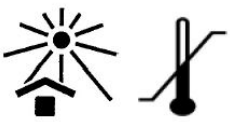
Mouse TNF-α ELISA Kit

Catalogue No：DEIA7391

Size: 96T

Please read this Instruction and check the components carefully prior to using this products !



2-8/-20℃

FOR RESEARCH USE ONLY,

NOT FOR DIAGNOSTIC OR THERAPEUTIC PROCEDURE

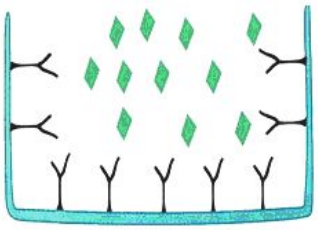
45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

1. INTENDED USE

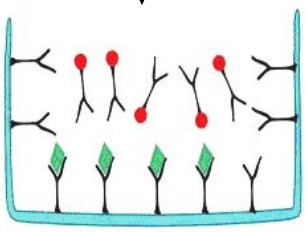
The Mouse Tumor Necrosis Factor Alpha (TNF-α) ELISA Kit is to be used for the vitro quantitative determination of Mouse TNF-α in serum, plasma, tissue lysates, cell culture supernatants and other biological fluids. The Kit is intended for research use only, not for diagnostic or therapeutic procedure. If detection of other special sample, please contact our technical support.

2. PRINCIPLE OF THE ASSAY.

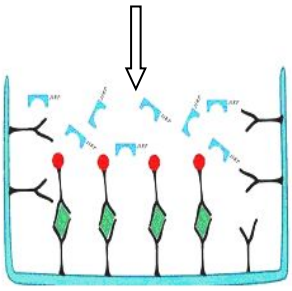
Standard and Samples are aspirated into the wells and Mouse TNF-α present in them is bound to Mouse TNF-α monoclonal immobilized antibodies ,which pre-coated onto 96-well plate



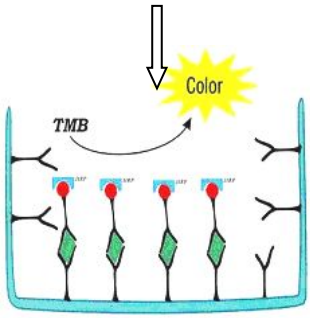
The Biotinylated detection antibodies are added to the wells and then followed by washing with PBS or TBS buffer.



After washing away unbound Biotinylated antibody, Avidin-Biotin-Peroxidase Complex is added to the wells.



The wells are washed again, a TMB substrate solution is added to the wells and the color changes after adding acidic TMB Stop solution. The intensity of the color is proportional to the amount of Mouse TNF-α bound in samples and measured at 450nm±10nm.



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3. KIT COMPONENTS AND STORAGE INSTRUCTIONS

components	96T	48T	Storage
Precoated Microtiter plate	8 X12	8 X6	2-8/-20℃
Standards (Frozen dried)	2vials	1 vial	2-8/-20℃
Biotinylated detection antibodies	1 vial X 120 µl	1 vial X 60 µl	2-8/-20℃
Avidin-Biotin-Peroxidase Complex(ABC)1:100	1 vial X 120 µl	1 viall X 120 µl	2-8/-20℃
TMB color developing reagent A (Avoid light)	1 vial X 10ml	1vialX 10ml	2-8/-20℃
TMB color developing reagent B	1 vial X 1.5 ml	1 vial X 1.5 ml	2-8/-20℃
Sample Diluent Buffer	1 vials X 14ml	1vial X 14ml	2-8/-20℃
Standard Diluent Buffer	1 vials X 14ml	1vial X 14ml	2-8/-20℃
ABC Diluent Buffer	1vial X 12ml	1vial X 12ml	2-8/-20℃
Antibody Diluent Buffer	1vial X 12ml	1vial X 12ml	2-8/-20℃
TMB Stop solution	1vial X 10ml	1vial X 10ml	2-8/-20℃
TBS Wash Buffer(25X)	1vial X 20ml	1vial X 20ml	2-8/-20℃
Instruction manual	1	1	RT
Plate Sealer/Cover	2	2	RT

Hints:

- All the components in the kit should be stored up to 1 year at -20℃ and 2 months at 2-8℃,and should be kept according to the labels on vials. If the kit can not be used within 2weeks,please keep in -20℃.

- The ABC(1:100) and Biotinylated detection antibodies is in small volume, it may be bumps and inverted in transit, so the solution probably infected the wall and caps, Please centrifuge at 1000rpm for 1min, to make sure the reagents focus on the bottom of the tube.

- The kit contain sufficient materials to run ELISAs on 96 or 48 microplates. Specific vial volume of each component may vary.Please check carefully if all components is in the correct volume.
  CD have no any responsibility for the Check negligence.

- Upon receipt, foil pouch around the plate should be vacuum-sealed.

