



## ClinMax™ Human TNF-alpha Quick ELISA Kit

Catalog Number: CEA-C246

Assay Tests: 96 tests

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

**IMPORTANT: Please carefully read this user guide before performing your experiment.**

## Product information

This kit is specifically designed for the accurate quantitation of human TNF-alpha from cell culture supernates, serum and plasma.

The principle of this assay employs a quantitative sandwich enzyme immunoassay approach. Initially, a microplate is coated with a capture antibody. Then, samples and biotinylated capture antibody are added to the wells. After the removal of any unbound materials through washing, streptavidin-HRP (SA-HRP) conjugate is added to the wells. Streptavidin has a very high affinity for biotin, so it binds to the biotinylated capture antibody that is already bound to the target antigen. After washing, a substrate specific to HRP is added to the wells. HRP catalyzes a reaction that converts the substrate into a detectable signal, often a color change or luminescence, depending on the substrate used. This enzymatic reaction amplifies the signal, allowing for higher sensitivity in detecting the target analyte. The intensity of the signal is measured using a spectrophotometer.

### NOTE:

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Please do not use the kit after the expiration date indicated on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.

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## Contents

The kit contains sufficient reagents for 96 wells.

Catalog	Contents	Amount
CEA246-C01	Pre-coated Anti-TNF- $\alpha$ Antibody Microplate	1 plate
CEA246-C02	Human TNF- $\alpha$ Standard	12.5 ng $\times$ 2
CEA246-C03	Biotin-Anti-TNF- $\alpha$ Antibody	20 $\mu$ g
CEA246-C04	Biotin-Antibody Dilution Buffer	8 mL
CEA246-C05	Streptavidin-HRP Con. Solution	500 $\mu$ L
CEA246-C06	Streptavidin-HRP Dilution Buffer	15 mL
CEA246-C07	20 $\times$ Washing Buffer	50 mL
CEA246-C08	Sample Dilution Buffer	15 mL $\times$ 2
CEA246-C09	Substrate Solution	12 mL
CEA246-C10	Stop Solution	6 mL

## Storage

Keep the unopened kit stored at 2-8  $^{\circ}$ C. Avoid using the kit beyond its expiration date.

For opened kit and reconstituted reagents, with the exception of the two contents listed in following table, others can be stored for up to 30 days at 2-8  $^{\circ}$ C.

Contents	Storage conditions
Pre-coated Anti-TNF- $\alpha$ Antibody Microplate	Return unused wells to the foil pouch, reseal along entire edge. May be stored for up to 1 month at 2-8 $^{\circ}$ C.
Human TNF- $\alpha$ Standard	Aliquot and store for up to 1 month at -70 $^{\circ}$ C in a freezer. Avoid repeated freeze-thaw cycles.
Biotin-Anti-TNF- $\alpha$ Antibody	Aliquot and store for up to 1 month at -70 $^{\circ}$ C in a freezer. Avoid repeated freeze-thaw cycles.

