Basic TNF alpha Mouse ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of mouse TNF alpha

Catalog Numbers ECM008 (96 tests)

Pub. No. MAN1000060 Rev. B (31)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Basic TNF alpha Mouse ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of mouse TNF alpha. Cell culture supernatant, serum, and plasma (EDTA, citrate) have been tested with this assay.

TNF alpha is a multi-functional pro-inflammatory cytokine that is part of the tumor necrosis factor (TNF) superfamily. This cytokine is primarily secreted by macrophages and binds to its receptors, TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. TNF alpha plays a role in regulating immune cells, cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. TNF alpha exists as a multimer of two, three, or five non-covalently linked units, but displays a single 17 kDa band after SDS-PAGE under non-reducing conditions. Knockout studies in mice have also suggested a neuroprotective function for TNF alpha, and it has been observed to cause tumor necrosis when injected into tumor-bearing mice. Other roles of TNF-alpha include its involvement in the immune response to bacterial, viral, parasitic, and certain fungal infections, as well as its role in the necrosis of specific tumors. TNF alpha induces cytolysis or cytostasis of certain transformed cells, being synergistic with interferon-gamma in its cytotoxicity. This cytokine has been associated with a range of diseases, including autoimmune diseases, insulin resistance, and cancer.

For literature updates, go to thermofisher.com.

Contents and storage

- Store kit reagents at 2–8°C.
- Immediately after use, return remaining reagents to cold storage (2–8°C).
- See the expiration date on the package.
- The kit components' expiry is guaranteed only if they are stored properly and not contaminated during repeated use.
- Do not mix components from other lots.

Components	Amount
Coated Microwell Strips	1 pouch (12 strips with 8 wells each)
Biotin-Conjugate (100X/200X)	70 µL
Streptavidin-HRP (100X)	150 µL
Mouse TNF alpha Standard, lyophilized (2 ng/mL upon reconstitution)	2 vials
Sample Diluent	12 mL
Calibrator Diluent	5 mL
Assay Buffer Concentrate 20X	5 mL
Wash Buffer Concentrate 20X	50 mL
Substrate Solution (Tetramethyl-benzidine)	15 mL
Stop Solution (1M Phosphoric acid)	15 mL
Adhesive Film	4

Required materials not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm (620 nm as optional reference wavelength)
- Beakers, flasks, and cylinders for preparation of reagents
- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Statistical calculator with program to perform regression analysis
- Microplate shaker

Before you begin

- Equilibrate the buffer concentrates to room temperature (18–25°C), then dilute before use.
- If crystals have formed in the buffer concentrates, warm gently to dissolve the crystals.

Prepare Wash Buffer (1X)

1. Transfer the entire contents (50 mL) of the Wash Buffer Concentrate (20X) to a clean 1,000-mL graduated cylinder, then add 950 mL of glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle, then label as 1X Wash Buffer.
3. Store Wash Buffer (1X) at 2–25°C for up to 30 days.

Prepare Assay Buffer (1X)

1. Transfer the entire contents (5 mL) of the Assay Buffer Concentrate (20X) to a clean 100-mL graduated cylinder, then add 95 mL of distilled water. Mix gently to avoid foaming.
2. Label as 1X Assay Buffer.
3. Store Assay Buffer (1X) at 2–8°C for up to 30 days.

Prepare 1X Biotin-Conjugate

IMPORTANT! Prepare Biotin-Conjugate within 30 minutes of usage.

- For serum and plasma samples, make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1X) in a clean plastic tube.
  - Dilute 0.06 mL of Biotin-Conjugate with 5.94 mL of Assay Buffer (1X), then mix thoroughly.
- For cell culture supernatant samples, make a 1:200 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1X) in a clean plastic tube.
  - Dilute 0.03 mL of Biotin-Conjugate with 5.97 mL of Assay Buffer (1X), then mix thoroughly.

Prepare 1X Streptavidin-HRP

Make a 1:100 dilution of the concentrated Streptavidin-HRP Conjugate in a clean plastic tube.

IMPORTANT! Prepare 1X Streptavidin-HRP within 30 minutes of usage.

Dilute 0.12 mL of concentrated Streptavidin-HRP conjugate with 11.88 mL of Assay Buffer (1X), then mix thoroughly.

Prepare Mouse TNF alpha Standard

Prepare fresh standard on each day of use as it cannot be stored.

1. Reconstitute Mouse TNF alpha standard by addition of Calibrator Diluent (for subsequent measurement of serum or plasma samples) or Sample Diluent (for subsequent measurement of cell culture supernatant samples).
2. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to ensure complete and homogeneous solubilization.

   Note: The concentration of the reconstituted standard is 2 ng/mL.
3. Before making dilutions, allow the standard to reconstitute for 10–30 minutes, then mix well.

External standard dilution

1. Label 6 tubes, one for each standard point: S2, S3, S4, S5, S6, and S7 (the reconstituted standard serves as S1).
2. Prepare 1:2 serial dilutions for the standard curve as follows: Pipette 130 µL of Calibrator Diluent (for subsequent measurement of serum or plasma samples) or Sample Diluent (for subsequent measurement of cell culture supernatant samples) into each tube.
3. Pipette 130 µL of reconstituted standard (S1 = 2 ng/mL) into the first tube, labeled S2, and mix (concentration of S2 = 1 ng/mL).
4. Pipette 130 µL of this dilution into the second tube, labeled S3, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 4 more times thus creating the points of the standard curve (see Figure 1).

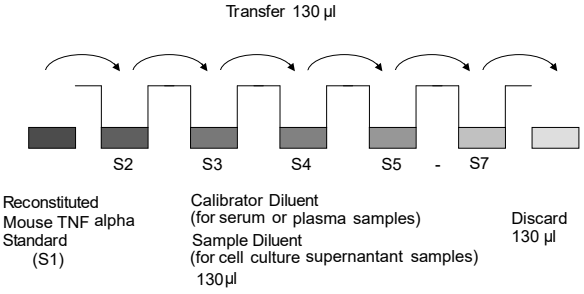
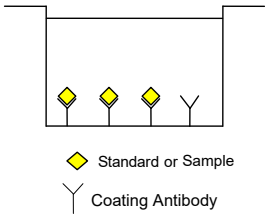


Figure 1 Dilute standards - tubes

Perform ELISA protocol

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Shaking is absolutely necessary for optimal test performance.

1 Bind antigen



1. Wash the microwell strips twice with approximately 400 µL of 1X Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10–15 seconds before aspiration. Do not scratch the surface of the microwells.

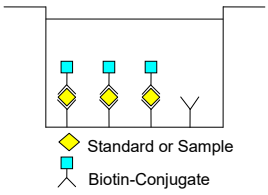
   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively, microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

Table 1 Example of the arrangement of blanks, standards, and samples in the microwell strips

	1	2	3	4	5	6	7	8	9	10	11	12
	Standard		Sample									
A	1	1	1	1	9	9	17	17	25	25	33	33
B	2	2	2	2	10	10	18	18	26	26	34	34
C	3	3	3	3	11	11	19	19	27	27	35	35
D	4	4	4	4	12	12	20	20	28	28	36	36
E	5	5	5	5	13	13	21	21	29	29	37	37
F	6	6	6	6	14	14	22	22	30	30	38	38
G	7	7	7	7	15	15	23	23	31	31	39	39
H	Blank	Blank	8	8	16	16	24	24	32	32	40	40

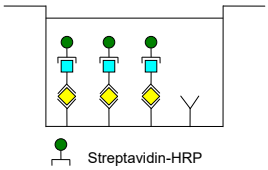
2. Add 50 µL of Sample Diluent to all wells.
3. Add 50 µL of external diluted standards (see “External standard dilution” on page 2) in duplicate to the corresponding standard wells.
4. Add 50 µL of Calibrator Diluent (for serum or plasma samples) or Sample Diluent (for cell culture supernatant samples) in duplicate to the blank wells.
5. Add 50 µL of each sample in duplicate to the sample wells.

2 Add 1X Biotin Conjugate



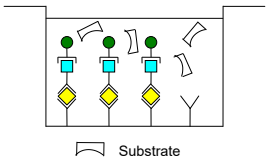
1. Add 50 µL of 1X Biotin-Conjugate (see “Prepare 1X Biotin-Conjugate” on page 2) to all wells.
2. Cover the plate with an adhesive film and incubate for 2 hours at room temperature on a microplate shaker set at 400 rpm.
3. Prepare 1X Streptavidin-HRP as mentioned in “Prepare 1X Streptavidin-HRP” on page 2.
4. Remove adhesive film and empty wells. Thoroughly aspirate the solution and wash wells 6 times with 1X Wash Buffer. Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration.

3 Add 1X Streptavidin-HRP Conjugate solution



1. Add 100 µL of 1X Streptavidin-HRP Conjugate (see “Prepare 1X Streptavidin-HRP” on page 2) to all wells, including the blanks wells.
2. Cover the plate with an adhesive film and incubate for 1 hour at room temperature on a microplate shaker set at 400 rpm.
3. Remove adhesive film and empty wells. Thoroughly aspirate the solution and wash wells 6 times with 1X Wash Buffer. Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration.

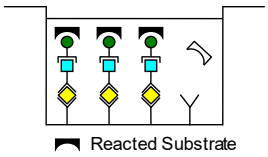
4 Add TMB Substrate Solution



1. Add 100 µL TMB Substrate Solution to all wells.
2. Incubate the microwell strips at room temperature (18–25°C) for about 30 minutes. Avoid direct exposure to intense light.

   Note: The color development on the plate should be monitored and the substrate reaction stopped (see next step) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

5 Add Stop Solution



It is recommended to add the stop solution when the highest standard develops a dark blue color.

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

IMPORTANT! It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme.

Calculation of results

Read the absorbance on a spectrophotometer using 450 nm as the primary wavelength (optionally 620 nm as the reference wavelength; 610 nm to 650 nm is acceptable as well). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the controls.

IMPORTANT! Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2–8°C in the dark.

Note: If instructions in this protocol have been followed samples have not been diluted, the concentration read from the standard curve must not be multiplied by a dilution factor.

A representative standard curve is shown in Figure 2.

Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Mouse TNF alpha was diluted in serial 2-fold steps in Sample Diluent.

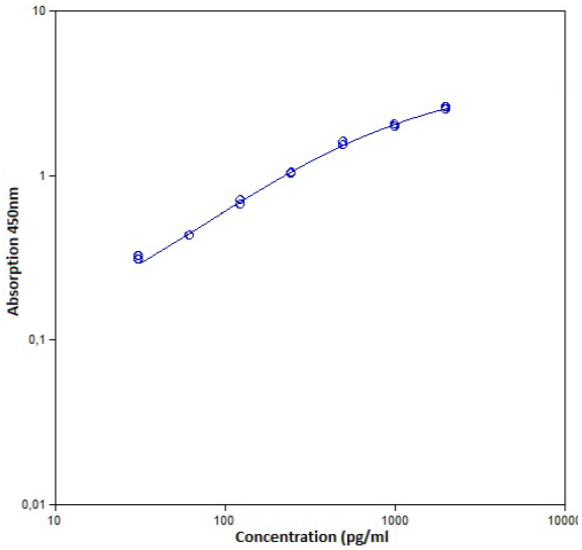


Figure 2 Representative standard curve for mouse TNF alpha ELISA

Table 2 Typical data using the mouse TNF alpha ELISA (measuring wavelength of 450 nm, reference wavelength of 620 nm)

Standard	mouse TNF alpha Concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	2000	2.492 2.560	2.526	1.4
2	1000	1.998 1.960	1.979	1.0
3	500	1.521 1.580	1.551	1.9
4	250	1.018 1.031	1.024	0.6
5	125	0.657 0.695	0.676	2.8
6	62.2	0.424 0.427	0.426	0.3
7	31.3	0.320 0.306	0.313	2.2
Blank	0	0.071 0.074	0.073	2.4

The OD values of the standard curve may vary according to the conditions of assay performance (for example, operator, pipetting technique, washing technique, or temperature effects).

Performance characteristics

Sensitivity

The limit of detection of mouse TNF alpha defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 3.7 pg/ml (mean of 6 independent assays).

Specificity

The assay detects both natural and recombinant mouse TNF alpha. The interference of circulating factors of the immune systemwas evaluated by spiking these proteins at physiologically relevant concentrations into mouse IL-10 positive serum. There was no cross-reactivity detected.

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  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN1000060 B (31)

Revision	Date	Description
B (31)	30 January 2025	Contents and storage (“Contents and storage” on page 1), ELISA protocol (“Perform ELISA protocol” on page 3), and Calculation of results (“Calculation of results” on page 4) sections were updated. Version format was changed to B (31) in conformance with internal document control procedures.
A00 (30)	25 April 2024	New document for Basic TNF alpha Mouse ELISA Kit.