

## 小鼠 TNF- $\alpha$ 定量分析酶联免疫检测试剂盒

本试剂盒仅供科研使用。用于体外定量检测小鼠血清、血浆或细胞培养上清液中的 TNF- $\alpha$  浓度。**使用前请仔细阅读说明书并检查试剂组分是否完整**，如有疑问请与合肥博美生物科技有限责任公司联系，我们将提供力所能及的帮助。如您有其它需求，请登录合肥博美生物科技有限责任公司网站 [www.bomeibio.com](http://www.bomeibio.com) 或致电本公司 400-688-3412。

### TNF- $\alpha$ 简介：

肿瘤坏死因子 (TNF- $\alpha$ ) 是由单核细胞和巨噬细胞产生的多肽类细胞因子，在炎症反应、免疫系统的发展、细胞程序性死亡和脂代谢中起重要的作用。其功能主要是在免疫反应中是一个多功能的调节器甚至作为一个强烈的热原性物质刺激中性粒细胞，改变血管内皮细胞的特性，调节其它组织的代谢活性。TNF- $\alpha$  也可通过抑制脂蛋白脂肪酶的活性而导致恶液病。爱泼斯坦病毒引起的 B 细胞活化也可被 TNF- $\alpha$  所抑制。

巨噬细胞表面的淋巴因子和肉毒素也可介导包括上皮细胞、内皮细胞和肿瘤细胞等产生 TNF- $\alpha$ 。据报道干扰素能显著提高 TNF- $\alpha$  的分泌量。

TNF- $\alpha$  在关节炎和其它组织的炎症的发病机理中起重要作用。TNF- $\alpha$  也参与了包括哮喘、克罗恩氏病、类风湿性关节炎、神经性疼痛、肥胖症、II型糖尿病、自身免疫病和肿瘤等疾病的发生。

### 检测原理：

本试剂盒采用双抗体夹心 ELISA 法检测样本中小鼠 TNF- $\alpha$  的浓度。小鼠 TNF- $\alpha$  捕获抗体已预包被于酶标板上，当加入标本或参考品时，其中的小鼠 TNF- $\alpha$  会与捕获抗体结合，其它游离的成分通过洗涤的过程被除去。当加入生物素化的抗小鼠 TNF- $\alpha$  抗体后，抗小鼠 TNF- $\alpha$  抗体与小鼠 TNF- $\alpha$  接合，形成夹心的免疫复合物，其它游离的成分通过洗涤的过程被除去。随后加入辣根过氧化物酶标记的亲合素。生物素与亲合素特异性结合，亲合素连接的酶就会与夹心的免疫复合物连接起来；其它游离的成分通过洗涤的过程被除去。最后加入显色剂，若样本中存在 TNF- $\alpha$  将会形成免疫复合物，辣根过氧化物酶会催化无色的显色剂氧化成蓝色物质，在加入终止液后呈黄色。通过酶标仪检测，读其 450nm 处的 OD 值，小鼠 TNF- $\alpha$  浓度与 OD<sub>450</sub> 值之间呈正比，通过参考品绘制标准曲线，对照未知样本中 OD 值，即可算出标本中 TNF- $\alpha$  浓度。

### 人定量分析酶联免疫检测试剂盒组成：

组分	规格
预包被板	12条 或 6条
样本分析缓冲液	1瓶5ml/3 ml
标准品稀释液	10ml/5ml
标准品	2/1支(冻干)*
生物素化抗体	1瓶10ml/5ml
HRP连接的酶结合物	1瓶10ml/5ml
浓缩洗涤液 20 $\times$	30ml/瓶
TMB底物	1瓶10ml/5 ml
终止液	1瓶5ml/3 ml
封板胶纸	3/2张
说明书	1份

### 标本收集：

- 标本的收集请按下列流程进行操作：
  - 细胞上清标本离心去除悬浮物后即可；
  - 血清标本应是自然凝固后，取上清，避免在冰箱中凝固血液；

- C. 血浆标本，推荐用EDTA的方法收集；
- D. 若待测样本不能及时检测，标本收集后请分装，冻存于-20℃，避免反复冻融。
2. 血清标本不应添加任何防腐剂或抗凝剂；
  3. 标本应清澈透明，检测前样本中如有悬浮物应通过离心去除。
  4. 请勿使用溶血，高血脂或污染的标本检测，否则结果将不准确。

**注：正常小鼠血清或血浆样本请用标本缓冲液做倍比稀释后再检测。**

### 注意事项：

1. 试剂盒请保存在2~8℃。
2. 浓缩洗涤液因在低温下可能有结晶，请水浴加热使结晶完全溶解后再配制工作液。
3. 若标准品复溶后，请在三天内用完。
4. 底物请勿接触氧化剂和金属。
5. 加样时，请及时更换枪头，避免交叉污染。
6. 严禁混用不同批号的试剂盒组份。
7. 充分混匀对保证反应结果的准确性很重要，在加液后请轻轻叩击边缘以保证混匀。
8. 室温反应，请严格控制在25~28℃。
9. 洗涤过程是至关重要的，洗涤不充分会使精确度下降并导致结果误差较大。
10. 试验中标准品和样本检测时建议作双复孔。
11. 加样过程中避免气泡的产生。
12. 血清和血浆标本的检测时，检测抗体的孵育时间应当适当延长。