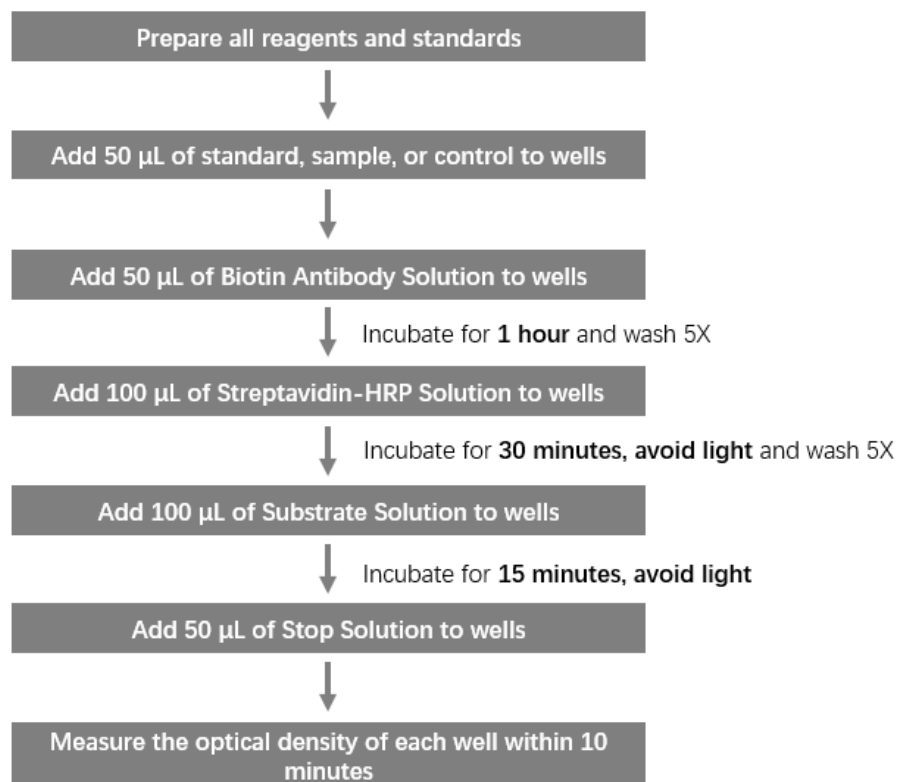
**NOTE:** Streptavidin-HRP Con. Solution and Substrate Solution should avoid light.

### Required materials not supplied.

<b>Instrument</b>	Microplate reader capable of measuring absorbance at 450 nm
<b>Reagents</b>	Deionized, ultrapure or distilled water
<b>Consumables</b>	50 mL and 500 mL graduated cylinders
	Pipettes and pipette tips
	Tubes to prepare standard dilutions.

### Workflow

#### Analyte: TNF- $\alpha$



**NOTE:** Incubation temperature is 18  $^{\circ}\text{C}$ -25  $^{\circ}\text{C}$

### Prepare the working buffers and standard dilutions.

**IMPORTANT:** Bring all reagents to room temperature before use. If crystals have formed in buffer solution, place the buffer solution in an 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

#### Prepare the reconstruct antibody stock solution.

Add 200 µL ultrapure water to the provided lyophilized product (CEA246-C03) , dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. The concentration of reconstituted TNF-α antibody stock solution is 100 µg/mL.

**NOTE:** *Avoiding vigorous shaking or vortexing. The reconstituted solution should be stored at -70°C. The freeze-thaw cycle should not exceed 1 time, and the size of the aliquot should not be less than 10 µg.*

#### Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.
2. Biotin Antibody Solution: Add 30 µL of the reconstituted TNF-α antibody stock solution to 6 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
3. Streptavidin-HRP Solution: Add 400 µL of Streptavidin-HRP Con. Solution to 12 mL of Streptavidin-HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

#### Prepare the reconstituted standard.

Add 1 mL ultrapure water to the provided lyophilized product (CEA246-C02) , dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. The concentration of reconstituted human TNF-α Standard is 12.5 ng/mL.

**NOTE:** *Avoiding vigorous shaking or vortexing. The reconstituted solution should be stored at -70°C. The freeze-thaw cycle should not exceed 1 time.*

#### Prepare the standard serial dilutions.

1. Label a tube "Std.-1". Add 100 µL of the reconstituted human TNF-α Standard and 900 µL of Sample Dilution Buffer to tube Std.-1, gently mix well (Std.-1 =1250 pg/mL).
2. Label 6 tubes, one for each standard point: Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7.
3. Prepare serial dilutions for the standard curve as follows: Add 500 µL of Sample Dilution Buffer to each tube (Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7).
4. Transfer 500 µL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 625 pg/mL).
5. Continue to transfer 500 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-7.
6. Sample Dilution Buffer serves as zero standard (blank).

## PROCEDURE OF ASSAY

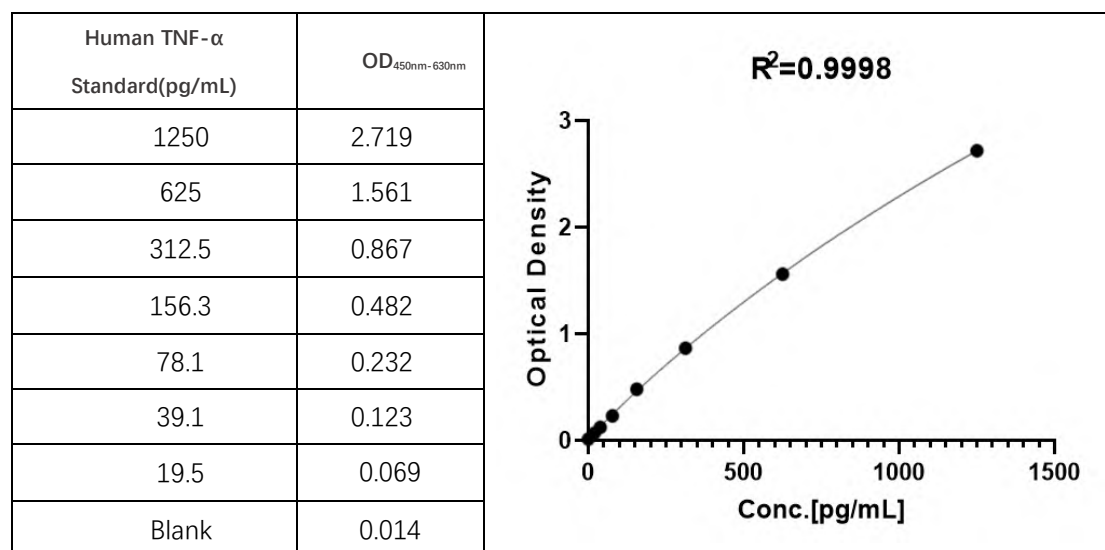
1. Add 50  $\mu$ L of TNF- $\alpha$  Standard, sample, or control to wells.
2. Add 50  $\mu$ L Biotin Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25  $^{\circ}$ C) for **1 hours**.
3. Aspirate each well and add 300  $\mu$ L of 1 $\times$ Washing Buffer to each well, gently tap the plate for **1 minute**. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Repeat the wash process four times for a total of five washes.
4. Add 100  $\mu$ L of Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25  $^{\circ}$ C) for **30 minutes, avoid light**.
5. Repeat step 3.
6. Add 100  $\mu$ L of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25  $^{\circ}$ C) for **15 minutes, avoid light**.
7. Add 50  $\mu$ L of Stop Solution to each well. Tap the plate gently to ensure thorough mixing.  
*Note: the color in the wells should change from blue to yellow.*
8. Read the absorbance at 450nm and 630nm using Microplate reader within 10minutes.  
*Note: To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

## CALCULATION OF RESULTS

1. Compute the average of the duplicated readings for every standard, control, and sample. Then, subtract the average optical density (O.D.) of the zero standard(blank).
2. Establish a standard curve by processing the data using computer software capable of executing a four-parameter logistic (4-PL) curve fitting.
3. Normal range of Standard curve:  $R^2 \geq 0.9900$ .
4. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.

### Typical data

**Note:** For each experiment, a standard curve needs to be set for each microplate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.



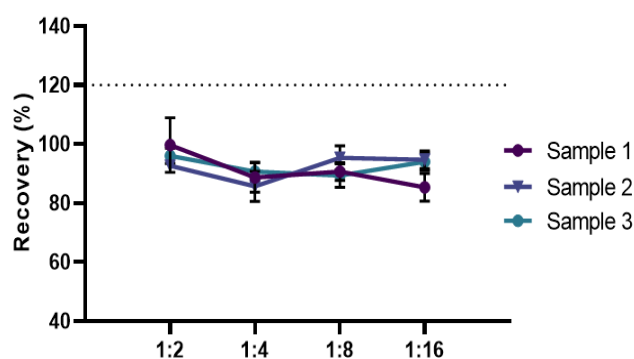
## PERFORMANCE CHARACTERISTICS

### 1. Sensitivity

The minimum detectable concentration (MDC) of TNF- $\alpha$  is typically less than 2.0 pg/mL. The MDC was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## 2. Linearity

Three samples (Serum) spiked with high concentrations of TNF- $\alpha$  were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of TNF- $\alpha$  for serum samples is 91.9%.



## 3. Intra-Assay Precision

Ten replicates of each of 3 samples containing different TNF- $\alpha$  concentrations were tested in one assay. Acceptable criteria: CV < 10%.

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV (%)
500	491.17	19.12	10	3.9
100	100.91	7.30	10	7.2
30	31.48	1.60	10	5.1

## 4. Inter-Assay Precision

3 samples containing different concentrations of TNF- $\alpha$  were tested in independent assays. Acceptable criteria: CV < 15%.

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV (%)
500	489.67	21.35	9	4.4
100	101.74	7.15	9	7.0
30	29.97	2.76	9	9.2



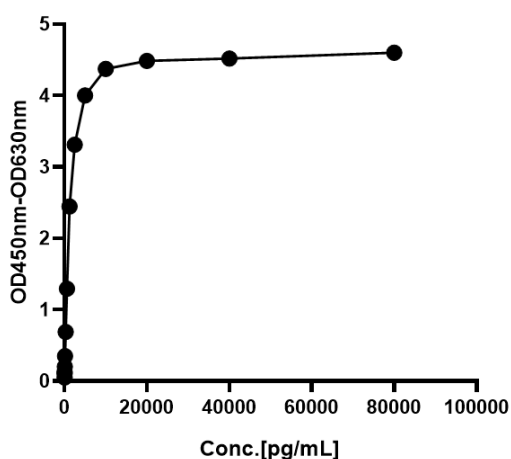
## 5. Recovery

Recombinant TNF- $\alpha$  was spiked into 3 human serum samples, and then analyzed. The average recovery of TNF- $\alpha$  for serum samples is 91.3%.

Sample ID	Conc Measured (pg/mL)	Conc Added (pg/mL)	Conc Recovered (pg/mL)	Recovery (%)
1	613.40	625.0	613.40	98.1
	281.80	312.5	281.80	90.2
	152.61	156.3	152.61	97.7
	74.15	78.1	74.15	94.9
	-	-		
2	581.92	625.0	581.92	93.1
	296.43	312.5	296.43	94.9
	135.44	156.3	135.44	86.7
	71.64	78.1	71.64	91.7
	-	-		
3	571.42	625.0	571.42	91.4
	268.88	312.5	268.88	86.0
	133.34	156.3	133.34	85.3
	66.66	78.1	66.66	85.3
	-	-		

## 6. Hook Effect

Not be affected by the concentration of TNF- $\alpha$  up to 10 ng/mL.



## 7. Sample Values

Human peripheral blood mononuclear cells (PBMCs) obtained were plated at a density of  $1 \times 10^6$  cells/mL in complete RPMI 1640 medium containing 10% fetal bovine serum, 2 mM L-glutamine, and antibiotic supplements (penicillin 100 U/mL with streptomycin sulfate 100  $\mu$ g/mL). Following a 5-day incubation period, experimental groups were either maintained as untreated controls or stimulated with 10  $\mu$ g/mL phytohemagglutinin (PHA). Subsequent analysis involved quantitative measurement of TNF-alpha concentrations in collected culture supernatants using standardized immunoassays.

Condition	Day 3 Mean (pg/mL)	Day 5 Mean (pg/mL)
Unstimulated	ND	ND
Stimulated with PHA	248	983

ND= Non-detectable

## TROUBLESHOOTING GUIDE

Problem	Cause	Solution
<b>Poor standard curve</b>	* Inaccurate pipetting	* Check pipettes
<b>Large CV</b>	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
<b>High background</b>	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
<b>Very low readings across the plate</b>	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
<b>Samples are reading too high, but standard curve looks fine</b>	* Samples contain cytokine levels above assay range	* Dilute samples and run again
<b>Drift</b>	* 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 the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts