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## Mouse TNF- $\alpha$ Immunoassay

Catalog Number: SEKM-0034

For the quantitative determination of mouse TNF- $\alpha$  concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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**REPEATABILITY:** The coefficient of variation of both intra-assay and inter-assay were less than 10%.

**RECOVERY:** The recovery of TNF- $\alpha$  spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of TNF- $\alpha$  in two matrices

Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	90	88-103
Cell culture supernatants	102	92-114

**LINEARITY:** To assess the linearity of the assay, three samples were spiked with high concentrations of TNF- $\alpha$  in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

The linearity of the assay

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	93	96
	Range (%)	84-95	93-112
1:4	Average% of Expected	92	106
	Range (%)	89-102	101-115
1:6	Average% of Expected	93	97
	Range (%)	83-108	88-106
1:8	Average% of Expected	105	94
	Range (%)	97-113	89-103

### Performance Characteristics

**SENSITIVITY:** The minimum detectable dose was 3.9 pg/mL.

**SPECIFICITY:** This assay recognizes both natural and recombinant mouse TNF- $\alpha$ . The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant mouse	Recombinant rat	Recombinant human
G-CSF		TNF- $\alpha$
GM-CSF		TNF-sRI
IL-1 $\alpha$		
IL-1 $\beta$		

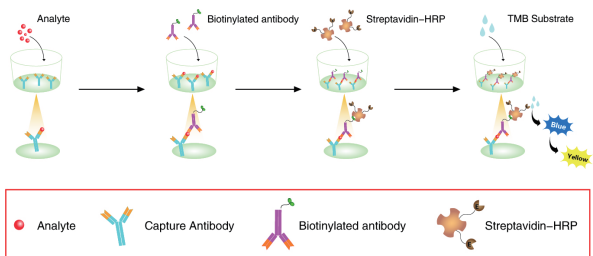
### BACKGROUND

Mouse TNF- $\alpha$  is synthesized as a 26 kDa type II transmembrane protein that consists of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 179 aa extracellular domain (ECD). Within the ECD, mouse TNF- $\alpha$  shares 95% aa identity with rat, and 80% aa identity with canine, equine, feline, human, rabbit, and porcine TNF- $\alpha$ . It is produced by a wide variety of immune, epithelial, endothelial, and tumor cells. TNF- $\alpha$  is assembled intracellularly to form a noncovalently linked homotrimer which is expressed on the cell surface. Cell surface TNF- $\alpha$  can both induce the lysis of tumor cells and virus infected cells, and generate its own downstream cell signaling following ligation by soluble TNF RI. Shedding of membrane bound TNF- $\alpha$  by TACE/ADAM17 releases the bioactive cytokine, a 55 kDa soluble trimer containing the TNF- $\alpha$  extracellular domain.

### PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TNF- $\alpha$  has been pre-coated onto a microplate. Standards and samples are pipetted into the wells; any TNF- $\alpha$  present is captured by the coated antibody after incubation. After washing away any unbound substances, a biotin-conjugate antibody specific for TNF- $\alpha$  is added to detect the captured TNF- $\alpha$  protein in the sample. Following a wash to remove any unbound combination, horseradish peroxidase (HRP)-conjugated Streptavidin is added to the wells. After extensive washing, a tetramethyl-benzidine (TMB) reagent is added to the wells for signal development. Solution containing sulfuric acid is used to stop color development. The color intensity, proportional to the quantity of bound protein, is then measurable at 450nm.

## DESCRIPTION



## TECHNICAL HINTS AND LIMITATIONS

1. This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

## PRECAUTIONS

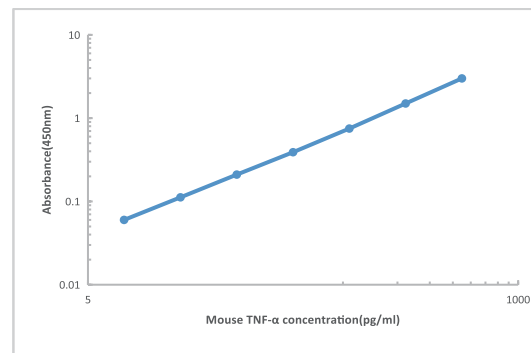
The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

## DESCRIPTION

4. 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.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the TNF- $\alpha$  ELISA

Standardized(pg/ml)	OD.	OD.	Average	Corrected
0	0.109	0.112	0.111	-
7.81	0.178	0.180	0.179	0.069
15.63	0.259	0.262	0.260	0.150
31.25	0.385	0.390	0.387	0.277
62.5	0.611	0.619	0.615	0.505
125	0.993	1.006	0.999	0.889
250	1.616	1.637	1.627	1.516
500	2.633	2.668	2.651	2.540



Representative standard curve for TNF- $\alpha$  ELISA.

**ASSAY PROCEDURE**

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.



Add 100µl standard or samples to each well, incubate 90 minutes, 37°C.



Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-mouse TNF-α antibody to each well, incubate 60 minutes, 37°C.



Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes, 37°C.



Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 15 minutes, 37°C. Protect from light.



Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

**CALCULATION OF RESULTS**

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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.

**KIT COMPONENTS & STORAGE CONDITIONS**

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells x12 strips)	1 plate	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 2 – 8°C**
Standard - lyophilized, 2000 pg/ml upon reconstitution	2 vials	Store at 2-8°C ***for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 µl/vial	1 vial	Store at 2-8°C ***for six months
Concentrated Streptavidin-HRP solution(100X) - 120 µl/vial	1 vial	Store at 2-8°C ***for six months
Standard/Sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C ***for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C ***for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C ***for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C ***for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C ***for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C ***for six months
Plate Cover Seals	4 pieces	

\*\*Provided this is within the expiration date of the kit.

**OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED**

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirrt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

**SPECIMEN COLLECTION & STORAGE**

**Cell Culture Supernates** - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at  $\leq -20\text{ }^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

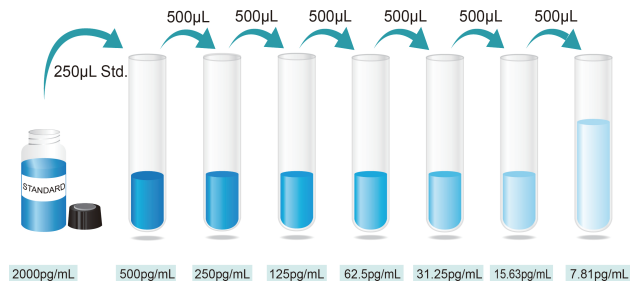
**Serum** - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at  $\leq -20\text{ }^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

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

**REAGENTS PREPARATION**

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25°C) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard/Sample** - Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 2000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 750µL of Standard/Sample Diluent into the 500pg/mL tube, and add 250µL stock solution of 2000pg/mL into it to get the 500pg/mL. Use the 500pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 500 pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).

Preparation of TNF- $\alpha$  standard dilutions

\*If you do not run out of re-melting standard, store it at  $-20\text{ }^{\circ}\text{C}$ . Diluted standard shall not be reused.

4. **Working solution of Biotin-Conjugate anti-mouse TNF- $\alpha$  antibody**- Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.  
\*The working solution should be used within one day after dilution.
5. **Working solution of Streptavidin-HRP**: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.