

Murine TNF- α ELISA Development Kit

(Catalog #K1051-1000; Store kit at -20°C)

I. Description:

Murine TNF- α ELISA Development Kit contains the key components required for the quantitative measurement of natural and/or recombinant murine TNF- α in a sandwich ELISA format within the range of 8-2000 pg/ml. Using the ELISA protocol described below, the recommended microplates, reagents and solutions, the components supplied in this kit are sufficient to assay mTNF- α in approximately 1000 ELISA plate wells.

II. Kit Components:

Capture Antibody: 100 μ g of affinity purified anti-mTNF- α . Reconstitute in 1-ml sterile water for a concentration of 100 μ g/ml.

Detection Antibody: 25 μ g of biotinylated goat antigen-affinity purified anti-mTNF- α . Reconstitute in 0.25 ml sterile water for a concentration of 100 μ g/ml.

Murine TNF- α Standard: 1 μ g of recombinant mTNF- α . Reconstitute in 1 ml sterile water for a concentration of 1 μ g/ml.

III. Storage Conditions:

The reconstituted Capture Antibody and Detection Antibody can be stored at 4°C for up to 2 months or aliquoted and stored frozen at \leq -20°C for up to 6 months. Reconstituted mTNF- α standard can be kept at 4°C for 1 week, or aliquoted and stored frozen at \leq -20°C for up to 2 months.

IV. Recommended Materials:

ELISA microplates (NUNC Maxisorp, Cat.# 4420404);
Tween-20 (BioVision, Cat.# 2109-100);
BSA (BioVision, Cat.# 2119-10);
Avidin Peroxidase Conjugate (Sigma, Cat.# A-7419);
ABTS Liquid Substrate Solution (Sigma, Cat.# A3219);
PBS [10X] (BioVision, Cat.# 2113-500).

Recommended Solutions:

PBS: Dilute 10X PBS to 1PBS in ddH₂O
Wash Buffer: 0.05% Tween-20 in PBS
Block Buffer: 1% BSA in PBS
Diluent: 0.05% Tween-20, 0.1% BSA in PBS
Substrate Solution: ABTS liquid substrate solution

V. Plate Preparation:

1. Dilute Capture antibody with PBS to a concentration of 1 μ g/ml. 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 plates 4 times using 300 μ l of wash buffer per well. After the last wash invert plate to remove residual buffer and blot on paper towel.
3. Add 300 μ l block buffer to each well. Incubate for at least 1 hour at room temperature.
4. Aspirate and wash plate 4 times.

VI. ELISA Protocol:

Standard/Sample: Serial dilute standard from 2 ng/ml to zero in diluent. Immediately add 100 μ l of standard or sample to each well in triplicate. Incubate at room temperature for at least 2 hours.

Detection: Aspirate and wash plate 4 times. Dilute detection antibody in diluent to a concentration of 0.25 μ g/ml. Add 100 μ l per well. Incubate at room temperature for 2 hours.

Avidin Peroxidase: Aspirate and wash plate 4 times. Dilute Avidin Peroxidase 1:2000 in diluent. Add 100 μ l per well. Incubate 30 minutes at room temperature.

Substrate: Aspirate and wash plate 4 times. Add 100 μ l to each well. Incubate at room temperature for color development. Monitor color development with an ELISA plate reader at 405 nm with wavelength correction set at 650 nm.

Note: Reliable standard curve are obtained when either O.D. readings do not exceed 0.2 units for the zero standard concentrations, or 1.2 units for the highest standard concentration. The plate should be monitored at 5 minute intervals for approximately 25 minutes.

IV. Featured Products:

Apoptosis Detection Kits & Reagents

- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents

Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

Cell Proliferation & Senescence

- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- LDH-Cytotoxicity Assay Kit

Cell Damage & Repair

- HDAC & HAT Activity Assays & Drug Discovery Kits
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Activity Assay Kits

Signal Transduction

- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

Adipocyte & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin
- CETP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

Molecular Biology & Reporter Assays

- siRNA Expression Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits
- 5 Minutes DNA Ligation Kit