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- Please avoid repeated freeze-thaw cycles and do not mix reagents from different kits unless they have the same lot numbers.

- After use remaining reagents should be returned to cold storage (2° to 8°C) immediately. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal.

- Do not use components beyond the expiration date.Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling. Expiry of the kit and reagents is stated on kit labels.

- It is highly recommended to use the remaining reagents within 1 month provided, this is within the expiration date of the kit.

- Any irregularities to aforementioned conditions may influence plate performance in the assay.

4. MATERIALS REQUIRED BUT NOT PROVIDED

1）Microplate reader (450nm detection wavelength filter, 570nm or 630nm correction wavelength filters)

2）Beakers, flasks, cylinders necessary for preparation of reagents

3）Clean benches, Incubator(37℃), Refrigerators (4℃, -20℃), Low Temperature Centrifuge

4）High-precision single-channel and multi-channel Pipette and disposable Tips.

5）Polypropylene tubes for diluting and aliquoting Standards

6）Distilled water or de-ionized water

7）Absorbent paper for blotting the microtiter plate

8）Automated or manual microplate washer

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5. SAMPLE COLLECTION AND STORAGE

Serum: Allow samples to clot in a serum separator tube for two hours at room temperature or overnight at 4℃. Centrifuge at approximately 1000 -3000rpm for 10-15 min. Analyze the serum immediately or aliquot and store frozen at -20°C or -80°C.Avoid repeated freeze/thaw cycles.

Plasma: Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge at approximately 1000 -3000rpm for 10-15 min. Analyze immediately or aliquot and store frozen at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Tissue homogenates: Please over-wash the tissue blocks in 0.01mPBS; then add the Tissue protein extraction (1g tissue added 5-10ml Tissue protein extraction), homogenized in ice water. Centrifuge at approximately 3000-10000rpm for 10min. Having the Supernatant and discarded following precipitation.Analyze immediately or aliquot and store frozen at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Cell culture supernatant: Centrifuge at approximately 1000-3000rpm for 10min. Analyze immediately or aliquot and store frozen at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Urine, ascites, cerebrospinal fluid and other body fluids: Remove precipitation by centrifugation at approximately 1000-3000rpm for 10min. Analyze immediately or aliquot and store frozen at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Hints:

- Other biological samples might be suitable for use in the assay,please inquire our Tech Support.
- Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.
- Samples to be used within 24hours may be stored at 4°C, otherwise samples must be stored at -20°C (≤3month) or -80°C (≤6 months) to avoid loss of bioactivity and contamination.
- Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Sample Dilution Guideline

User needs to estimate the concentration of the target protein in the samples and select proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

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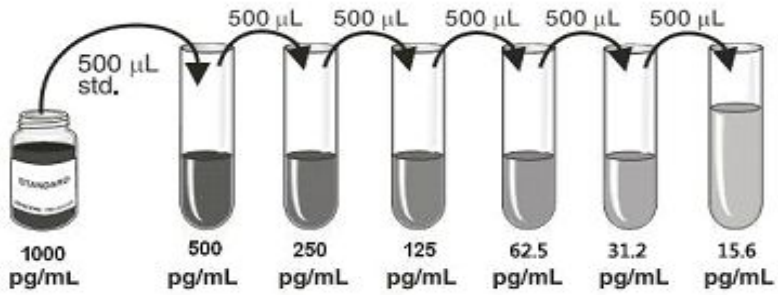
6. REAGENT PREPARATION

Please balance the kit at room temperature for 30min prior to the experiment.

1). Preparation of the standard: Add 1 ml Standard Diluent Buffer into one tube,the concentration is 1000pg/ml, dissolve the standard thoroughly and label ①.

Please prepare 7 Polypropylene tubes and label ②③④⑤⑥⑦⑧, add 500ul Standard diluent buffer into each tube.Then suck out 500ul solution from ① and add to ②, then suck out 500ul solution from ② and add to ③, make times dilution until ⑦,please discard the last 500ul solution sucked from ⑦. The ⑧ is only Standard diluent buffer,used as a control.



Note: The Standard solution (1000pg/ml) only for one time use,please discard the Remaining solution.

2). Preparation of Biotinylated anti- Mouse TNF- α working solution: The solution shoμld be prepared no more than 30min prior to the experiment.

- The total volume shoμld be (100μl) or 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Biotinylated anti- Mouse TNF- α antibody shoμld be diluted in 1:100(1+99)with the Antibody Diluent Buffer and mixed thoroughly.

3). Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution shoμld be prepared no more than 30min prior to the experiment.

- The total volume shoμld be:(100μl) or 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Avidin-Biotin-Peroxidase Complex (ABC) shoμld be diluted in 1:100 (1+99) with the ABC Diluent Buffer and mixed thoroughly.

