Mouse TNF-α ELISA Kit

Product description

Tumor necrosis factor-alpha (TNF-α), also known as cachexin or TNFSF1A, is a multifunctional cytokine that plays a central role in inflammation, apoptosis, and immune system development. It is produced by various immune cells, epithelial cells, endothelial cells, and tumor cells. TNF-α exerts its effects through receptors TNFR1 and TNFR2, activating signaling pathways involving caspase 8, transcription factor NF-κB, and kinase JNK.

Human TNF-α shares 79% amino acid homology with mouse TNF-α, suggesting that its biological functions may not exhibit significant species specificity. TNF-α has been shown to confer resistance against certain types of infections while paradoxically inducing inflammation in pathological processes. It may also affect glucose uptake and insulin resistance. Additionally, TNF-α plays a critical role in tumor proliferation, migration, invasion, and angiogenesis.

The Arcegen Mouse TNF-α ELISA assay kit is an in vitro enzyme-linked immunosorbent assay (ELISA) kit designed to quantitatively measure mouse TNF-α in serum and plasma samples. The assay utilizes high-affinity anti-mouse TNF-α antibodies pre-coated onto an ELISA plate. Standard samples and test samples are added to the plate wells and incubated, allowing mouse TNF-α present in the samples to bind to the solid-phase antibodies. After washing to remove unbound substances, a detection antibody is added for incubation, followed by addition of enzyme conjugate (Streptavidin-HRP). After washing again, a colorimetric substrate (TMB) is added for color development, and the intensity of the color reaction is proportional to the concentration of mouse TNF-α in the samples. The reaction is terminated, and absorbance is measured at 450 nm wavelength (with a reference wavelength of 570-630 nm).

Specifications

Item Number	P162006S / P162006E
Specification	48 T / 96 T
Detection Range	31.25-2000 pg/mL
Detection Method	Sandwich ELISA
Species Detected	Mouse
Detection Time	4.5 hours
Sensitivity	7.82 pg/mL
Dilution Linearity	84 - 124%
Recovery Rate	81 - 119%
Intra-assay Variability	3.8%
Inter-assay Variability	5.3%

Components

Component Number	Component Names	Storage Temperature	P162006S	P162006E
P162006-A	ELISA plate	2~8°C	48 T	96 T
P162006-B	Standard sample	2~8°C	1 tube	2 tubes
P162006-C	Detection antibody	2~8°C	120 µL	240 µL
P162006-D	Enzyme conjugate	2~8°C(Avoid Light)	30 µL	60 µL
P162006-E	5× dilution buffer	2~8°C	8 mL	15 mL
P162006-F	20× wash buffer	2~8°C	25 mL	50 mL
P162006-G	Substrate solution	2~8°C(Avoid Light)	8 mL	15 mL
P162006-H	Stop solution	Room Temperature	5 mL	10 mL
P162006-I	Plate sealing film	Room Temperature	3 pieces	5 pieces

Storage

The kit can be stored at 2~8°C, or according to the storage conditions of individual components to avoid contamination and repeated freeze-thaw cycles. Diluted reagents prepared to working concentration should be used immediately and discarded; they should not be reused. The shelf life is 1 year.

Table 1. Reagent Storage Table After Initial Use

Component Names	Storage Conditions
ELISA plate	Unused strips can be returned to the aluminum foil bag, tightly sealed, and stored at 2~8°C to avoid moisture absorption.
Standard sample	Use within 48 hours after dissolution, store at 2~8°C to avoid contamination.
Detection antibody	Use within 48 hours after dilution, store at 2~8°C to avoid contamination.
Enzyme conjugate	
5× dilution buffer	Store at 2~8°C for 1 month, avoiding contamination.
20× wash buffer	
Substrate solution	Store at 2~8°C for 1 month, avoiding light exposure.
Stop solution	Can be stored at room temperature.
Plate sealing film	

Instructions for use

1. Used for quantitative detection of TNF-α content in serum, plasma, and cell culture supernatants.

2. Please read the instructions carefully before using this product.

Operating precautions

1. This product is for research use only.
2. For your safety and health, please wear lab coat and disposable gloves when handling.
3. The kit should be used within the shelf life. Mixing related reagents from different batches is prohibited.
4. This product is only intended for detecting target antigens and samples as indicated in the instructions. Other applications should be validated and evaluated for reliability and accuracy by the user.
5. Do not mix or substitute reagents or materials from different kit batches or suppliers.

Common Technical Tips

1. If the sample OD value is higher than the S1 OD value, further dilution should be done in an appropriate diluent.
2. Avoid generating foam during mixing.
3. When adding standard solutions, samples, and others, replace tips in a timely manner to avoid cross-contamination.
4. Ensure proper sealing of the microplate or complete covering with plate sealer during the incubation period.
5. Completely remove all solutions and buffers during the washing steps.
6. Before dissolving the standard solutions, do not invert the vials randomly. After inverting the vials, thoroughly mix by gentle inversion after adding buffer and then centrifuge at low speed.
7. Place reagents according to the instructions during the experiment.
8. Discard buffer solutions promptly after completing the experiment; use once and discard.
9. Different kits have different compositions; do not mix components from different kits.

Other Preparation Materials

1. ELISA reader, measure absorbance at 450 nm (reference wavelength 630 nm).
2. Incubator, automated microplate washer.
3. Pipettes, 1 μL to 1 mL pipettes with corresponding tips.
4. 100 mL and 1 L graduated cylinders.
5. Standard or sample dilution tubes.
6. Blotting paper.
7. Distilled water or deionized water.
8. Computer and analysis software.

Pre-Experiment Preparation

1. Sample Collection and Processing

1) Cell culture supernatant: Centrifuge at 1, 000×g for 10 minutes to remove precipitates. Test immediately or aliquot and store at -20°C or below.

2) Serum samples: Collect serum using tubes free from pyrogens and endotoxins. Allow blood to clot for 30 minutes, then centrifuge at 1, 000×g for 10 minutes. Test immediately after collection or aliquot and store at -20°C or below.

3) Plasma samples: Collect plasma using EDTA, sodium citrate, or heparin anticoagulants. Centrifuge at 1, 000×g for 30 minutes to collect the sample. Test immediately or aliquot and store at -20°C or below.

This assay kit may be applicable to other biological samples. Serum and cell culture supernatant have been validated.

[Note]Remove visible precipitates from samples before testing. Do not use severely hemolyzed or lipemic samples. Samples should be aliquoted and stored at -20°C to prevent loss of TNF-α activity. If testing within 24 hours, samples can be stored at 2~8°C to avoid repeated freeze-thaw cycles. Before testing, slowly bring frozen samples to room temperature (25°C±3°C) and gently mix.

If samples need dilution, use the specified sample dilution buffer for dilution.

Recommended dilutions for normal serum/plasma samples (for reference only): 1:1 with 1× dilution buffer.

Recommended dilution for cell culture supernatant (for reference only): Undiluted.

Due to variations in the content of the target protein in samples, the dilution ratio for each sample is recommended based on pre-experimental results or actual conditions.

2. Preparation of the ELISA Plate

The ELISA plate should be equilibrated to room temperature before use. Unused strips should be promptly sealed with a desiccant and stored at 2~8°C. It is recommended to perform duplicate assays for each sample.

3. Reagent Preparation

Before use, all reagents and samples should be equilibrated to room temperature. To ensure experimental accuracy, complete the process within 15 minutes prior to use.

1) Preparation of 1×Wash Buffer: Equilibrate the concentrate to room temperature and dissolve completely without crystallization. Mix well, take 25 mL of 20×Wash Buffer and dilute with distilled water to a final volume of 500 mL. The specific volume can be adjusted according to the amount needed for each use.

2) Preparation of 1× Dilution Buffer: Equilibrate the concentrate to room temperature and

dissolve completely without crystallization. Mix well, take 10 mL of 5×Dilution Buffer and dilute with distilled water to a final volume of 50 mL. The specific volume can be adjusted according to the amount needed for each use. 1×Dilution Buffer is used to dilute standard samples, test samples, detection antibodies, and enzyme conjugates.

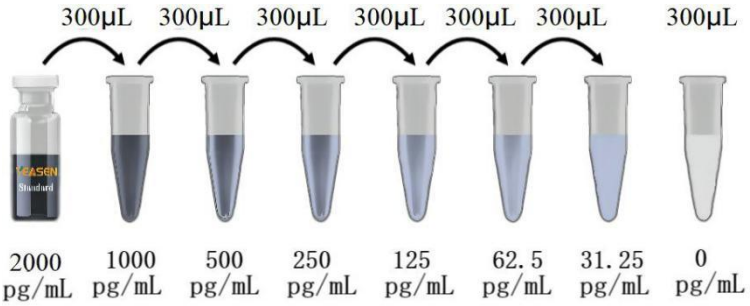
3) Preparation of Detection Antibody: Before use, centrifuge at 10, 000 rpm for 20 seconds, then dilute with 1×Dilution Buffer at a 1:50 ratio for working concentration. For example, take 120 μL and dilute with 1×Dilution Buffer to a final volume of 6 mL. The specific volume can be adjusted according to the amount needed for each use, and mix well.

4) Preparation of Enzyme Conjugate: Before use, centrifuge at 10, 000 rpm for 20 seconds, then dilute with 1×Dilution Buffer at a 1:200 ratio for working concentration. For example, take 30 μL and dilute with 1×Dilution Buffer to a final volume of 6 mL. The specific volume can be adjusted according to the amount needed for each use, and mix well.

5) Preparation of Standard Curve: Prepare 7 sterile 1.5 mL centrifuge tubes and label them according to the standard concentrations. For S1 preparation, dissolve the specified amount of standard lyophilized powder with distilled water and mix well. Label it as 2000 pg/mL. Add 300 μL of 1×Dilution Buffer to each tube, starting with 300 μL of S1, transfer to the next tube, and repeat until the last tube is labeled with the lowest concentration of 31.25 pg/mL. Each assay requires preparation of the corresponding standard curve, and standard curves from different kits or different times should not be mixed. For sample testing, 100 μL of standard is required per well, and ensure the prepared volume is sufficient to avoid insufficient usage.

Table 2. Preparation of TNF-α Standard System (31.25 - 2000 pg/mL)

Standard Curve	Diluent(μL)	Volume of Standard Added(μL)	Final Concentration of Standard(pg/mL)
S1	As labeled	/	2000
S2	300	300	1000
S3	300	300	500
S4	300	300	250
S5	300	300	125
S6	300	300	62.5
S7	300	300	31.25
Blank	300	0	0



Operating Instructions

Using all reagents and test samples requires equilibration to room temperature before use. It is strongly recommended to perform duplicate well testing for all standard and test samples.

1. Reagent preparation: Prepare various reagents for testing, dilute standard samples, and prepare test samples.
2. ELISA plate determination: Calculate the number of enzyme-labeled plate strips required for test samples and standard samples. Remove the enzyme-labeled plate strips from the aluminum foil bag, return the remaining strips to the bag, and seal the bag for storage at low temperature.
3. Soak the ELISA plate: Add 350 μL of 1×wash solution per well to soak the enzyme-labeled plate. After 30 seconds, discard the liquid from the wells and pat dry the enzyme-labeled plate. The liquid volume has a significant impact on the test results; ensure there is no residual wash solution after the final pat.
4. Incubation with samples: Add various gradient standard samples and diluted test samples, 100 μL per well, ensuring completion within 15 minutes. Incubate at room temperature for 2 hours.
5. Washing the enzyme-labeled plate: Discard the liquid from the wells and wash the plate 5 times with 1×wash solution (350 μL per well), pat dry the enzyme-labeled plate.
6. Incubation with detection antibody: Add the detection antibody pre-prepared to the working concentration to the enzyme-labeled plate, 100 μL per well, and incubate at room temperature for 2 hours.
7. Washing the enzyme-labeled plate: Discard the liquid from the wells and wash the plate 5 times with 1×wash solution (350 μL per well), pat dry the enzyme-labeled plate.
8. Incubation with enzyme conjugate: Add the enzyme conjugate pre-prepared to the working concentration to the enzyme-labeled plate, 100 μL per well, and incubate at room temperature for 20 minutes.
9. Washing the enzyme-labeled plate: Discard the liquid from the wells and wash the plate 5 times with 1×wash solution (350 μL per well), pat dry the enzyme-labeled plate.
10. Color development: Allow the substrate solution to equilibrate to room temperature for 10 minutes before use. Add the substrate solution to the enzyme-labeled plate, 100 μL per well, and

incubate at room temperature in the dark for 15 minutes.

11. Termination: Add 50 μL per well of the stop solution to the enzyme-labeled plate. At this point, the color changes from blue to yellow. Gently shake the enzyme-labeled plate to ensure uniform color development.

12. Reading: Read the absorbance values at 450 nm/630 nm within 10 minutes.

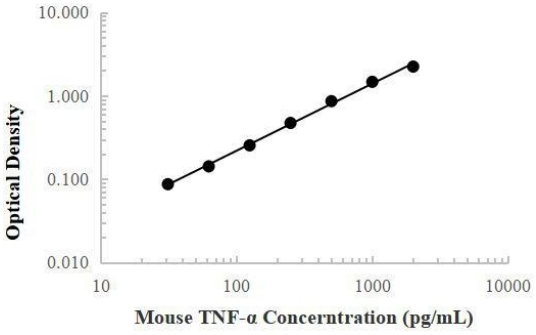
Standard Curve Establishment

Calculate the average OD values of duplicate wells for both standard samples and test samples, and subtract the average OD value of blank wells to obtain the calibrated OD value. Plot the standard curve with the logarithm of the standard sample concentration on the horizontal axis and the logarithm of the calibrated OD value on the vertical axis. Various plotting and statistical software can be used to assist in drawing the standard curve and calculating the concentration of unknown samples. The four-parameter fitting method often yields better fitting results, while other methods such as linear fitting may also yield good fitting results, depending on the specific experimental data.

Experimental Data

1. Standard Curve Data

Fitting data to generate a standard curve for analysis of experimental data.



Standard Curve Graph

Concentration(pg/mL)	Absorbance		Mean Value	Calibration Value
2000	2.270	2.199	2.235	2.236
1000	1.472	1.478	1.475	1.469
500	0.835	0.868	0.852	0.862
250	0.478	0.473	0.476	0.472
125	0.260	0.257	0.258	0.254
62.5	0.142	0.145	0.144	0.142
31.25	0.084	0.081	0.083	0.087
0	0.014	0.014	0.014	/

2. Sensitivity Detection

The minimum detectable limit of TNF-α is 7.82 pg/mL, calculated by repeating the measurement of the OD value of the blank wells 20 times and determining the mean and standard deviation.

3. Precision Detection

Intra-assay Precision

Three samples of known concentrations were measured in duplicate on the same microplate for 8 repetitions to assess intra-assay precision.

Inter-assay Precision

Three samples of known concentrations were measured in duplicate across 24 repetitions on different microplates to assess inter-assay precision.

Project	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
	8	8	8	24	24	24
Mean Value	940.0	241.4	57.9	942.6	236.5	59.6
Standard Deviation	40.0	9.5	1.9	64.0	9.2	3.2
Coefficient of Variation (%)	4.3	3.9	3.3	6.8	3.9	5.3

4. Recovery Rate Detection

The recovery rate of TNF-α was determined by adding TNF-α at different levels to the samples, and the recovery rates are as follows:

Sample Types	Average Recovery Rate (%)	Range (%)
Serum	112.3	103.0-119.1
Plasma	94.0	81.0-104.5
Cell culture supernatant	90.0	82.6-98.0

5. Dilution Linearity Test

Serum Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	104.4	102.0-107.1
1:04	106.0	98.0-112.4
1:08	116.9	108.1-122.1
1:16	97.6	88.1-108.7

Plasma Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	107.1	95.2-115.2
1:04	109.4	105.2-114.0
1:08	118.1	118.2-124.2
1:16	107.2	99.8-113.9

Cell Culture Supernatant Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	107.1	94.5-122.9
1:04	104.0	88.3-120.2
1:08	104.7	84.3-123.2
1:16	94.2	85.7-106.1

6. Sample Values

Using this assay kit to test samples from several healthy volunteers, with no details available regarding the medication history of the volunteers.

Sample Types	Sample Number	Mean Value(pg/mL)	Sample Value(pg/mL)
Serum	4	n.d.	n.d.
Plasma	4	n.d.	n.d.
Cell Culture Supernatant	4	n.d.	n.d.

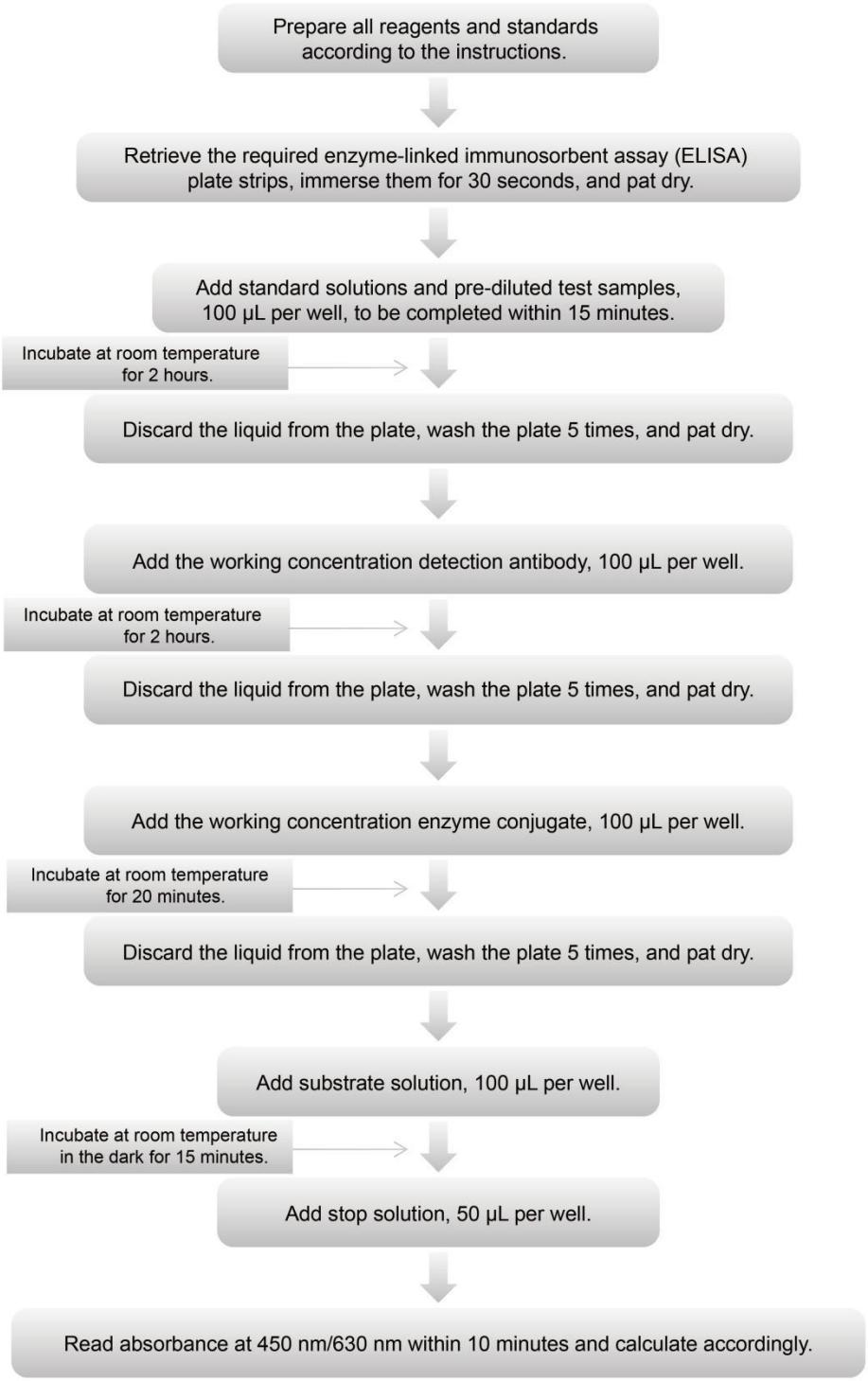
n.d. refers to sample concentrations lower than the detection range of 31.25 pg/mL.

7. Specificity Assay

This assay kit identifies both natural and recombinant mouse tumor necrosis factor alpha (TNF-α). The following factors have undergone specific evaluation, and no significant cross-reactivity or interference effects have been observed.

Recombinant human:		Recombinant Mouse:	
IL-1α	IL-10	IL-1α	IL-10
IL-1β	IL-12	IL-1β	IFN-γ
IL-2	IFN-γ	IL-2	VEGF
IL-4	TNF-α	IL-4	RANKL
IL-5	LIF	IL-5	TRAIL
IL-6	RANKL	IL-6	
IL-8	TRAIL		

Detection Schematic



Frequently Asked Questions

Issues	Causes	Solution
Poor standard curve	Inaccurate pipetting volumes	Check the pipette, calibrate it regularly, handle it carefully, tightly cap the tip while mixing thoroughly, and minimize foam formation.
	Inappropriate dilution methods	
Low color intensity	Insufficient incubation time	Allow sufficient incubation time, and replace samples and reconstituted standards overnight.
	Inadequate pipetting volumes or improper dilution	Calibrate pipettes and standardize operations.
High coefficient of variation (CV)	Improper washing of the ELISA plate	Use the correct washing procedure; if using a plate washer, check all ports for blockages.
	Contaminated washing solution	Prepare fresh washing solution.
Low sensitivity	Improper storage of the assay kit	Store according to the product component table.