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TNF-α (Mouse) ELISA Kit

rev 04/21

(Catalog # K1051-100, 100 assays; Store at -20°C)

I. Introduction:

BioVision's mouse TNF-α ELISA Kit is based on the standard sandwich enzyme-linked immunosorbent assay technology. This assay employs a monoclonal antibody (mAb) specific for mouse TNF-α coated on a 96-well plate. Standards and test samples are added to the wells and TNF-α present in a sample is bound to the wells by the immobilized antibody. A biotinylated detection polyclonal antibody from goat specific for TNF-α is added subsequently. After washing away the unbound biotinylated antibody with PBS or TBS buffer, Avidin-Biotin-Peroxidase Complex is added to the wells. The wells are again washed with PBS or TBS buffer to remove the unbound conjugates. HRP substrate TMB is used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow color is proportional to the mouse TNF-α captured onto the plate. This ELISA kit shows no cross-reactivity with other relevant proteins. Detection Range: 15.6 pg/ml – 1000 pg/ml (cell culture supernates), 7.8 pg/ml – 500 pg/ml (mouse serum, plasma). Sensitivity: < 1 pg/ml.

II. Application:

Quantitative measurement of TNF-α in mouse serum, plasma, cell lysates, and cell culture supernatants

III. Specificity:

Native and recombinant mouse TNF-α

IV. Sample Type:

- Serum & plasma (heparin, EDTA)
- Cell culture supernatants

V. Kit Contents:

Components	K1051-100	Part No.
TNF-α mAb coated plate, 96 wells	12 strips x 8 wells	K1051-100-1
Mouse TNF-α standard (10 ng/vial)	2 vials	K1051-100-2
Biotinylated anti-mouse TNF-α Ab (100X)	100 µl	K1051-100-3
Avidin-Biotin-Peroxidase Complex (ABC) (100X)	100 µl	K1051-100-4
Sample diluent buffer	30 ml	K1051-100-5
Antibody diluent buffer	12 ml	K1051-100-6
ABC diluent buffer	12 ml	K1051-100-7
TMB	10 ml	K1051-100-8
Stop solution	10 ml	K1051-100-9
Wash Buffer (25X)	20 ml	K1051-100-10
Plate Sealers	4	K1051-100-11

VI. User Supplied Reagents and Equipment:

- 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.

VII. Storage Conditions and Reagent Preparation:

Store kit at 4°C for 6 months or at -20°C for 12 months. Avoid repeated freeze-thaw cycles. Spin tubes briefly to bring down all components to the bottom of tubes.

- **Reconstitution of the mouse TNF-α standard**: Two vials of **TNF-α standard** (10 ng per vial) are included in each kit. Use one vial for each experiment. Prepare 10,000 pg/ml of mouse TNF-α 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.
  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.

VIII. Sample Preparation and Storage:

For **cell culture supernates**, centrifuge to remove particulates, assay immediately or aliquot and store at -20°C. **For serum samples**, 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. **For Plasma samples**, 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. **For**

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.

Notes:

a. Samples with hyperlipoidemia and haemolyticus is not suitable for this kit.
b. 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.
c. Sample dilution guidelines: 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.

IX. 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.
   Notes:
   a. We recommend that each mouse TNF-α 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.
   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-α 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**.
8. 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-α concentration of the samples can be interpolated from the standard curve. **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-α*
(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

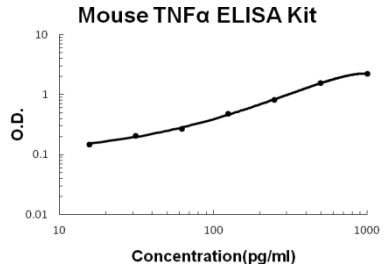

Figure: Standard Curve: This standard curves is for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

Human CellExp™ TNF alpha, human recombinant (6482)
TNF alpha (rat) ELISA Kit (K1052)
TNF alpha, murine recombinant (1051)
TNF alpha Antibody (3052, 3052R, 3054, 3053R)
TNF alpha (human) ELISA Kit (K4779)
TNF alpha, human recombinant (1050)
TNF alpha, rat recombinant (1052)

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