Mouse TNF-alpha ELISA Kit

Catalog Number：CEA-M255

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 mouse 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.

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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.

Manufactured and distributed by
ACRODiagnostics Inc.
TEL: 010-67855298-8147

Distributed by:
ACROBiosystems Inc.
US & Canada TEL: +1 800-810-0816
Asia & Pacific TEL: +86 400-682-252

Contents

The kit contains sufficient reagents for 96 wells.

Catalog	Contents	Amount
CEA255-C01	Pre-coated Anti-mouse TNF-alpha Antibody Microplate	1 plate
CEA255-C02	Mouse TNF-alpha Standard	5 ng×2
CEA255-C03	Biotin-Anti-mouse TNF-alpha Antibody Con. Solution	100 µL
CEA255-C04	Biotin-Antibody Dilution Buffer	8 mL
CEA255-C05	Streptavidin-HRP Con. Solution	500 µL
CEA255-C06	Streptavidin-HRP Dilution Buffer	15 mL
CEA255-C07	20× Washing Buffer	50 mL
CEA255-C08	Sample Dilution Buffer	15 mL×2
CEA255-C09	Substrate Solution	12 mL
CEA255-C10	Stop Solution	6 mL

NOTE: Bubbles in microplate wells do not affect the experiment and require no action. Proceed with the experimental procedures and methods described below.

Storage

Keep the unopened kit stored at 2-8 °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 °C.

Contents	Storage conditions
Pre-coated Anti-mouse 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°C.
Mouse TNF-alpha Standard	Aliquot and store for up to 1 month at -70°C in a freezer. Avoid repeated freeze-thaw cycles.

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

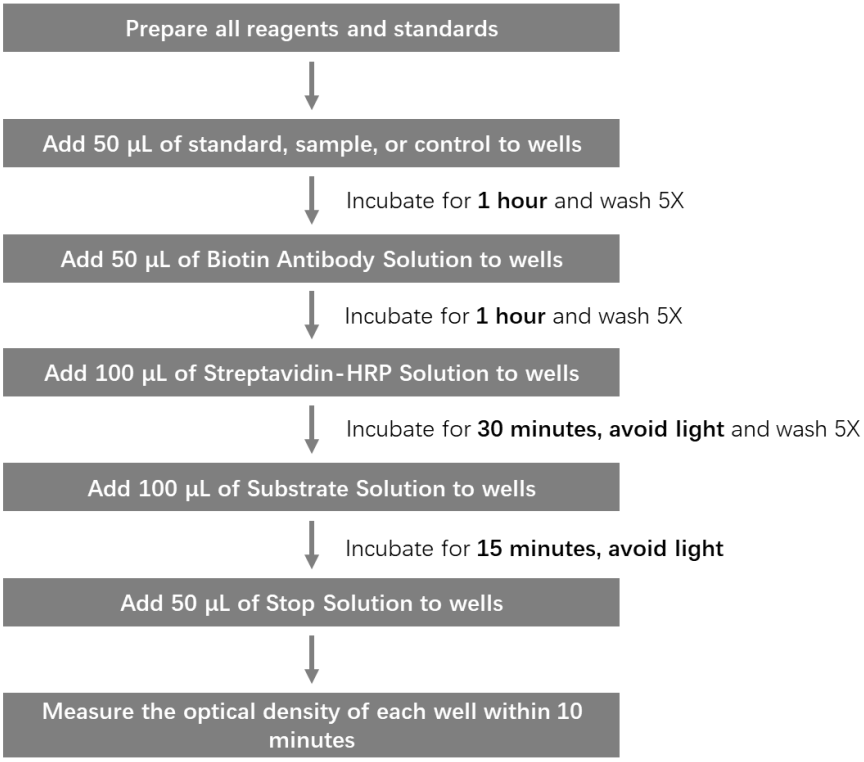
IMPORTANT: Bring all reagents to room temperature before use. If crystals have formed in buffer solution, place the buffer solution in an 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

Required materials not supplied.

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

Workflow

Analyte:Mouse TNF-alpha



NOTE： Incubation temperature is 18 ℃-25 ℃

Prepare the working buffers and standard dilutions.

Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.
2. Biotin-Anti-mouse TNF-alpha Antibody Solution: Add 60 µL of Biotin-Anti-mouse TNF-alpha Antibody Con. Solution to 6 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
3. Mouse TNF-alpha 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（CEA255-C02）, dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. The concentration of reconstituted mouse TNF-alpha Standard is 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 7 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7.
2. Add 100 µL of the liquid from reconstituted mouse TNF-alpha Standard and 900 µL of Sample Dilution Buffer to tube Std.-1, thoroughly mix (Std.-1 =500 pg/mL).
3. Prepare 1:1 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 = 250 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 µL of mouse TNF-alpha Standard, sample, or control to wells. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hour**.
2. Aspirate each well and add 300 µL of 1×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.
3. Add 50 µL Biotin-Anti-mouse TNF-alpha Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hour.**
4. Repeat step 2.
5. Add 100 µL of Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for 3**0 minutes, avoid light.**
6. Repeat step 2.
7. Add 100 µL of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.
8. Add 50 µL 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.*
9. 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.*

