Human Tumor Necrosis Factor Alpha (TNF-α) ELISA Kit

(Enzyme-Linked Immunosorbent Assay)

Catalog Number：CRS-A002

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

INTENDED USE

The kit is developed for quantitative detection of TNF-α in human serum and cell culture supernates. It is intended for research use only (RUO).

BACKGROUND

Tumor Necrosis Factor alpha (TNF-α), also known as cachectin, is the prototypic ligand of the TNF superfamily. It is produced primarily by activated macrophages , also secreted by other cells such as CD4+ lymphocyte、NK cell、Neutrophils, mast cells, eosinophils and neurons. It is a pleiotropic molecule and play a central role on inflammation, immune system development, apoptosis and lipid metabolism. Its overexpression related to a series of pathological state including Cachexia, septic shock, and autoimmune disorders.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of human Tumor Necrosis Factor alpha (TNF-α) by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-TNF-α Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-TNF-α Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450nm and 630nm. The OD Value reflects the amount of TNF-α bound.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for cell supernatant, serum and plasma samples.
3. Do not use reagents past their expiration date.
4. Do not mix or substitute reagents with those from other kits or other lot number kits.
5. If samples generate values higher than the highest standard, dilute the samples with the appropriate

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calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other intermediate dilutions can be in cell culture medium.

6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.

7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
CRS002-C01	Pre-coated Anti-TNF-α Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
CRS002-C02	Human TNF-α Standard	20 μg	Powder	2-8°C	-70°C
CRS002-C03	Biotin-Anti- TNF-α Antibody	20 μg	Powder	2-8°C	-70°C
CRS002-C04	Streptavidin-HRP	50 μL	Liquid	2-8°C, avoid light	2-8°C, avoid light
CRS002-C05	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
CRS002-C06	2xDilution Buffer	50 mL	Liquid	2-8°C	2-8°C
CRS002-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
CRS002-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

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KIT STORAGE AND EXPIRATION DATE

1. The unopened kit is stable for 18 months from the date of manufacture if stored at 2°C to 8°C.
2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

Note: *a. Do not use reagents past their expiration date.*

b. Find the expiration date on the outside packaging.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection; requirements;

37° C Incubator;

Single or dual wavelength microplate reader with 450nm and 630nm filter;

Tubes: 1.5mL,10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vortexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, and the size of the aliquot should not be less than 10 μg.

Table 2. Preparation method

Catalog	Components	Size (96 T)	Storage solution concentration.	Reconstituted water Vol.
CRS002-C02	Human TNF-α Standard	20 μg	100 μg/mL	200 μL
CRS002-C03	Biotin-Anti-TNF-α Antibody	20 μg	100 μg/mL	200 μL

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RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Anti-TNF-α Antibody working fluid:

Dilute Biotin-Anti-TNF-α Antibody with 1×Dilution Buffer to 0.5 μg/mL. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

1.5 Sample preparation

a. If the sample to be tested is the serum or plasma, dilute test sample at 1:2 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:1.

b. If the sample to be tested is the cell supernatant, dilute test sample at 1:2 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:1.

2. Preparation of Standard curve

The concentration of the reconstituted human TNF-α Standard (CRS002-C02) is 100 μg/mL，prepare (Std.-0) by diluting 5 μL the reconstituted human TNF-α Standard into 495 μL Sample Dilution Buffer, mix gently well. Then prepare Std.- 1' by diluting 5 μL Std.-0 into 495 μL Sample Dilution Buffer. At last, prepare the highest concentration of standard curve, Std.-1 (1250 pg/mL), by diluting 75 μL Std.- 1' into 525 μL Sample Dilution Buffer. Prepare 1:1 serial dilutions for the standard curve as follows: Pipette 300 μL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.

