

ab285327 – Mouse TNF alpha ELISA Kit

For the quantitative measurement of TNF alpha in mouse serum, plasma, cell lysates, and cell culture supernatants.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab285327>

Storage and Stability

On receipt entire assay kit should be stored at 4°C for 6 months or -20°C for 12 months. Protected from light.

Materials Supplied

Item	Quantity	Storage Condition
TNF-alpha mAb coated plate, 96 wells	12 strips x 8 wells	4°C
Mouse TNF-alpha standard (10 ng/vial)	2 vials	4°C
Sample diluent buffer	30 mL	4°C
Biotinylated anti-mouse TNF-alpha Ab (100X)	100 µL	4°C
Antibody dilution buffer	12 mL	4°C
Avidin-Biotin-Peroxidase Complex (ABC) (100X)	100 µL	4°C
ABC diluent buffer	12 mL	4°C
TMB	10 mL	4°C
Stop Solution	10 mL	4°C
Wash buffer (25X)	20 mL	4°C
Plate sealers	4 units	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.

Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

Reconstitution of the mouse TNF-alpha standard: Two vials of TNF-alpha standard (10 ng per vial) are included in each kit. Use one vial for each experiment. Prepare 10,000 pg/ml of mouse TNF-alpha standard solution by adding 1ml of Sample diluent buffer into one of the vials. Keep the tube at room temperature for 10 min. and mix thoroughly. Label 8 tubes as follows:

Tube 1 – 1000 pg/ml

Tube 2 – 500 pg/ml

Tube 3 – 250 pg/ml

Tube 4 – 125 pg/ml

Tube 5 – 62.5 pg/ml

Tube 6 – 31.25 pg/ml

Tube 7 – 15.625 pg/ml

Tube 8 – 0 pg/ml (Sample diluent buffer only).

To prepare Standard 1, mix 0.1 ml of reconstituted standard stock solution (10,000 pg/ml) with 0.9 ml of Sample diluent buffer. Add 0.3 ml of Sample diluent buffer to Tubes 2-7.

To prepare Standard 2, add 0.3 ml from Tube 1 to Tube 2.

To prepare Standard 3, add 0.3 ml from Tube 2 to Tube 3.

Continue serial dilution for Tubes 4-7.

Δ Note: The standard solutions are best used within 2 hrs. The 10 ng/ml standard solution should be stored at 4°C for up to 12 hrs, or at -20°C for up to 48 hrs. Avoid repeated freeze-thaw cycles.

Biotinylated antibody working solution (1X): Dilute the antibody 1:100 with the Antibody diluent buffer. Prepare 0.1 ml of Biotinylated antibody working solution (1X) by adding 1 µl of Biotinylated antibody (100X) to 99 µl of Antibody diluent buffer for each well. Mix well. Solution should be prepared no more than 2 hrs prior to the experiment.

Avidin-Biotin-Peroxidase Complex (ABC) working solution (1X): Dilute Peroxidase complex 1:100 with the ABC dilution buffer. Prepare 0.1 ml of ABC working solution (1X) by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100X) to 99 µl of ABC diluent buffer for each well. Mix well. Solution should be prepared no more than 2 hrs prior to the experiment.

Wash Buffer (1X): Prepare 500 ml of Wash Buffer (1X) by diluting 20 ml of Wash Buffer (25X) with 480 ml of deionized water. If crystals are present in Wash Buffer (25X) concentrate, warm the bottle to room temperature and mix gently until all crystals are dissolved completely.

Sample Preparation

Samples with hyperlipidemia and haemolyticus is not suitable for this kit.

Store samples to be assayed within 24 hrs at 2-8°C.

For long-term storage, aliquot and freeze samples at -20°C.

Avoid repeated freeze-thaw cycles.

Serum:

Allow the serum to clot in a serum separator tube (about 4 hrs) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

Plasma:

Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min. at 1000 x g within 30 min. of collection. Analyze immediately or aliquot and store frozen at -20°C.

Cell Lysates:

Lyse the cells completely and make sure there are no visible cell sediments. Centrifuge the lysates at 10,000 x g for 5 min. Collect the supernatant and perform the assay.

Cell culture supernatant:

Centrifuge to remove particulates, assay immediately or aliquot and store at -20°C.

Other biological fluids:

Centrifuge samples for 20 min at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.

Δ Note: The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The sample must be well mixed with the diluents buffer. Several trials may be necessary in practice.

Assay Protocol

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min. before use.

When diluting samples and reagents, they must be mixed completely and evenly.

Don't let 96-well plate dry, as it will inactivate active components on plate.

1. Aliquot 100 µl of standards, samples, or controls in each well. Add 100 µl of the sample diluent buffer into the control well (Zero well). See "Sample Dilution Guideline" for details.

Δ Notes:

- a. We recommend that each mouse TNF-alpha standard solution and each sample is measured in duplicate.
 - b. We recommend doing a pilot experiment using standards and a small number of samples to inspect the validity of experiment operation and the appropriateness of sample dilution proportion.
2. Seal the plate with the cover and incubate at 37°C for 90 min or 120 mins at room temperature (RT). Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do not let the wells completely dry at any time.
 3. Add 100 µl of biotinylated antibody working solution (1X) into each well and incubate the plate at 37°C for 60 min or 90 mins at RT. Wash plate 3 times with Wash buffer (1X), and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 300 µl Wash buffer (1X) for 1~2 min. Repeat this process two additional times for a total of three washes.
 4. Add 100 µl of ABC working solution (1X) into each well and incubate the plate at 37°C for 30 min or 40 mins at RT. Wash plate 5 times with Wash buffer (1X), and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 3 for plate washing method).
 5. Add 90 µl of TMB into each well and incubate plate at 37°C in dark for 15-25 min or 30 mins at RT.
Δ Note: For reference only, the optimal incubation time should be determined by end user. The shades of blue can be seen in the wells with the four most concentrated mouse TNF-alpha standard solutions; the other wells show no obvious color.
 6. Add 100 µl of Stop solution into each well. The color changes into yellow immediately.
 7. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the Stop solution.

Calculation

Relative O.D.450 = O.D.450 of each well – O.D.450 of Zero well. The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The mouse TNF-alpha concentration of the samples can be interpolated from the standard curve.

Δ Note: if the samples were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical Data Obtained from mouse TNF-alpha

(TMB reaction incubated at 37°C for 20 min.)

Concentration (pg/mL)	0	15.6	31.2	62.5	125	250	500	1000
O.D.	0.028	0.090	0.156	0.281	0.543	0.923	1.484	2.258

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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