

## HUMAN TNF- $\alpha$ ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF TNF- $\alpha$  CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND PLASMA.



FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

### PURCHASE INFORMATION:

ELISA NAME	HUMAN TNF- $\alpha$ ELISA
Catalog No.	SK00109-01
Lot No.	
Formulation	96 T
Standard range	3.9-500 pg/ml
Sensitivity	1.9 pg/ml
Sample Volume	100 $\mu$ l
Sample Dilution	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human TNF- $\alpha$ only
Intra-assay Precision	6-8%
Inter-assay Precision	10-12%
Storage	2°C - 8°C

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## INTRODUCTION

Human TNF- $\alpha$  immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure TNF- $\alpha$  in cell culture supernates, serum and plasma. It contains recombinant TNF- $\alpha$  and antibodies raised against this protein. It has been shown to accurately quantify recombinant TNF- $\alpha$ . Results obtained with naturally occurring TNF- $\alpha$  samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural TNF- $\alpha$ .

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human TNF- $\alpha$  has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF- $\alpha$  present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for human TNF- $\alpha$  is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, Avidin link HRP is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of TNF- $\alpha$  bound in the initial step. The color development is stopped and the intensity of the color is measured.

## LIMITATIONS OF THE PROCEDURE

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\_ The kit should not be used beyond the expiration date on the kit label.

\_ Do not mix or substitute reagents with those from other lots or sources.

\_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

\_ If samples generate values higher than the highest standard, dilute the samples with dilution buffer and repeat the assay.

\_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

\_ 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.

## MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
<b>TNF-<math>\alpha</math> Microplate</b> - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human TNF- $\alpha$ .	<b>109-01-01</b>	<b>1 plate</b>
<b>TNF-<math>\alpha</math> Standard</b> – 4000 pg/vial of recombinant TNF- $\alpha$ in a buffered protein base with preservatives; lyophilized.	<b>109-01-02</b>	<b>1 vial</b>
<b>Detection Antibody Concentrate</b> – 105 $\mu$ L/vial, 100-fold concentrated of biotinylated antibody against human TNF- $\alpha$ with preservatives; lyophilized.	<b>109-01-03</b>	<b>1 vial</b>
<b>Positive Control</b> - one vial of recombinant TNF- $\alpha$ , lyophilized	<b>109-01-04</b>	<b>1 vial</b>
<b>Avidin-HRP Conjugate</b> - 50 $\mu$ L/vial, 250-fold concentrated solution of Avidin conjugate to HRP with preservatives	<b>AVHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> - 60mL of buffered protein based solution with preservatives	<b>DB07</b>	<b>1 bottle</b>
<b>Wash Buffer</b> - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	<b>WB01</b>	<b>1 bottle</b>
<b>TMB Substrate Solution</b> - 11 mL of TMB substrate solution	<b>TMB01</b>	<b>1 bottle</b>
<b>Stop Solution</b> – 11 mL of 0.5M HCl	<b>S-STOP</b>	<b>1 bottle</b>
<b>Plate Sealer</b>	<b>EAPS</b>	<b>1 piece</b>

## STORAGE

**Unopened Kit:** Store at 2 - 8°C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.

**Opened / Reconstituted Reagents:** Reconstituted Standard and Antibody Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Avidin-HRP Conjugate 250-fold concentrate and other components may be stored at 2 - 8°C for up to 6 months.

**Microplate Wells:** Return unused wells to the plastic pouch containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

**OTHER SUPPLIES REQUIRED**

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

**PRECAUTIONS FOR USE**

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

**SAMPLE COLLECTION AND STORAGE**

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  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  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.**

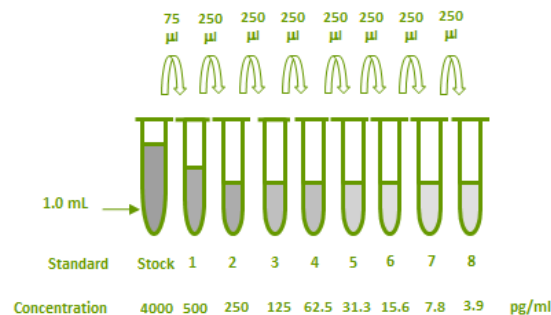
**REAGENT PREPARATION**

**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 50 ml of Wash Buffer Concentrate into deionized or distilled water (450 ml) to prepare 500 ml of Wash Buffer.

**TNF- $\alpha$  Standard - Refer to vial label for reconstitution volume.** Reconstitute the TNF- $\alpha$  standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipet 525  $\mu$ L of Dilution Buffer into tube #1, then pipet 75  $\mu$ L of stock solution into tube #1 to produce the high standard of 500 pg/mL. Pipet 250  $\mu$ L of Dilution Buffer into tubes #2 to #8, and use the high standard solution to produce a dilution series (see below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1000 $\mu$ l	4000 pg/ml
# 1	75 $\mu$ l of stock	525 $\mu$ l	500 pg/ml
# 2	250 $\mu$ l of 1	250 $\mu$ l	250 pg/ml
# 3	250 $\mu$ l of 2	250 $\mu$ l	125 pg/ml
# 4	250 $\mu$ l of 3	250 $\mu$ l	62.5 pg/ml
# 5	250 $\mu$ l of 4	250 $\mu$ l	31.25 pg/ml
# 6	250 $\mu$ l of 5	250 $\mu$ l	15.6 pg/ml
# 7	250 $\mu$ l of 6	250 $\mu$ l	7.8 pg/ml
# 8	250 $\mu$ l of 7	250 $\mu$ l	3.9 pg/ml



**Detection Antibody Concentrate** - Reconstitute the Detection Antibody Concentrate with 105  $\mu$ L of Dilution Buffer to produce a 100-fold concentrated

stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105  $\mu$ L of 100-fold concentrated stock solution to prepare working solution.

**Avidin-HRP Conjugate** - Pipette 11.952 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 48  $\mu$ L of 250-fold concentrated stock solution to prepare working solution. **Note:** 1X working solution of Avidin-HRP Conjugate should be used within a few days.

**Positive Control** - Reconstitute the positive control with 2.0 mL of Dilution Buffer to make positive control working solution. **Note:** Positive control working solution should be used within a few days.

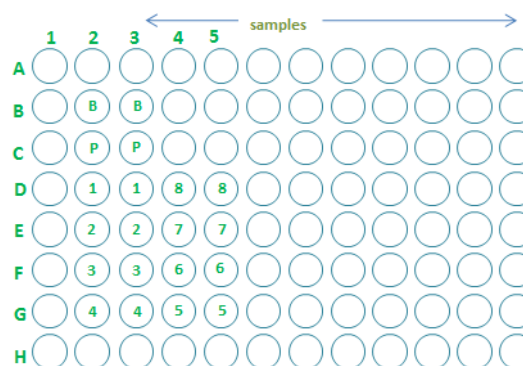
### ASSAY PROCEDURE

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

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Add 100  $\mu$ L of Dilution Buffer to Blank well (B2, B3).
4. Add 100  $\mu$ L of Standard (from D2, D3 to G2, G3 and D4, D5 to G4, G5), sample, or positive control (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300  $\mu$ L) using a squirt bottle, manifold dispenser, 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.
6. Add 100  $\mu$ L of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100  $\mu$ L of Avidin-HRP Conjugate working solution to each well. Incubate for 1 hour on

micro-plate shaker at room temperature. **Protect from light.**

9. Repeat the aspiration/wash as in step 5.
10. Add 100  $\mu$ L of Substrate Solution to each well. Incubate for 2-6 minutes at room temperature. **Protect from light.**
11. Add 100  $\mu$ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



### CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the TNF- $\alpha$  concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

### TYPICAL DATA

A standard curve should be generated for each set of samples assayed.

TNF- $\alpha$ (PG/ML)	CORRECTED O.D. (450NM)
Blank	0 (0.085)
3.9	0.036
7.8	0.071
15.6	0.165
31.25	0.306
62.5	0.526
125	1.060
250	1.968
500	3.086

- **Lot No.:**
- **Positive Control : 100 - 186 pg/mL**

**CALIBRATION**

This immunoassay is calibrated against a highly purified recombinant human TNF- $\alpha$ .

**SENSITIVITY**

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of TNF- $\alpha$  was 1.9 pg/mL.

**SPECIFICITY**

This assay recognizes both natural and recombinant human TNF- $\alpha$ . The factors listed below were prepared at 50 ng/ml in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/ml in a mid-range rh TNF- $\alpha$  control were assayed for interference. No significant cross-reactivity or interference was observed.

**Human Recombinant Proteins:** IL-4, IL-1 $\alpha$

**Mouse Recombinant Proteins:** TNF- $\alpha$

**SUMMARY OF ASSAY PROCEDURE**