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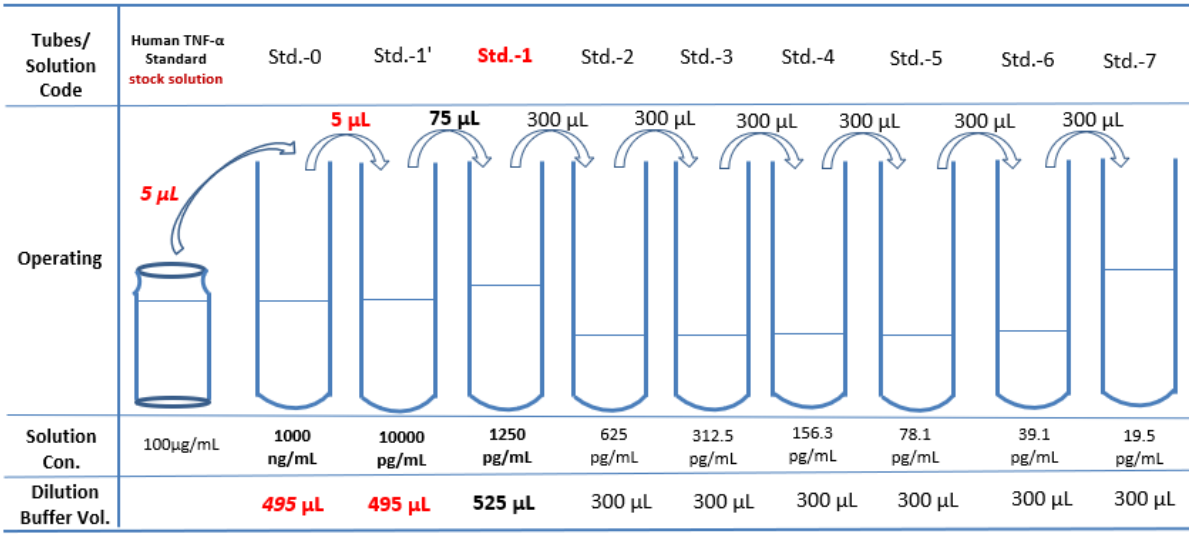

3. Add Samples

Add 100 µL Calibrator and samples to each well. For blank Control wells, please add 100 µL Dilution Buffer.

***Note**: It is recommended to set doeble holes for samples and standard curves to be tested.*

4. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 10s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

6. Add Biotin-Anti-TNF-α Antibody

For all wells, add 100 µL Biotin- Anti-TNF-α Antibody (0.5 µg/mL) working solution. Please prepare it for one-time use only.

7. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

8. Washing

Repeat step 5.

9. Add Streptavidin-HRP

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For all wells, add 100 µL Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

10. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 30 min.

11. Washing

Repeat step 5.

12. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

13. Termination

Add 50 µL Stop Solution to each well, and tap the plate gently to allow thorough mixing.

Note: *The color in the wells should change from blue to yellow.*

14. Data Recording

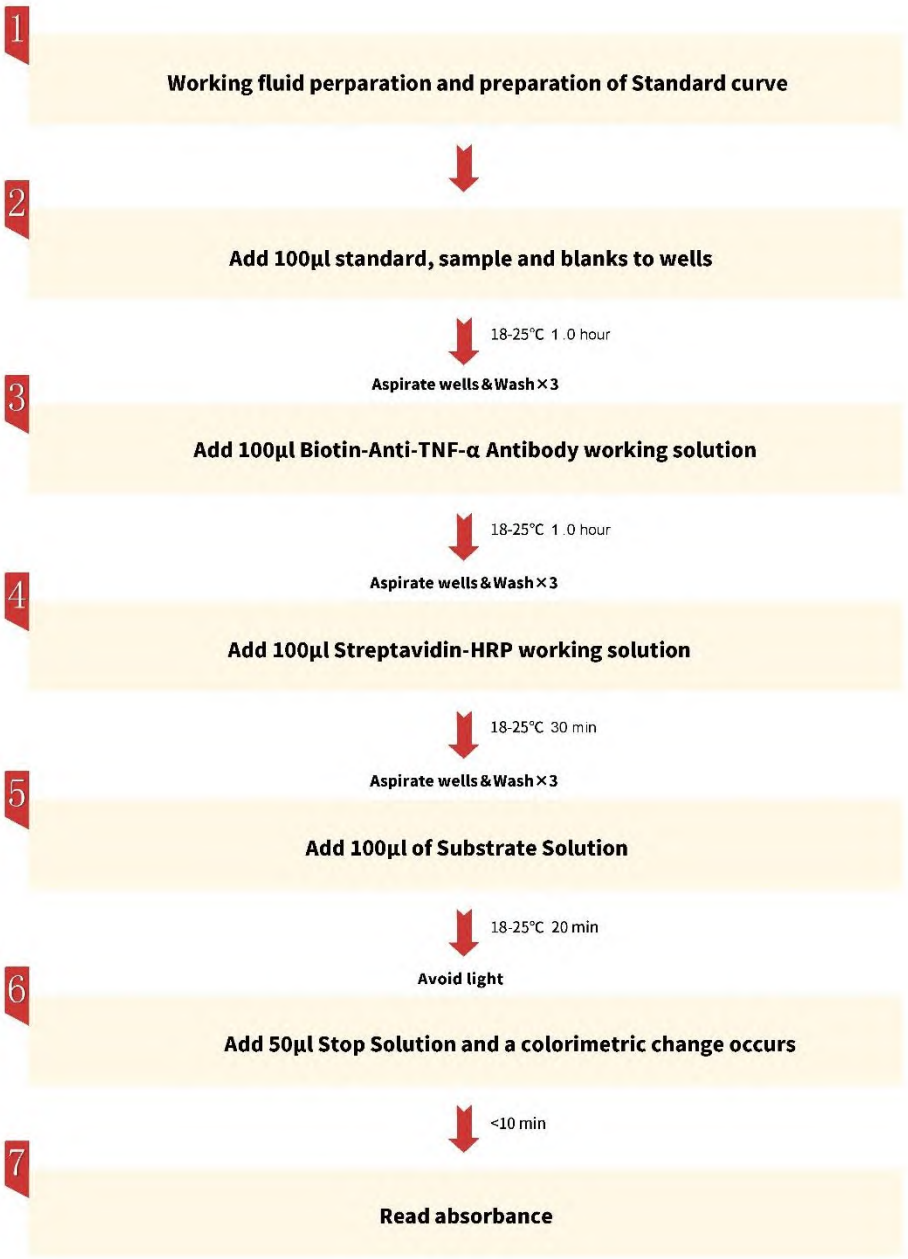
Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

