



TNF α (rat) ELISA Kit

(Catalog # K1052-100, 100 assays; Store at -20°C)

rev 3/19

I. Introduction:

BioVision's rat TNF alpha ELISA Kit is based on the standard sandwich enzyme-linked immunosorbent assay technology. This assay employs a monoclonal antibody specific for rat TNF alpha coated on a 96-well plate. Standards and test samples are added to the wells and TNF alpha present in a sample is bound to the wells by the immobilized antibody. A biotinylated detection polyclonal antibody from goat specific for TNF alpha 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 rat TNF alpha 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 (rat serum, plasma). Sensitivity: < 1 pg/ml.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Natural and recombinant rat TNF alpha.

IV. Sample Type:

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

V. Kit Contents:

Components	K1052-100	Part No.
TNF alpha Microplate coated with anti-rat monoclonal Ab against TNF alpha, 96 wells	12 stripsx8 wells	K1052-100-1
Lyophilized recombinant rat TNF alpha standard (10 ng/vial)	2 vials	K1052-100-2
Biotinylated anti-rat TNF alpha antibody	130 μ l	K1052-100-3
Avidin-Biotin-Peroxidase Complex (ABC)	130 μ l	K1052-100-4
Sample diluent buffer	30 ml	K1052-100-5
Antibody diluent buffer	12 ml	K1052-100-6
ABC diluent buffer	12 ml	K1052-100-7
TMB color developing agent (Colorless)	10 ml	K1052-100-8
TMB stop solution	10 ml	K1052-100-9
Plate sealers	4	K1052-100-10

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.
- Washing buffer (neutral PBS or TBS).
 - Preparation of 0.01M TBS: Add 1.2 g Tris, 8.5 g NaCl; 450 μ l of purified acetic acid or 700 μ l of concentrated hydrochloric acid to 1000 ml H₂O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
 - Preparation of 0.01 M PBS: Add 8.5 g sodium chloride, 1.4 g Na₂HPO₄ and 0.2 g NaH₂PO₄ to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

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 rat 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 rat 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. Prepare 1000 pg/ml of rat TNF alpha standard solution by adding 0.1 ml of the 10 ng/ml TNF alpha Standard solution into 0.9 ml sample diluent buffer and mix thoroughly. Label 6 Eppendorf tubes with 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml & 15.6 pg/ml respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the 1000 pg/ml TNF alpha standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

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.

- **Preparation of biotinylated anti-rat TNF alpha antibody working solution:** Dilute 1:100 with the antibody diluent buffer and mix thoroughly. Prepare 0.1 ml of TNF alpha antibody working solution for each well. Solution should be prepared no more than 2 hrs. prior to the experiment.
- **Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution:** Dilute 1:100 with the ABC dilution buffer and mix thoroughly. Prepare 0.1 ml of ABC working solution for each well. Solution should be prepared no more than 1 hr. prior to the experiment.

VIII. Sample Preparation and Storage:

Centrifuge cell culture supernates to remove particulates, assay immediately or aliquot and store at -20°C. Allow the serum to clot in a serum separator tube (about 4 hrs) at room temperature. Centrifuge at approximately 1500 X g for 15 min. Analyze the serum immediately



or aliquot and store frozen at -20°C. 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.

Notes:

- 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.
- 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. The following is a guideline for sample dilution. Several trials may be necessary in practice. For high target protein concentration (10-100 ng/ml): dilute 1:100. For medium target protein concentration (1-10 ng/ml): dilute 1:10. For low target protein concentration (15.6-1000 pg/ml): dilute 1:2. For very low target protein concentration (≤ 15.6 pg/ml). No dilution necessary or dilute 1:2.

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.

- Aliquot 0.1ml per well of the 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml and 15.6 pg/ml rat TNF alpha standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of rat cell culture supernates, serum or plasma to each empty well. See "Sample Dilution Guideline" for details.

Notes:

- We recommend that each rat TNF alpha standard solution and each sample is measured in duplicate.
 - 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.
- Seal the plate with the cover and incubate at 37°C for 90 min. 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.
 - Add 0.1ml of biotinylated anti-rat TNF alpha antibody working solution into each well and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS or 0.01M PBS, 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 0.3 ml PBS or TBS buffer for 1~2 min. Repeat this process two additional times for a total of three washes. Note: For automated washing, aspirate all wells and wash three times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)
 - Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS or 0.01 M PBS, 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).
 - Add 90µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 20-25 min.
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 rat TNF alpha standard solutions; the other wells show no obvious color.
 - Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
 - 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 rat TNF alpha 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 rat 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.	0.154	0.206	0.257	0.373	0.634	1.085	1.906	2.732

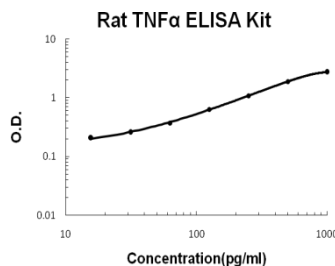


Figure: Standard Curve: This standard curves is for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

- Human CellExp™ TNF alpha, human recombinant (6482)
- TNF alpha (mouse) ELISA Kit (K1051)
- 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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