
TMB Liquid Substrate: Aspirate and wash plate 4 times. Add 100 μ L of TMB HRP Microwell Substrate Standard Kinetic One Component "Ready Use" to each well. Incubate at room temperature for 20-30 minutes and monitor color development. Exposure to direct light should be avoided.

Stop Solution: Add 50-100 μ L of Stop Solution to each well. Monitor color development with an ELISA plate reader at 450 nm with wavelength correction set at 540 nm or 570 nm.

Typical Standard Curve



T519



Precautions

Some of the required components may contain acid and/or cause allergic reactions. Breathing in product mist

or fumes should be avoided. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

References

1. El-Harith el-HA et al. (2004) Saudi Med J. 25: 135
2. Adolf GR et al. (1990) Infec Immun. 58: 3996