Mouse TNF- α ELISA Kit

For the quantitative determination of mouse tumour necrosis factor alpha (TNF-α) concentrations in mouse serum, cell culture supernatant

Catalogue Number: MEC1003

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (02) mTNF-α

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INTENDED USE

This Mouse TNF-alpha (α) ELISA Kit is to be used for the *in vitro* quantitative determination of mouse tumour necrosis factor alpha (mouse TNF-α) concentrations in serum and cell culture supernatant. This kit is intended FOR LABORATORY RESEARCH USE ONLY.

INTRODUCTION

Tumor Necrosis Factor alpha (TNF-α) is a multifunctional proinflammatory cytokine, mainly secreted by activated macrophages. The cytokine was named for its remarkable ability to cause hemorrhagic necrosis of tumors in mice. It is implicated with a variety of biological procedures including systemic inflammation, cell proliferation, apoptosis, lipid metabolism, and coagulation. It is well documented that TNF-α functions through its receptors, TNFR1 (p55) and TNFR2 (p75).

TNF-α plays an important role in the immune response to bacterial, and certain fungal, viral, parasitic invasions, in tissue remodeling, autoimmune-diseases and the necrosis of specific tumors. The pleiotropic effect of TNF-α regulation is attributed to its ability to trigger multiple signaling pathways simultaneously. TNF-α hyper-expression in response to the components of some bacteria such as LPS can cause life threatening septic shock. Recombinant TNF-α, in combination with chemotherapy, has been applied for synergistic treatment of soft sarcomas, melanomas and other irresectable tumors. Anti-TNF-α therapy has been used for treatment of rheumatoid arthritis.

This mouse TNF-α ELISA is a 3.5 hour solid phase immunoassay readily applicable to measure mouse TNF-α levels in serum, plasma, cell culture supernatant in the range of 0 to 1000pg/mL.

PRINCIPLE OF THE ASSAY

This mouse TNF-α enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to mouse TNF-α. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated monoclonal antibody preparation specific for mouse TNF-α and incubated. Mouse TNF-α if present, will bind and become immobilized by the antibody pre-coated on the wells and then be “sandwiched” by biotin conjugate. The microtiter plate wells are thoroughly washed to remove unbound mouse TNF-α and other components of the sample. In order to quantify the amount of mouse TNF-α present in the sample, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Avidin is

a tetramer containing four identical subunits that each has a high affinity-binding site for biotin. The wells are thoroughly washed to remove all unbound HRP-conjugated Avidin and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain mouse TNF-α, biotin-conjugated antibody, and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of mouse TNF-α in the samples, this kit includes two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing.) According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D) versus mouse TNF-α concentration (pg/mL). The concentration of mouse TNF-α in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **MOUSE TNF-α MICROTITER PLATE** (Part MEC03-1) ***96 wells***
   Pre-coated with anti-mouse TNF-α monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part MEC03-2) ***6 mL***
   Anti-mouse TNF-α monoclonal antibody conjugated to Biotin.

3. **AVIDIN CONJUGATE** (Part MEC03-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase.

4. **MOUSE TNF-α STANDARD** (Part MEC03-4) ***2 vials***
   Recombinant mouse TNF-α (1ng/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part MEC03-5) ***25 mL***
   Animal serum with buffer and preservative. *For serum testing.*

6. **CALIBRATOR DILUENT II** (Part MEC03-6) ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant testing.*

7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **TMB SUBSTRATE** (Part 30010) ***10 mL***
   Buffered solution with TMB. Ready for use.

9. **STOP SOLUTION** (Part 30008) ***14 mL***
   2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100μL and 50-200μL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm).
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Waste: Autoclave 60 min. at 121°C.
   Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

COLLECTION, HANDLING AND STORAGE

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Samples to be used immediately, or must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

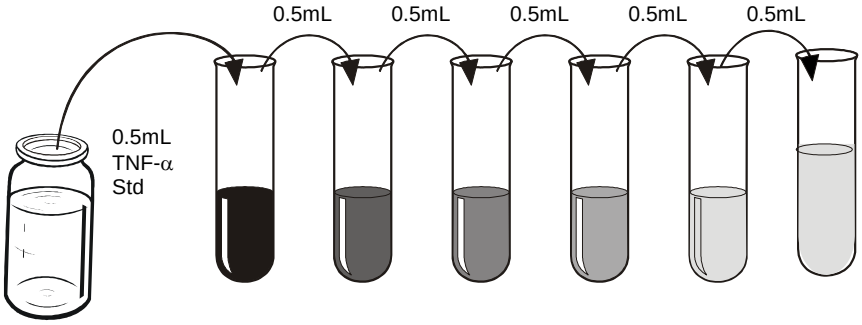
Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

2. **Mouse TNF-α Standard:**

   a) Two vials of Standard are provided in this kit to allow both serum and cell culture supernatant testing. Reconstitute mouse TNF-α Standard with 1.0 mL of the appropriate calibrator diluent. This reconstitution produces a stock solution of 1000 pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstitution. The mouse TNF-α standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles; aliquot if repeated use is expected.

   b) Use the above stock solution to produce a serial 2-fold dilution series, as described below, within the range of this assay (15.6 to 1000pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted mouse TNF-α stock solution will serve as the high standard (1000 pg/mL) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



Mouse TNF-α Standard	500 pg/mL	250 pg/mL	125 pg/mL	62.50 pg/mL	31.25 pg/mL	15.625 pg/mL	1000 pg/mL

ASSAY PROCEDURE

1. Prepare Wash Buffer and Mouse TNF-α Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards and Samples to the Microtiter Plate.*

Wells	Contents			**Wells**	Contents		
1A, 1B	Standard 1	**0 pg/mL**	(S1)	**2A, 2B**	Standard 4	**125 pg/mL**	(S5)
1C, 1D	Standard 2	**15.6 pg/mL**	(S2)	**2C, 2D**	Standard 5	**250 pg/mL**	(S6)
1E, 1F	Standard 3	**31.2 pg/mL**	(S3)	**2E, 2F**	Standard 6	**500 pg/mL**	(S7)
1G, 1H	Standard 4	**62.5 pg/mL**	(S4)	**2G, 2H**	Standard 7	**1000 pg/mL**	(S8)
3A, 3B	**Mouse TNF-alpha** samples						

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	1	5	9	13	17	21	25	29	33	37
B	S1	S5	1	5	9	13	17	21	25	29	33	37
C	S2	S6	2	6	10	14	18	22	26	30	34	38
D	S2	S6	2	6	10	14	18	22	26	30	34	38
E	S3	S7	3	7	11	15	19	23	27	31	35	39
F	S3	S7	3	7	11	15	19	23	27	31	35	39
G	S4	S8	4	8	12	16	20	24	28	32	36	40
H	S4	S8	4	8	12	16	20	24	28	32	36	40

2. Add 50µL of Standard or Sample to the appropriate well of the antibody pre-coated Microtiter Plate and incubate 1 hour at room temperature.

3. Without discarding the standards and samples, add 50μL mouse TNF-α Biotin conjugate to each wells. Mix well. Cover and incubate for 1 hour at room temperature.

4. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

5. Dispense 100μl of Avidin Conjugate to each well Mix well. Cover and incubate for 1 hour at room temperature.

6. Repeat wash procedure as described in Step 4.

7. Add 100μL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

8. Add 100μL Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader set within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of mouse TNF-α in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding mouse TNF-α concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of mouse TNF-α in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding mouse TNF-α concentration. If samples generate values higher than the highest standard, dilute the samples and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

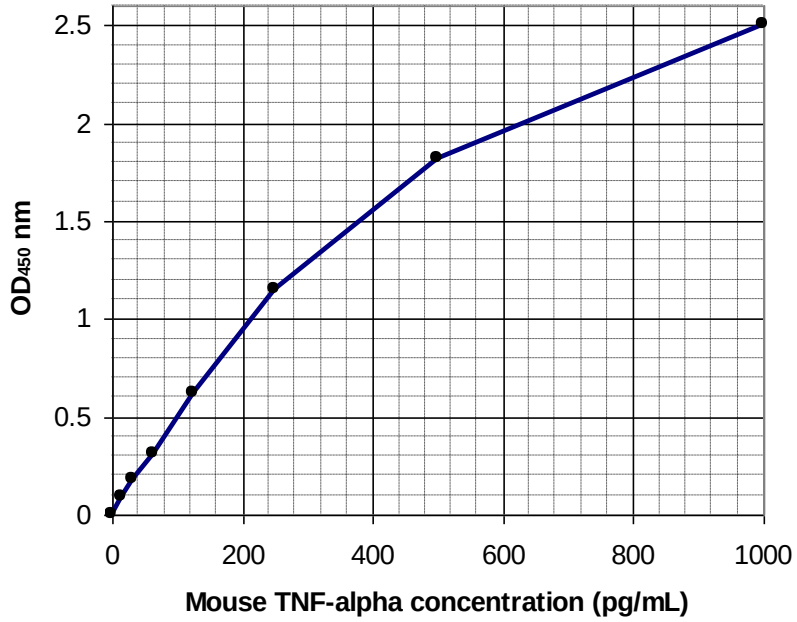
TYPICAL DATA

Results of a typical standard run of a mouse TNF-α ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of *illustration only*, and should not be used to calculate unknowns. Each user should obtain its own standard curve.

EXAMPLE ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

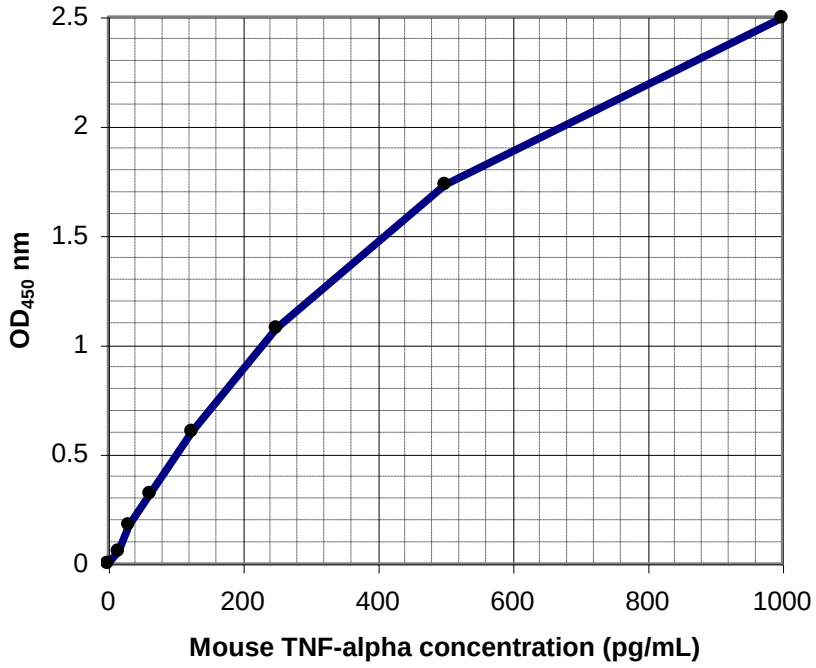
Standard (pg/mL)	O.D. (450 nm)	Zero Standard Subtracted
0	0.056	0
15.625	0.159	0.087
31.25	0.250	0.178
62.5	0.393	0.312
125	0.692	0.620
250	1.202	1.146
500	1.874	1.818
1,000	2.556	2.504



EXAMPLE TWO

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	O.D. (450 nm)	Zero Standard Subtracted
0	0.072	0
15.625	0.127	0.055
31.25	0.237	0.176
62.5	0.391	0.319
125	0.675	0.603
250	1.148	1.076
500	1.804	1.732
1,000	2.566	2.494



PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration were assayed by replicates of eighteen in 1 assay.

	Calibrator Diluent I Assay		
Sample	**1**	**2**	**3**
N	18	18	18
Mean (pg/mL)	153.1	282.8	455.6
Coefficient of Variation (%)	4.71	4.44	1.64

	Calibrator Diluent II Assay		
Sample	**1**	**2**	**3**
N	18	18	18
Mean (pg/mL)	129	257	510
Coefficient of Variation (%)	6.82	5.44	1.51

2. **INTER-ASSAY PRECISION**

To determine between-run precision, three different samples of known concentration were assayed in eight different assays.

	Calibrator Diluent II Assay		
Sample	**1**	**2**	**3**
N	8	8	8
Mean (pg/mL)	130	240	502
Coefficient of Variation (%)	10.8	4.41	4.07

3. **RECOVERY**

The recovery of mouse TNF-α spiked to cell culture media and serum were evaluated.

Sample Type	***Average Recovery (%)***
Cell Culture Media	102.7
Serum	75.5

4. **SENSITIVITY**

The minimum detectable dose of mouse TNF-α using a standard curve generated with Calibrator Diluent I or Calibrator Diluent II is 4.5pg/mL.

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