cellsciences.com

Mouse TNF-alpha ELISA Kit

Catalog No: CKM064

Size: 1 x 96 tests

Introduction:

TNF-α (tumor necrosis factor-α) is secreted by macrophages, monocytes, neutrophils, T-cells, NK-cells following their stimulation by bacterial lipopolysaccharides. Murine TNF-α is N-glycosylated. Homology with TNF-β is approximately 30 percent. TNF-α shows a wide spectrum of biological activities. It causes cytolysis and cytostasis of many tumor cell lines in vitro. Within hours after injection, TNF-α leads to the destruction of small blood vessels within malignant tumors. TNF-α also enhances phagocytosis and cytotoxicity in neutrophilic granulocytes and also modulates the expression of many other proteins.

The Mouse TNF-α ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Mouse TNF-α in serum, plasma and cell culture supernatants. This assay employs an antibody specific for Mouse TNF-α coated on a 96-well plate. Standards and samples are pipetted into the wells and TNF-α present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Mouse TNF-α antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of TNF-α bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents and materials supplied in the kit:

Items	Quantity
A. Microplate coated with Anti-Mouse TNF-alpha	96 wells
B. Wash Buffer Concentrate (20x)	25 mL
C. Recombinant Mouse TNF-alpha Standards	2 vials
D. Assay Diluent A: Standard/Sample – Serum/Plasma Diluent Buffer*	30 mL
E. Assay Diluent B (5x): Standard/Sample - Cell Culture Medium Diluent	15 mL
F. Detection Antibody: Anti-Mouse TNF-alpha Biotin Each vial is enough to coat ½ microplate.	2 vials
G. Streptavidin-HRP Concentrate (300x)	200 µl
H. TMB One-Step Substrate Reagent (3, 3', 5, 5'-tetramethylbenzidine in buffered solution)	12 mL
I. Stop Solution (0.2 M Sulfuric Acid) Caution: Acid, corrosive.	8 mL

** Contains 0.09% Sodium Azide as preservative. Precaution: Sodium Azide is a poisonous and hazardous substance which should be handled by trained staff only.*

This kit is configured for research use only. Not for diagnostic or therapeutic use.

Cell Sciences®
Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

Storage of Kit Reagents:
Stable for up to 6 months from date of shipment at 2-4°C. Store reconstituted standard (recombinant protein) at -80°C. Opened Microplate Wells and reagents are stable for 1 month at 2-4°C. Return unused wells to the pouch containing desiccant pack and reseal along the entire edge. Note: The kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

Additional Materials Required:
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µl to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- 100 mL and 1 liter graduated cylinders
- Absorbent paper
- Distilled or deionized water
- Log-log graph paper or computer/software for data analysis
- Tubes to prepare standard or sample dilution

Preparation of Kit Reagents:

1) Bring all reagents and samples to room temperature (18 - 25°C) before use.

2) Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) is used for dilution of serum/plasma samples, and Assay Diluent B (Item E) is used for dilution of culture supernatants.

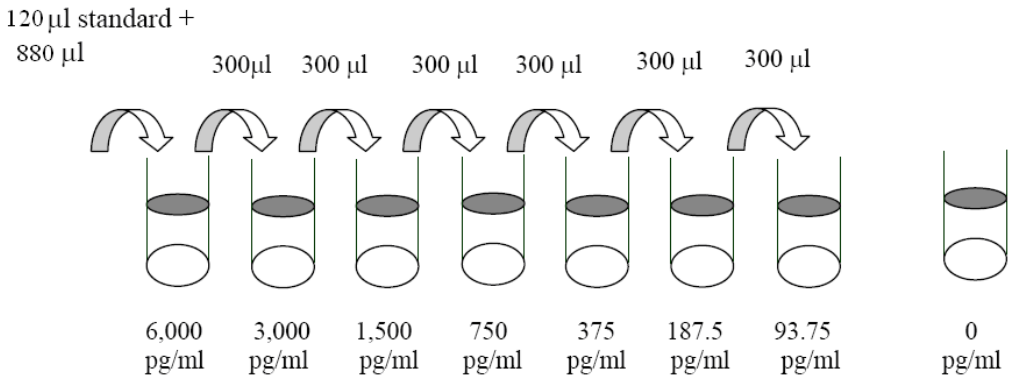
   Suggested dilution factor for normal serum/plasma: 2 fold*

   *Please note that levels of the target protein may vary between different specimens. Optimal dilution factors must be determined by the investigator.

3) Assay Diluent B should be diluted 5-fold with deionized or distilled water.

4) Preparation of standard: **Briefly spin the vial of Item C.** Add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) into Item C vial to prepare a 50 ng/ml standard. **Dissolve the powder thoroughly by a gentle mix.** Add 120 µl TNF-α standard from the vial of Item C, into a tube with 880 µl Assay Diluent A or 1x Assay Diluent B to prepare a 6,000 pg/ml stock standard solution. Pipette 300 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).

Figure 1



Cell Sciences®
Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

5) If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

6) Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of ELISA Method (below).

7) Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 300-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 40 µl of HRP-Streptavidin concentrate into a tube with 12 ml 1x Assay Diluent B to prepare a 300-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

ELISA Method:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Add 100 µl of each standard (see Preparation of Kit Reagents, step 4) and sample (see Preparation of Kit Reagents, step 2) into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.

3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µl of 1x prepared biotinylated antibody (see Preparation of Kit Reagents, step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash as in step 3.

6. Add 100 µl of prepared Streptavidin solution (see Preparation of Kit Reagents, step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution. Repeat the wash as in step 3.

8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Assay Procedure Summary:

1. Prepare all reagents, samples and standards as instructed.

⇩

2. Add 100 µl standard or sample to each well.
   Incubate 2.5 hours at RT or overnight at 2-4°C.

⇩

Cell Sciences®
Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

3. Add 100 µl prepared biotin antibody to each well.
   Incubate 1 hour at RT.

⇩

4. Add 100 µl prepared Streptavidin solution.
   Incubate 45 minutes at RT.

⇩

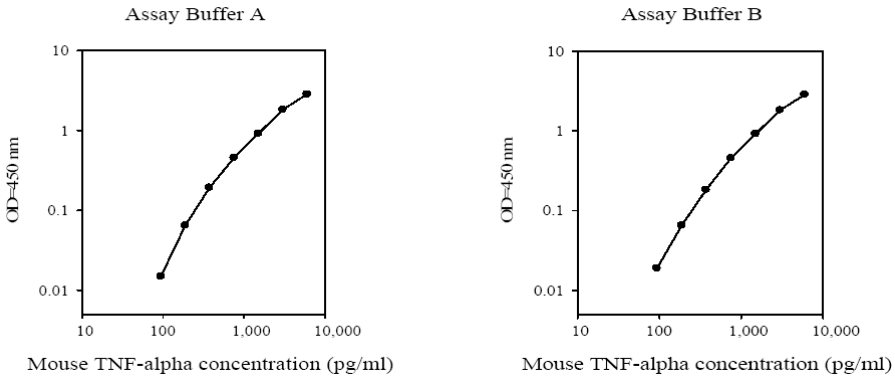
5. Add 100 µl TMB to each well.
   Incubate 30 minutes at RT.

⇩

6. Add 50 µl Stop Solution to each well.
   Read at 450 nm immediately.

Calculation of Results:
Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data:
Standard curve is for demonstration **ONLY**. A standard curve **MUST** be run with each assay.



Performance and Characteristics:

Sensitivity
The minimum detectable dose of TNF-alpha is typically less than 60 pg/mL.

Recovery

Recovery was determined by spiking various levels of Mouse TNF-alpha into mouse serum, plasma and cell culture media. Mean recoveries are as follows:

Cell Sciences®
Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

Sample Type	Average % Recovery	Range (%)
Serum	94.18	82-103
Plasma	93.82	83-102
Cell culture media	92.46	84-104

Linearity

Sample Type		Serum	Plasma	Cell culture media
1:2	Average % of Expected	93	94	92
	Range (%)	84-102	83-103	82-103
1:4	Average % of Expected	94	95	96
	Range (%)	82-103	84-104	83-104

Reproducibility

Intra-Assay: CV<10%
Inter-Assay: CV<12%

Specificity:
Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested *(e.g.*, Mouse CD30, L CD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin , Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-CFS, IFN- γ, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 α, IL-1β, IL-2, IL-3, IL-3 Rb, IL- 4, IL-5, IL-6, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, KC, Leptin R, LEPTIN(OB), LIX, L-Selectin, Lymphotactin, MCP-1, MCP-5, M-CSF, MIG, MIP-1α, MIP-1γ, MIP-2, MIP-3β, MIP-3α, PF-4, P-Selectin, RANTES, SCF, SDF-1α, TARC, TCA-3, TECK, TIMP-1, TNF RI, TNF RII, TPO, VCAM-1, VEGF).

Troubleshooting Guide:

Problem	Cause	Solution
1. Standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times	1. Ensure sufficient incubation time; change Step 2 to overnight.
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation.
3. Large CV	1. Inaccurate pipetting	1. Check pipettes.
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer.
5. Low sensitivity	1. Improper storage	1. Store your standard at <-20°C after reconstitution, others at 2-4°C. Keep substrate solution protected from light.
	2. Stop solution	2. Stop solution should be added to each well before measure.

Cell Sciences®
Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com