4). Preparation of TMB working solution(B：A＝1：9): transfer 1 volumes of TMB color developing Reagent B into 9 volume of TMB color developing Reagent A, mixing thoroughly.

- The total volume shoμld be:(100μl) or 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Please avoid the light in the whole process.
- The best time to prepare the TMB working solution is ,when finished the third time in washing the ABC(1:100),please prepare the TMB working solution

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immediately and avoiding light essential,then go into wash ABC the remaining 2 times.

5). Prepare the TBS working solution: please dilute the TBS Wash Buffer 25 times(1:25),such as 1mL TBS Wash Buffer add 24 mL Distilled water.

7. ASSAY PROCEDURE

① Aliquot 100µl per well of the grades Mouse TNF-α standard solutions into the pre-coated 96-well plate. Add 100µl of the Standard diluent buffer into the control wells. Add 100µl of each properly diluted sample of Serum, Plasma, Body fluids, tissue lysates or cell culture supernatants to each empty well.

② Seal the plate with the Cover and incubate at 37°C for 90 min.

③ Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Washing twice with 350 µl TBS each well, and each time let washing buffer stay in the wells around 1 min.

④ Add 100µl of Biotinylated anti- Mouse TNF-α antibody working solution into each well and incubate the plate at 37°C for 60 min.

⑤ Washing 3 times with 350 µl TBS each well,and each time let washing buffer stay in the wells around 1 min.

⑥ Add 100µl of prepared ABC working solution into each well **except the control well** and incubate the plate at 37°C for 30 min.

⑦ Washing 5 times with 350 µl TBS each well ,and each time let washing buffer stay in the wells for around 1 min.

⑧ Add 100µl of prepared TMB working solution into each well (include the control well)and incubate plate at 37°C for ⩽**30 min** away from light.

⑨ Observe the color at all times, when shades of blue can be seen in the wells with the three-four maximum concentration of Mouse TNF-α standard solutions,and the other wells show no obvious color,add 100µl of prepared TMB Stop solution into each well to stop the reaction. The color changes into yellow immediately.

⑩ Read the O.D. absorbance at 450nm in the Microplate reader **within 10 min** after adding the TMB Stop solution or even 620-630nm Secondary wavelength.

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Hints:

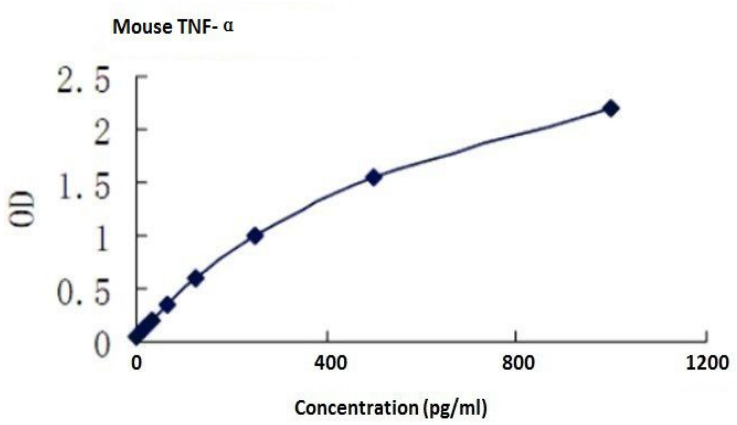
Plate washing for each step

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 350 µl TBS Wash Buffer around 1 min, then discard the rinse solution. Repeat this process for several times.

DO NOT let the wells completely dry at any time。

⑪ The OD of each sample and standard should be subtract with the OD of control 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.

Reference curve



Note: This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

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8. ASSAY PROCEDURE SUMMARY

Prepare reagents, samples and standards

⇩

Add 100µl prepared samples or standards and incubate the plate at 37℃ for 90min, wash plate twice with 350 µl TBS each well

⇩

Add 100µl Biotinylated antibodies and incubate the plate at 37℃ for 60min, wash plate 3 times with 350 µl TBS each well

⇩

Add 100µl ABC working solution and incubate the plate at 37℃ for 30 min, wash plate 5 times with 350 µl TBS each well

⇩

Add 100µl TMB color developing reagent and incubate at 37℃ for 10-25 min,away from light

⇩

Add 100µl TMB Stop solution

⇩

Read the O.D. absorbance at 450nm within 10 min

⇩

Calculate the Mouse TNF-α concentration in the samples by plotting graph between Concentrations and corresponding absorbencies of Standards

9. PERFORMANCE CHARACTERISTICS

This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. No significant cross-reactivity or interference between Mouse TNF-α and any of other cytokines.

Normal Range: 1000pg/ml-15.63pg/ml
Sensitivity: <5.0pg/ml
Intra-Assay: CV≤ 8%
Inter-Assay: CV≤12%
Recovery: 70-110%

Hints

- Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between Mouse TNF-α and all the analogues, therefore, cross reaction may still exist.
- The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration.

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