# Mouse TNF alpha Uncoated ELISA Kit

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

Catalog Number 88-7324

Pub. No. MAN0017423 Rev. C (40)

**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 information

| Symbol | Contents | Mouse TNF alpha Uncoated ELISA Kit |
|---|---|---|
| REF | Catalog number | 88-7324 |
| — | Sensitivity | 15.6 pg/mL |
| — | Standard curve range | 15.6–1000 pg/mL |
| | Temperature limit | Store at 2–8°C |
| LOT | Batch code | See vial |
| | Use by | See box label |
| | Caution | Contains preservatives |

### Description

This Mouse TNF alpha Uncoated ELISA Kit contains the necessary reagents, standards, buffers and diluents for performing quantitative enzyme-linked immunosorbent assays (ELISA). This ELISA set is specifically engineered for accurate and precise measurement of mouse TNF alpha protein levels from samples including serum, plasma, tissue homogenate, lavage fluid and supernatants from cell cultures.

The use of plasma isolated from heparinized blood is not recommended in this ELISA and may result in reduced detection of mouse TNF alpha.

## Components of 2-plate format (2 × 96 tests)

- **Capture Antibody**
  Pretitrated, purified anti mouse TNF alpha antibody
  1 vial (100 µL) Capture Antibody Concentrate (250X)
- **Detection Antibody**
  Pretitrated, biotin-conjugated anti mouse TNF alpha antibody
  1 vial (100 µL) Detection Antibody Concentrate (250X)
- **Standard**
  Recombinant mouse TNF alpha for generating standard curve and calibrating samples
  1 vial mouse TNF alpha Standard (10X) (lyophilized): 10 ng/mL upon reconstitution
- **Coating Buffer**
  1 vial (2.5 mL) Phosphate Buffered Saline Concentrate (PBS, 10X)
- **ELISA/ELISPOT Diluent**
  1 bottle (30 mL) Diluent Concentrate (5X)
- **Enzyme Solution**
  1 vial (250 µL) pretitrated Streptavidin-HRP (100X)
- **Substrate Solution**
  Tetramethylbenzidine (TMB) Substrate Solution; 1 bottle (20 mL)
- **96-well plates**
  2 plates

## Components of 10-plates format (10 × 96 tests)

- **Capture Antibody**
  Pretitrated, purified anti mouse TNF alpha antibody
  1 vial (500 µL) Capture Antibody Concentrate (250X)
- **Detection Antibody**
  Pretitrated, biotin-conjugated anti mouse TNF alpha antibody
  1 vial (500 µL) Detection Antibody Concentrate (250X)
- **Standard**
  Recombinant mouse TNF alpha for generating standard curve and calibrating samples
  1 vial mouse TNF alpha Standard (10X) (lyophilized): 10 ng/mL upon reconstitution
- **Coating Buffer**
  1 vial (12 mL) Phosphate Buffered Saline Concentrate (PBS, 10X)
- **ELISA/ELISPOT Diluent**
  1 bottle (150 mL) Diluent Concentrate (5X)
- **Enzyme Solution**
  1 vial (1.25 mL) pretitrated Streptavidin-HRP (100X)

- **Substrate Solution**
  Tetramethylbenzidine (TMB) Substrate Solution; 1 bottle (100 mL)
- **96-well plates**
  10 plates (**only** included with product catalog numbers ending in suffixes–86)
  20 plates (**only** included with product catalog numbers ending in suffixes–76)

  **Note:** Product catalog numbers ending in suffixes -77 and -88 do not contain any 96-well plates.

## Required materials not supplied

- Buffers
  - Wash Buffer: 1X PBS, 0.05% Tween™–20 or ELISA Wash Buffer Powder Cat. No. 00-0400-46
  - Stop Solution: 1 M $H_3PO_4$ or 2 N $H_2SO_4$ or Stop Solution Cat. No: SS03 or SS04
- Pipettes and pipettors
- Refrigerator
- 96-well plate (Nunc™ MaxiSorp™)

  **Note:** The use of ELISA plates that are not high-affinity protein-binding plates will result in suboptimal performance, for example, low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Nunc™ MaxiSorp™ 96-well plates provided or suggested.
- Microplate shaker
- 96-well ELISA plate reader (microplate spectrophotometer)
- Plate sealer
- *(Optional)* ELISA plate washer

## Stability

This kit is guaranteed to perform as defined if stored and handled according to instructions of this manual. Expiration date is indicated on the box label.

## Storage instructions for kit reagents

Store undiluted original kit reagents at 2–8°C unless otherwise described.

## Procedural guidelines

- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date.
- Do not expose kit reagents to strong light during storage or incubation.
- To avoid microbial contamination, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.

## Before you begin

- Equilibrate the buffer concentrates ( Wash Buffer, Coating Buffer, ELISA/ELISPOT Diluent, and Substrate Solution) to room temperature (18–25°C), then dilute before use.
- If crystals have formed in the buffer concentrates, warm gently to dissolve the crystals.
- ELISA/ELISPOT Diluent sometimes contains precipitates which do not harm the assay. In such a case dilute the buffer to 1X, warm in the water bath (at 37°C) and the precipitates/crystals may dissolve. Small precipitates may remain, however, these will not interfere in the assay.

## Prepare reagents

**IMPORTANT!**

- Diluted Wash Buffer and Coating Buffer are stable for 30 days if stored at 2–25°C.
- Diluted ELISA/ELISPOT Diluent is stable for one week if stored at 2–8°C.
- Enzyme and Detection Antibody should be diluted 30 minutes before use.
- After dilution return unused stock of Enzyme and Detection Antibody to the refrigerator.

### Prepare Coating Buffer (1X)

Make a 1:10 dilution of Coating Buffer (10X) in deionized water, then mix well.

**Table 1 Dilution for 1 plate of Coating Buffer (1X)**

| Number of plates (96 wells) | Coating Buffer concentrate (10X) | Distilled water |
|---|---|---|
| 1 plate | 1.2 mL | 10.8 mL |

### Prepare Capture Antibody Solution (1X)

Dilute capture antibody (250X) 1:250 in Coating Buffer (1X), then mix well.

**Table 2 Dilution for 1 plate of Capture Antibody Solution (1X)**

| Number of plates (96 wells) | Capture Antibody (250X) | Coating Buffer (1X) |
|---|---|---|
| 1 plate | 0.048 mL | 11.952 mL |

### Prepare ELISA/ELISPOT Diluent (1X)

Dilute ELISA/ELISPOT Diluent (5X) 1:5 in deionized water, then mix well.

**Table 3 Dilution for 1 plate of ELISA/ELISPOT Diluent (1X)**

| Number of plates (96 wells) | ELISA/ELISPOT Diluent (5X) | Distilled water |
|---|---|---|
| 1 plate | 15 mL | 60 mL |

### Prepare Detection Antibody Solution (1X)

Dilute Detection Antibody Concentrate (250X) 1:250 in ELISA/ELISPOT Diluent (1X), then mix well.

**Table 4 Dilution for 1 plate of Detection Antibody Solution (1X)**

| Number of plates (96 wells) | Detection Antibody Concentrate (250X) | ELISA/ELISPOT Diluent (1X) |
|---|---|---|
| 1 plate | 0.048 mL | 11.952 mL |

### Prepare Enzyme Solution (1X)

Dilute Streptavidin-HRP (100X) 1:100 in ELISA/ELISPOT Diluent (1X), then mix well.

**Table 5 Dilution for 1 plate of Enzyme Solution (1X)**

| Number of plates (96 wells) | Streptavidin-HRP (100X) | ELISA/ELISPOT Diluent (1X) |
|---|---|---|
| 1 plate | 0.12 mL | 11.88 mL |

### Prepare Standard (1X)

**IMPORTANT!** Make the 1:10 dilution for your standard curve just prior to use.

1. Reconstitute lyophilized standard (10X) by addition of distilled water.

   Reconstitution volume is stated on the label of the standard vial.

2. Allow the lyophilized standard to reconstitute for 10–30 minutes.

3. Swirl or mix gently to ensure complete and homogeneous solubilization (concentration of reconstituted standard = 10 ng/mL).

4. The reconstituted standard must be stored in single use aliquots at –20°C.

   **Note:**
   - When stored properly, the reconstituted standard is stable and usable for up to 6 months.
   - Avoid repeated freeze-thaw cycles.

5. The reconstituted concentrated thawed single use (10X) standard aliquot must be diluted 1:10 in ELISA/ELISPOT Diluent (1X) in a clean plastic tube.

   Shake gently to mix. (Concentration of diluted standard = 1000 pg/mL = S1).

**IMPORTANT!** Any remaining diluted standard must be discarded after 1 hour of use.

**Table 6 Dilution for S1 Standard concentration**

| Number of Standards | Single use Standard aliquot (10X) | ELISA/ELISPOT Diluent (1X) |
|---|---|---|
| 1 | 45 µL | 405 µL |

## Perform ELISA assay

**Note:**
- Shaking is necessary for all incubation steps to obtain optimal test performance values unless otherwise noted.
- In case of incubation without shaking, the obtained O.D. values may be decreased. Nevertheless the results are still valid.
- Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.