Note*: To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).
2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
3. Normal range of Standard curve: $R^2 \geq 0.9900$.
4. Detection range: 19.5 pg/mL-1250 pg/mL. If the OD value of the sample to be tested is higher than 1250 pg/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 19.5 pg/mL, the sample should be reported.

QUICK GUILD



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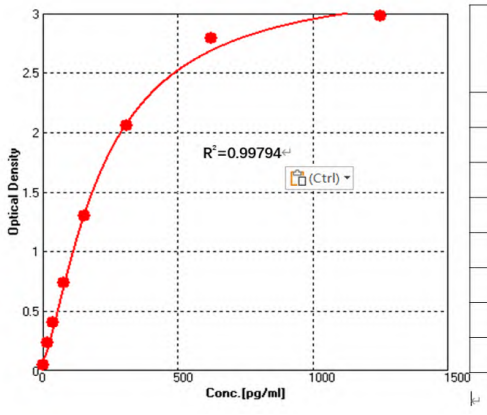
TYPICAL DATA

The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.



Sample Con.	OD-1	OD-2	OD-3	AVE	SD	CV
1250pg/ml	2.996	2.935	3.014	2.982	0.041	1%
625pg/ml	2.758	2.765	2.847	2.790	0.049	2%
312.5pg/ml	1.959	2.101	2.125	2.062	0.090	4%
156.3pg/ml	1.217	1.346	1.326	1.296	0.069	5%
78.1pg/ml	0.707	0.760	0.741	0.736	0.027	4%
39.1pg/ml	0.388	0.407	0.412	0.402	0.013	3%
19.5pg/ml	0.231	0.250	0.223	0.235	0.014	6%
0pg/ml	0.039	0.047	0.042	0.043	0.004	9%

SENSITIVITY

The minimum detectable concentration of human TNF-α is 2.321 pg/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

2. Precision

Three samples of known concentration were tested on 3 different plates, 10 replicates in each plate to assess inter-assay precision.

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	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	30	30	30
Mean (pg/mL)	346.018	172.273	89.184	359.774	184.266	97.260
SD	8.042	11.376	2.782	17.914	10.386	7.461
CV (%)	2.3	6.6	3.1	5.0	5.6	7.7

***Note**: The example data is for reference only.*

RECOVERY

Three parts of blank serum were added with different concentrations of human TNF-α, and the serum without human TNF-α was used as background to calculate the recovery rate. The Range of the recovery rate is 90%-111%, and the average recovery is 101%.

