HUMAN TNF-α ELISA KIT

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

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN TNF-α ELISA**
Catalog No.	SK00109-01
Lot No.	
Formulation	96 T
Standard range	3.9 - 500 pg/mL
Sensitivity	1-2 pg/mL
Sample Volume	100 µL
Sample Dilution	*Optimal dilutions should be determined by each laboratory for each application*
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human TNF-α only
Calibration	Human TNF-α recombinant
Intra-assay Precision	6 - 8%
Inter-assay Precision	10 - 12%
Storage	2 – 8°C for 1 month, more information check page 2 ~3
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human TNF-α ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human TNF-α from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human TNF-α and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural TNF-α samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human TNF-α. The capture antibody can bind to the human TNF-α in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human TNF-α is added to the wells. After another washing of the plate, Avidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human TNF-α bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
TNF-α Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human TNF-α .	**109-01-01**	**1 plate**
TNF-α Standard – refer to lot of recombinant TNF-α in a buffered protein base with preservative; lyophilized.	**109-01-02**	**1 vial**
Detection Antibody Concentrate – refer to lot of biotinylated antibody against human TNF-α with preservative; lyophilized.	**109-01-03**	**1 vial**
Positive Control - one vial of 5-fold concentrate of recombinant TNF-α; lyophilized.	**109-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 150 µL/vial of 80-fold concentrated solution of Streptavidin conjugate to HRP with preservative.	**SAHRP**	**1 vial**
Dilution Buffer – 50 mL of buffered protein based solution with preservative.	**DB10**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB108A**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl solution.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8°C for up to 1 month. For longer storage up to 10 months, unopened Standard, Positive Control and Detection Antibody Concentrate, Dilution Buffer (DB10) and HRP Diluent Solution (DB108A) should be stored at -20°C. **Streptavidin-HRP Conjugate** and **TMB Substrate Solution** should be stored only at 2 ~ 8 °C**.** Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - 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, 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 ≤ -20°C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Plasma and serum samples DO NOT NEED to be diluted.

Optimal dilutions should be determined by each laboratory for each application with a sample pretest.

Use polypropylene test tubes.

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 1x Wash Buffer.

TNF-α Standard - Reconstitute the TNF-α standard with refer to lot Dilution Buffer. Pipet 250 µL of Dilution Buffer into tubes #2 to #8, and use the high standard solution #1 (500 pg/mL) 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	Refer to lot	
# 1	Refer to lot	Refer to lot	500 pg/ml
# 2	250 µl of 1	250 µl	250 pg/ml
# 3	250 µl of 2	250 µl	125 pg/ml
# 4	250 µl of 3	250 µl	62.5 pg/ml
# 5	250 µl of 4	250 µl	31.25 pg/ml
# 6	250 µl of 5	250 µl	15.6 pg/ml
# 7	250 µl of 6	250 µl	7.8 pg/ml
# 8	250 µl of 7	250 µl	3.9 pg/ml

Positive Control - Reconstitute the positive control with refer to lot of **Dilution Buffer (DB10)** to make a 5-fold concentrated stock solution. To make positive control working solution, dilute five times (i.e. add 200 µL of **Dilution Buffer (DB10)** + 50 µL of positive control stock solution).

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with refer to lot of **Dilution Buffer (DB10)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Dilution Buffer (DB10)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 10.368 mL of **HRP Diluent Solution (DB108A)** into a 15 mL centrifuge tube and transfer 132 µL of 80-fold

concentrated stock solution to prepare working solution (protect from light).

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL of Dilution Buffer to Blank wells.
3. Add 100 µL of Standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) 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.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of **Streptavidin-HRP Conjugate** working solution to each well. Incubate for 1 hour on micro-plate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot on micro-plate shaker at room temperature. **Protect from light.**
10. Add 100 µL 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.
11. Determine the optical density of each well using a micro-plate reader set to 450nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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-α (PG/ML)	CORRECTED O.D. (450NM)
Blank	0 (refer to lot)
3.906	0.020
7.813	0.037
15.625	0.093
31.25	0.172
62.5	0.342
125	0.710
250	1.408
500	2.481

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human TNF-α	100
Human IL-4	0
Human IL-1α	0
Mouse TNF-α	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light**.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate refer to lot on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read at 450nm.