### 1 Coat and block the plate

1. Coat the ELISA plate with 100 µL/well of Capture Antibody Solution (1X) (dilute as noted in "Prepare Capture Antibody Solution (1X)"). Seal the plate and incubate overnight at 4°C without shaking.

2. Aspirate wells and wash 2 times with 400 µL/well Wash Buffer.

   Allowing time for soaking (10–15 seconds) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.

   **Note:** Do not let wells dry out.

3. Block wells with 200 µL of ELISA/ELISPOT Diluent (1X). Incubate at room temperature for 1 hour without shaking.

### 2 Add Standards and Samples

1. Prepare S1 Standard concentration (see "Prepare Standard (1X)").

2. Aspirate and wash once with Wash Buffer according to step 1.2.

## 2 *(continued)*

3. Perform 2-fold serial dilutions of the S1 Standard to make the standard curve for a total of 7 points.
   - Add 100 µL of ELISA/ELISPOT Diluent (1X) to all standard wells leaving the first wells empty.
   - Add 200 µL of S1 standard concentration to the first empty wells A1/A2.
   - Transfer 100 µL of S1 Standard from wells A1/A2 to standard wells B1/B2.
   - Mix the contents of the wells B1 and B2 by repeated aspiration and ejection and transfer 100 µL to wells C1/C2.
   - Do not scratch surface of the microwells. Continue this procedure 4 times.
   - Discard 100 µL from the last standard well to align the volume with the other standard wells.

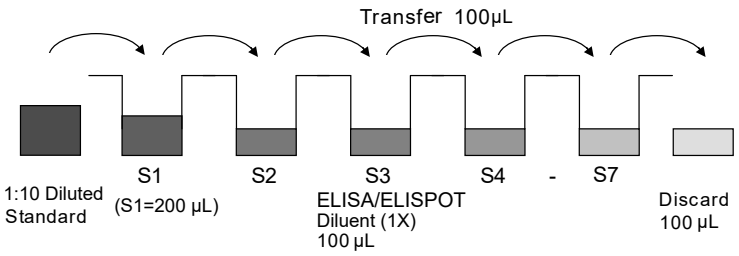


**Figure 1 Standard dilutions on the microwell plate**

**Table 7 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 | | Samples | | | | | | | | | |
| 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 |

4. Add 100 µL of ELISA/ELISPOT Diluent (1X) to the blank well.
5. Add 100 µL/well of samples to the designated sample wells.
6. Seal the plate and incubate on a microplate shaker at 400 rpm for 2 hours at room temperature.

## 3 Add Detection Antibody Solution (1X)

1. Prepare the Detection Antibody Solution (1X) (see “Prepare Detection Antibody Solution (1X)”).
2. Aspirate and wash according to step 1.2. Repeat for a total of 4 washes.
3. Add 100 µL of diluted Detection Antibody Solution (1X) to all wells used.
4. Seal the plate and incubate on a microplate shaker at 400 rpm for 1 hour at room temperature.

**4 Add Enzyme Solution (1X)**

1. Prepare the Enzyme Solution (1X) (see "Prepare Enzyme Solution (1X)").
2. Aspirate and wash according to step 1.2. Repeat for a total of 5 washes.
3. Add 100 µL of diluted Enzyme Solution (1X) to all wells used.
4. Seal the plate and incubate on a microplate shaker at 400 rpm for 30 minutes at room temperature.

**5 Add Substrate Solution**

1. Aspirate and wash according to step 1.2. Repeat for a total of 5 washes.
2. Add 100 µL of TMB Substrate Solution to all wells used.
3. Incubate for approximately 15–30 minutes at room temperature without shaking, until S1 has developed a dark blue color.

**6 Add Stop Solution**

1. Add 100 µL of Stop Solution to all wells used.
2. Read plate at 450 nm. If wavelength substraction is available, substract the values of 620 nm from those of 450 nm and analyze data.

## Troubleshooting and FAQs

Visit our online FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated to ensure accurate and thorough content.

- For troubleshooting information and FAQs for this product: **https://www.thermofisher.com/trizolfaqs**
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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

## Limited product warranty

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

**Revision history:** Pub. No. MAN0017423 C (40)

| Revision | Date | Description |
|---|---|---|
| C (40) | 6 May 2025 | • Removed Standard curve. Updated Prepare Reagent ("Prepare reagents" on page 2), Perform ELISA assay ("Perform ELISA assay" on page 3), and Troubleshooting and FAQs sections ("Troubleshooting and FAQs" on page 5).<br>• Minor updates were made throughout for consistency of style and terminology. |
| B.0 (32) | 21 October 2018 | Baseline document. |