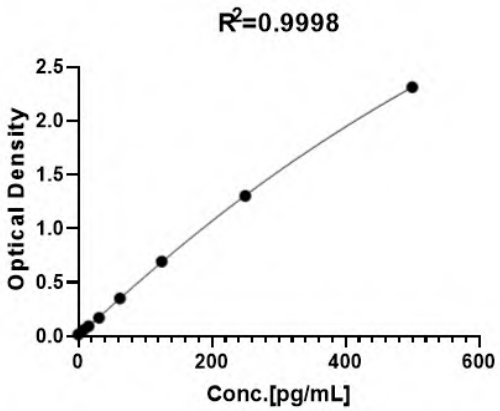
CALCULATION OF RESULTS

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: $R^2 \geqslant 0.9900$.
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.*

Mouse TNF-alpha Standard (pg/mL)	$OD_{450nm-630nm}$
500	2.311
250	1.301
125	0.690
62.5	0.348
31.25	0.168
15.625	0.090
7.8125	0.056
Blank	0.013



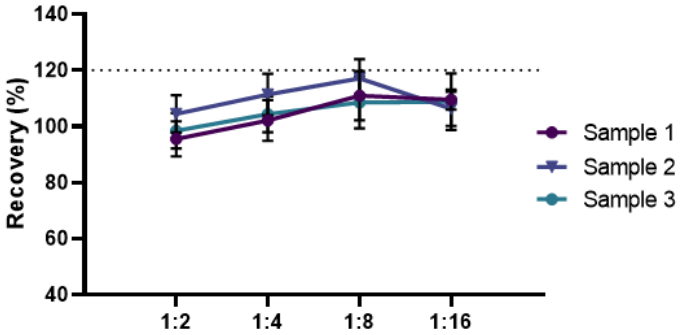
PERFORMANCE CHARACTERISTICS

1. Sensitivity

The minimum detectable concentration (MDC) of mouse TNF-alpha is typically less than 4 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 mouse 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 mouse TNF-alpha for serum samples is 106.4%.



3. Intra-Assay Precision

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

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV (%)
300	304.75	7.99	10	2.6
80	79.96	1.55	10	1.9
20	19.63	0.89	10	4.5

4. Inter-Assay Precision

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

Sample Concentration (pg/mL)	Mean (pg/mL))	SD	Numbers	CV (%)
300	322.74	23.37	9	7.2
80	81.96	5.34	9	6.5
20	19.81	1.19	9	6.0

5. Recovery

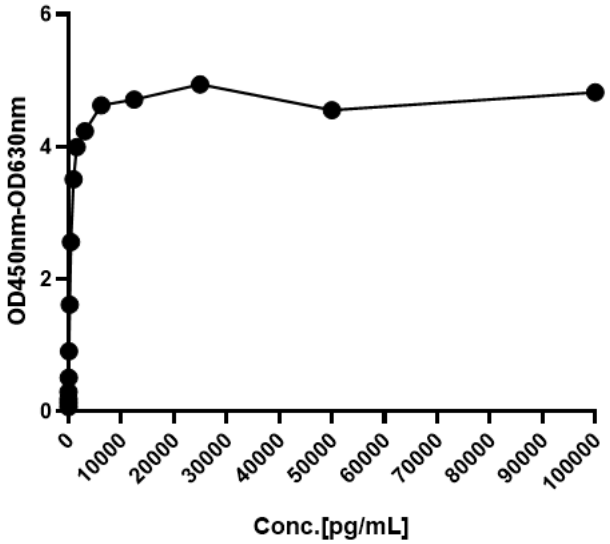
Recombinant mouse TNF-alpha was spiked into 3 mouse serum samples, and then analyzed. The average recovery of mouse TNF-alpha for serum samples is 90.1%.

Sample ID	Conc Measured (pg/mL)	Conc Added (pg/mL)	Conc Recovered (pg/mL)	Recovery (%)
1	307.53	250	217.88	87.2
	194.27	125	104.61	83.7
	141.17	62.5	51.52	82.4
	99.62	-		
	305.46	250	231.98	92.8

2	182.52	125	109.04	87.2
	131.42	62.5	57.95	92.7
	81.64	-		
3	358.14	250	271.67	108.7
	188.91	125	102.44	82.0
	145.52	62.5	59.05	94.5
	96.08	-		

6. Hook Effect

Not be affected by the concentration of mouse TNF-alpha up to 2 ng/mL.



7. Sample Values

RAW 264.7 mouse monocyte/macrophage cells (3.5 x 10^6 cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 ug/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with5.0 ug/mL LPS and 100 ng/mL rmIL-10 for 5 and 7 days. Subsequent analysis involved quantitative measurement of mouse TNF-alpha concentrations in collected culture supernatants using standardized immunoassays.

Condition	Day 5 Mean (pg/mL)	Day 7 Mean (pg/mL)
Unstimulated	391.12	218.37
Stimulated with LPS and rmIL-10	2165.97	915.16

8. Specificity

No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines at up to 1 μg/mL.

Mouse	IL-1β、IL-2、IL-6、IFN-γ
Human	TNF-alpha

9. CALIBRATION

This immunoassay is calibrated against highly purified recombinant mouse TNF-alpha produced at ACROBiosystems. The NIBSC/WHO International Standard for mouse TNF-alpha (88/532), which was intended as a potency standard, was evaluated in this kit.

NIBSC/WHO (88/532) approximate value (U/mL) = 0.2 x ACRO Mouse TNF-α (pg/mL)

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 the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts