Mouse Tumor Necrosis Factor alpha (TNF-α) ELISA Kit

Catalog No. EMC102a

(96 wells)

- This immunoassay kit allows for the *in vitro* quantitative determination of **mouse TNF-α** concentrations in **serum, plasma**, **cell culture supernatants.**
- This package insert must be read in its entirety before using this product.
- **For research use only. Not for use in diagnostic or therapeutic procedures.**

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PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TNF-α has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any TNF-α present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for TNF-α is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of TNF-α bound in the initial step. The color development is stopped by the addition of a hydrochloric acid and the intensity of the color is measured at a wavelength of 450nm. The concentration of TNF-α in the sample is then determined by comparing the OD of samples to the standard curve.

MATERIALS PROVIDED & STORAGE CONDITION

The unopened kit may be stored at 4°C for up to 6 months. Use the kit before expiration date.

COMPONENT	QUANTITY	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse TNF-α Precoated Microplate	1 strip plate (8*12 strips)	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 4 °C.
Mouse TNF-α Standard	1.0ng/vial*3 vials	-20 °C. Use a new standard for each assay. Discard after use.
Biotin-antibody Conjugate **Concentrate**	1 vial	Concentrate can be stored for up to 1 month at 4 °C. Discard after use.
HRP-Streptavidin **Concentrate** (Protect from light)	1vial	

Assay Diluent	1 bottle 20 ml	1 month at 4 °C
Biotin-antibody Conjugate Concentrate **Diluent**	1 bottle 16 ml	1 month at 4 °C
HRP-Streptavidin Concentrate **Diluent**	1 bottle 16 ml	1 month at 4 °C
20× Wash Buffer **Concentrate**	1 bottle 50 ml	1 month at 4 °C
Substrate(TMB)	1 bottle 12 ml	1 month at 4 °C (Protect from light)
Stop Solution	1 bottle 12 ml	1 month at 4 °C
Plate Sealers	6 strips	

The kit contains sufficient materials to run an ELISA on one 96 well plate.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 50 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and

assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARATION

- The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

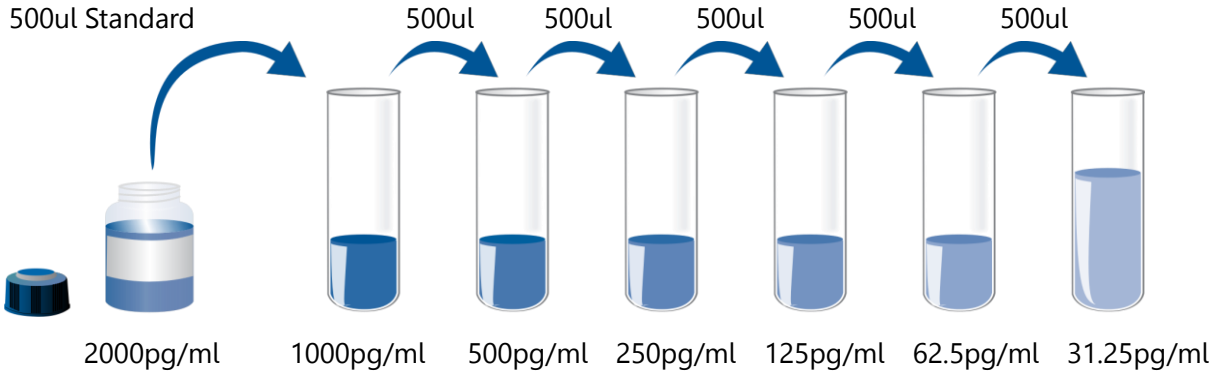
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute the **20×** Wash Buffer **Concentrate** into deionized or distilled water to prepare **1×** working solution.

Mouse TNF-α Standard - Centrifuge vial prior to opening. Reconstitute the TNF-α Standard with 0.5 ml **Assay Diluent**. The reconstitution produces a stock solution of 2000 pg/ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. The **Assay Diluent** serves as the zero standard (0 pg/ml). Pipette 500ul **Assay Diluent** into the remaining tubes; use the stock solution to produce a dilution series as below. Mix each tube thoroughly before the next transfer.

(Standard Curve: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/ml)



Biotin-Antibody - Dilute the **30×**Biotin-antibody Conjugate **Concentrate** into **1×** working solution using the Biotin-antibody Conjugate Concentrate **Diluent**.

HRP-Streptavidin - Dilute the **30×**HRP-Streptavidin **Concentrate** into **1×** working solution using the HRP- Streptavidin Concentrate **Diluent**.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100µl of Standard, Blank (Assay Diluent) or Sample per well. Cover with Plate Sealer. Incubate for **90** minutes at 37° C.
3. Aspirate each well and wash, repeating the process four times for a total five washes. Wash by filling each well with **1×** Wash Buffer(350ul) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot if against clean paper towels.
4. Prepare **Biotin-antibody** working solution 20 minutes in advance. Add 100µl of **Biotin-antibody** working solution into standard and sample wells and 100ul Biotin-antibody Conjugate Concentrate **Diluent** into Blank well.

Cover the microtiter plate with new Plate Sealer. Incubate for **1** hour at 37°C.

5. Aspirate each well and wash as step 3.
6. Prepare **HRP-Streptavidin** working solution 20 minutes in advance and protect from light. Add 100µl of **HRP-Streptavidin** working solution into standard and sample wells and HRP-Streptavidin Concentrate **Diluent** into Blank well. Cover the microtiter plate with new Plate Sealer. Incubate for **30** minutes at 37°C.
7. Repeat the aspiration and wash as step 3.
8. Add 100µl of **TMB Substrate** to each well. Incubate for **15** minutes at 37°C. **Protect from light**.
9. Add 100ul of **Stop Solution** to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well within **3** minutes, using a microplate reader set to 450nm.

CALCULATION OF RESULTS

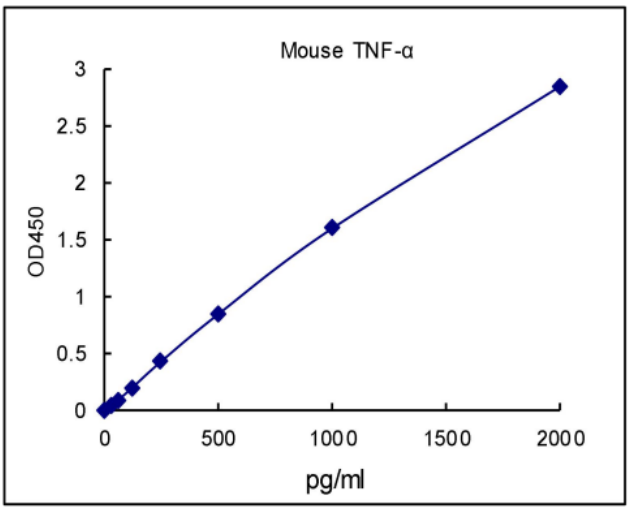
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Plot he standard curve on log-log graph paper or using plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

In case samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Plot mouse TNF-α standard curve



pg/ml	O.D.	Average
0	0.093 0.090	**0.092**
31.25	0.140 0.132	**0.136**
62.5	0.186 0.183	**0.185**
125	0.296 0.295	**0.296**
250	0.524 0.521	**0.523**
500	0.894 0.981	**0.938**
1000	1.675 1.711	**1.693**
2000	2.924 2.962	**2.943**

PRECISION

Intra-assay Precision: CV (%) <10%
Inter-assay Precision: CV (%) <10%

DETECTION RANGE

31.25pg/ml-2000pg/ml. The standard curve concentrations used for the ELISA's were 2000pg/ml, 1000pg/ml, 500pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml.

SENSITIVITY

The minimum detectable dose of mouse TNF-α was determined to be 15.6 pg/ml. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

SPECIFICITY

This assay recognizes natural and recombinant mouse TNF-α. No significant cross-reactivity or interference was observed with human IL-2, IL-4, IL-6, IL-8,

IL-10, TNF-α, TNF RI, TNF RII; mouse IL-2, IL-4, IL-6, IL-10, TNF-β, LIF, RANK, Fas and porcine TNF-α.

LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with **Assay Diluent** and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- Centrifuge vials before opening to collect contents.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is important.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

NOTE

Kit Lot No._____ Exp.Date _____ Date _____Tech _____

1st Incub: Start Time _____ Temp _____ Note:_____
End Time _____ Temp _____ Note:_____

2nd Incub: Start Time _____ Temp _____ Note:_____
End Time _____ Temp _____ Note:_____

3rd Incub: Start Time _____ Temp _____ Note:_____
End Time _____ Temp _____ Note:_____