Sample Type	Average% Recovery	Range
Serum(n=5)	101	90-111%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human TNF-α were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium	Serum	Citrate plasma
1:2	Average Recovery (%)	109	105	102
	Range (%)	102-116	96-114	96-111
1:4	Average Recovery (%)	110	101	96
	Range (%)	105-114	95-113	91-102
1:8	Average Recovery (%)	97	89	105
	Range (%)	95-101	86-96	96-112

***Note**: The example data is for reference only.*

SPECIFICITY

This assay recognizes natural and recombinant human TNF-α. No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines.

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Human	
IL-1β	IL-10
IL-2	IL-15
IL-4	IL-21
IL-7	GM-CSF
IL-6	IFN-gamma

SAMPLE VALUES

a. 80 human healthy sample were evaluated for the presence of human TNF-α in this assay. No medical histories were available for the donors used in this study.

Sample	n	Concentration (pg/mL)	Detection percentage (%)	Detection average Concentration (pg/mL)
Serum	80	n.d. - 295.711	35	62.534

Note: n.d. means that the concentration value cannot be detected which is lower than the sensitivity. The example data is for reference only.

b. In this assay, the sample is the cell supernatant of PBMC proliferation stimulated by CD28/OKT3 antibody.

Sample	n	Concentration (pg/mL)	Detection percentage (%)	Detection average Concentration (pg/mL)
cell supernatant	2	102.613-230.675	100	166.644

Note: The example data is for reference only.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human TNF-α (17/232). Reference Reagent is calibrated by NIBSC/WHO in June 2020.

NIBSC/WHO (17/232) approximate value (U/mL) = 0.110 × Human TNF-α value (pg/mL)

PLATE LAYOUT

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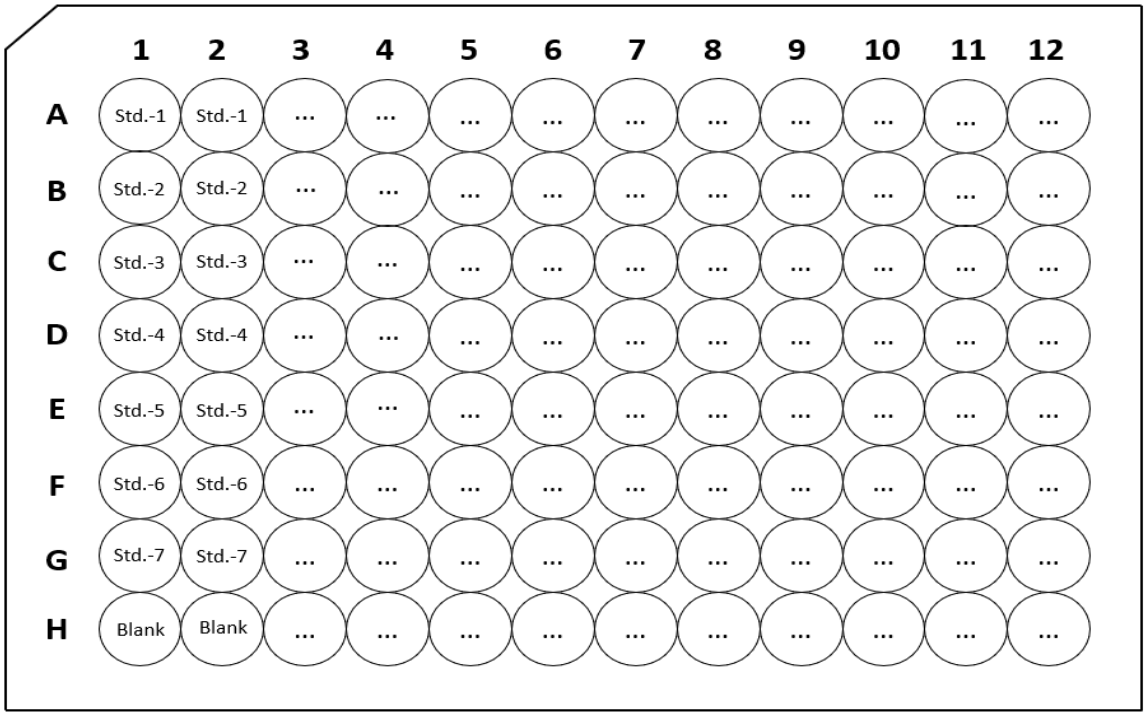

Note: Blank is a Blank Dilution Buffer hole.

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TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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