

Feline TNF- α ELISA Kit Immunoassay

Catalog Number: SEKF-0145

For the quantitative determination of Feline TNF- α concentrations in cellculture supernates, serum, and plasma.

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

MANUFACTURED AND DISTRIBUTED BY:

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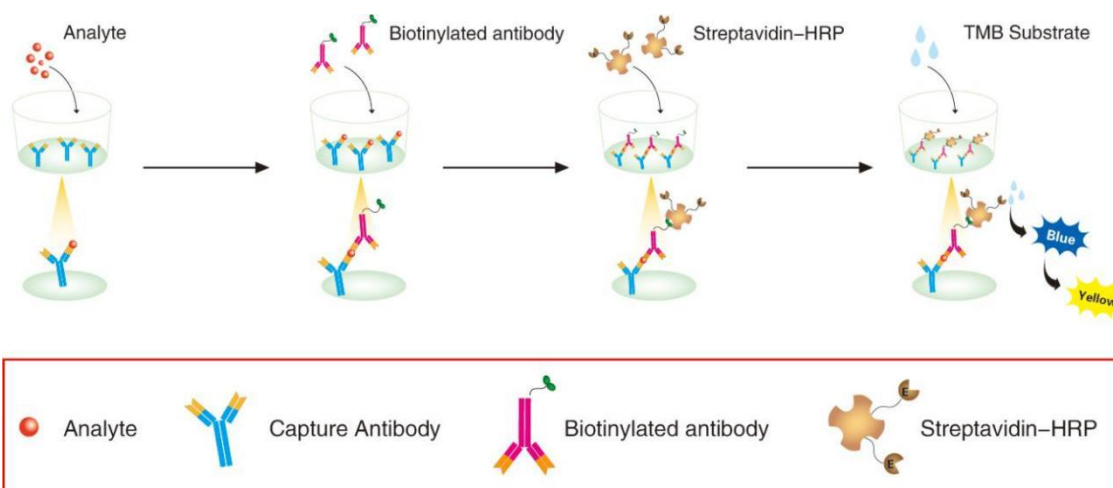
BACKGROUND

TNF- α , the prototypical member of the TNF protein superfamily, is a homotrimeric type-II membrane protein. Membrane bound TNF- α is cleaved by the metalloprotease TACE/ADAM17 to generate a soluble homotrimer. Both membrane and soluble forms of TNF- α are biologically active. TNF- α is produced primarily by macrophages, but it is produced also by a broad variety of cell types including lymphoid cells, mast cells, endothelial cells, cardiac myocytes, adipose tissue, fibroblasts, and neuronal tissue. Cellular response to TNF- α is mediated through interaction with receptors TNF-R1 and TNF-R2 and results in activation of pathways that favor both cell survival and apoptosis depending on the cell type and biological context. Activation of kinase pathways (including JNK, ERK (p44/42), p38 MAPK and NF- κ B) promotes the survival of cells, while TNF- α mediated activation of caspase-8 leads to programmed cell death.

PRINCIPLE OF THE ASSAY

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

Schematic diagram:



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.

KIT COMPONENTS & STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate -antibody coated 96-well Microplate (8 wells ×12 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, 2000pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Concentrated Biotin-Conjugated antibody (100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution (100X) - 120 ul/vial	1 vial	Store at 2-8°C** for six months
Standard /Sample Diluent - 16ml/vial	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent - 16ml/vial	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent - 16ml/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. Squirt 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 ≤ -20 °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 ≤ -20 °C. Avoid repeated freeze-thaw cycles.

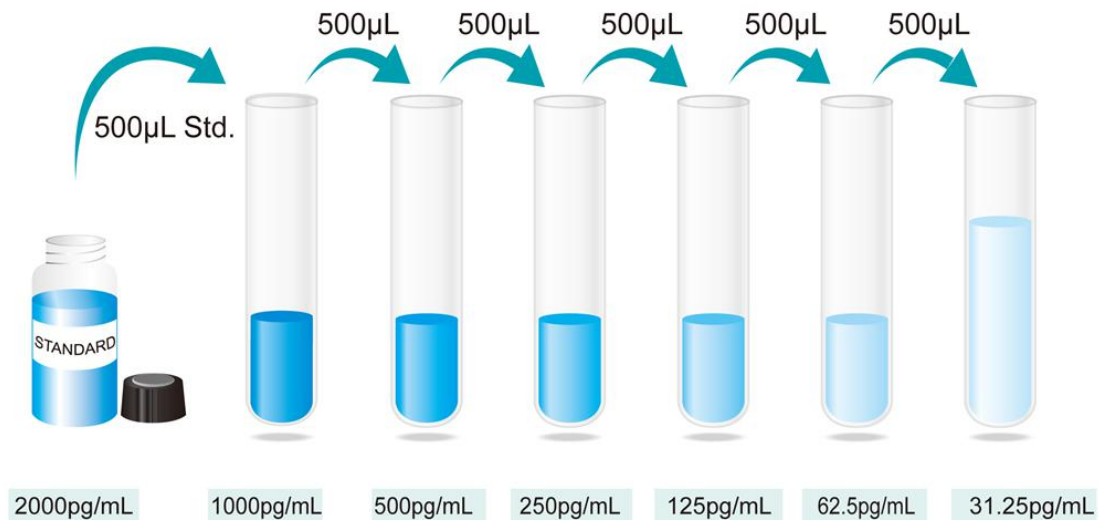
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: The normal Fish serum or plasma samples are suggested to make a 1:2 dilution.

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/Specimen**- 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. Use the stock solution of 2000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer.

The 2000pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).



Preparation of Feline TNF- α standard dilutions

***If you do not run out of re-melting standard, store it at -20°C. Diluted standard shall not be reused.**

- Working solution of Biotin-Conjugate anti-Feline TNF- α 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.**

- 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.

***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

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



Add 100µl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature($25 \pm 2^\circ\text{C}$).



Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-Feline TNF- α antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room



Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 30 minutes at room temperature($25 \pm 2^\circ\text{C}$).



Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 10-20 minutes (depending on signal) at room temperature($25 \pm 2^\circ\text{C}$).Protect from light.



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

CALCULATION OF RESULTS

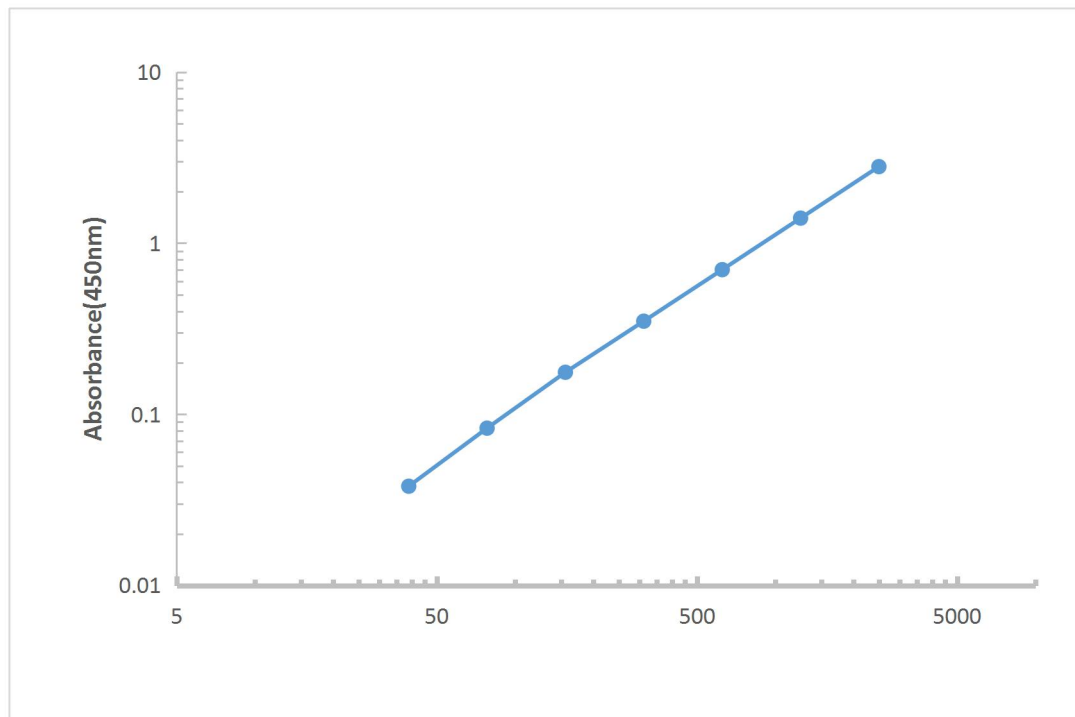
1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using 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.
4. The data may be linearized by plotting the log of the GH 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.

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

Typical data using the Feline TNF- α ELISA

标准品浓度(pg/ml)	OD值1	OD值2	平均值	矫正值
0	0.043	0.041	0.042	-----
31.25	0.088	0.082	0.085	0.043
62.5	0.109	0.110	0.109	0.068
125	0.184	0.186	0.185	0.143
250	0.339	0.335	0.337	0.295
500	0.642	0.645	0.643	0.602
1000	1.268	1.263	1.265	1.224
2000	2.491	2.495	2.493	2.451



Representative standard curve for Feline TNF- α ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 25pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant Feline TNF- α . The following recombinant feline proteins prepared at 1 ng/ml were tested and exhibited no cross-reactivity or interference.

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

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

BMP1, BMP2, BMP4, HGF, IL-1 β , IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15

Recovery of GH in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	90	81-115
Cell culture supernatants	93	90-120

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of **Feline TNF- α** in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	90	101
	Range (%)	80-103	96-113
1:4	Average% of Expected	92	108
	Range (%)	90-112	101-114
1:8	Average% of Expected	95	110
	Range (%)	83-104	96-111
1:16	Average% of Expected	90	103
	Range (%)	88-101	94-113

REFERENCES

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