### 检测前准备工作：

1. 试剂盒自冰箱中取出后应置室温平衡20分钟；每次检测后剩余试剂请及时于2~8℃保存。
2. 将浓缩洗涤液用双蒸水或去离子水稀释（1份加19份水）。
3. 标准品：加入标准品稀释液0.5ml至冻干标准品瓶中使TNF- $\alpha$ 终浓度达到2000pg/ml，静置15分钟后轻轻混悬待彻底溶解，用标准品稀释液倍比梯度稀释后依次加入检测孔中。（标准曲线取七个点，最高浓度为2000 pg/ml，标准品稀释液直接加入作为0浓度。）



### 洗涤方法：

自动洗板机或人工洗板：每孔洗涤液为300ul，注入与吸出间隔15-30秒。洗板5次。最后一次洗板完成后将板倒扣着在厚吸水纸上用力拍干。

### 实验过程需自备的材料：

1. 不同规格的加样枪及相应的枪头；
2. 酶标仪；
3. 自动洗板机；
4. 去离子水或双蒸水；

### 操作步骤：

1. 通过计算并确定一次性实验所需的板条数，取出所需板条放置在框架内，暂时用不到板条请放回铝箔袋密封，保存于4℃。
2. 建议设置本底校正孔，即空白孔，设置方法为该孔只加TMB显色液和中止液。每次实验均需做标准品对照并画出标准曲线。
3. 分别将标本或不同浓度标准品（100ul/孔）加入相应孔中，用封板胶纸封住反应孔，室温孵育120分钟。对于血清或血浆标本，请加入50 ul样本分析缓冲液后加50 ul标本，如稀释量大，请将样本与样本分析缓冲液等量加入，不足部分用标准品稀释液补充至100ul。
4. 洗板5次，且最后一次置厚吸水纸上拍干。

5. 加入生物素化抗体工作液(100u1/孔)。用封板胶纸封住反应孔，室温孵育60分钟。
6. 洗板5次，且最后一次置厚吸水纸上拍干。
7. 加入酶结合物工作液(100u1/孔)。用封板胶纸封住反应孔，避光室温孵育20分钟。
8. 洗板5次，且最后一次置厚吸水纸上拍干。
9. 加入显色剂TMB100u1/孔，避光室温孵育20分钟。
10. 加入终止液50u1/孔，混匀后即刻测量OD<sub>450</sub>值。

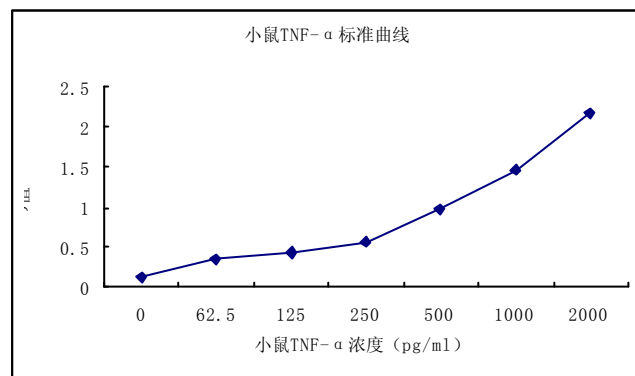
### 结果判断:

1. 复孔的值在20%的差异范围内结果才有效，复孔的值平均后可作为测量值。
2. 每个标准品或标本的OD值应减去本底校正孔的OD值。
3. 手工绘制标准曲线。以标准品浓度作横坐标，OD值作纵坐标，以平滑线连接各标准品的坐标点。通过标本的OD值可在标准曲线上查出其浓度。
4. 若标本 OD 值高于标准曲线上限，应适当稀释后重测，计算浓度时应乘以稀释倍数。

### 典型数值和参考曲线

浓度pg/ml	典型OD值1	典型OD值2	OD平均值
0	0.1123	0.1279	0.1201
62.5	0.3321	0.3764	0.35425
125	0.4143	0.4545	0.4344
250	0.5423	0.5761	0.5592
500	0.9524	0.9803	0.96635
1000	1.4201	1.5021	1.4611
2000	2.1121	2.2231	2.1676

### 小鼠TNF-α 参考标准曲线



**注意：本图仅供参考，应以同次试验标准品所绘标准曲线计算标本含量。**

### 灵敏度，特异性和重复性：

1. 灵敏度：多次重复结果表明，最小检出量为4.9pg/ml。
2. 特异性：与人TNF-α、sTNF RI s、TNF RII等没有交叉反应。
3. 重复性：板内，板间变异系数均<10%。

### 参考文献：

- Vet Immunol Immunopathol. 1995 Aug;47(3-4):187-201.  
 Immunobiology. 1995 Jul;193(2-4):186-92.  
 Annu Rev Med. 1994;45:491-503.

Int J Cardiol. 1993 Dec 31;42(3):231-8.

J Periodontol. 1993 May;64(5 Suppl):445-9.

## **ELISA Kit for the Quantitative Analysis of Mouse TNF- $\alpha$**

The mouse TNF- $\alpha$  ELISA (enzyme-linked immunosorbent assay) kit is used for detection of mouse TNF- $\alpha$  in cell culture supernatants, human serum and plasma. THE ELISA KIT IS FOR RESEARCH USE ONLY. Please read this instruction manual carefully and check out the material provided before use, and you can contact with our company if any questions. You can enter our website or call us for other aim.

### **Introduction**

Tumor Necrosis Factor  $\alpha$  (TNF- $\alpha$ ) is a polypeptide cytokine produced by monocytes and macrophages. TNF- $\alpha$  plays an important role in inflammation, immune system development, apoptosis, and lipid metabolism. It functions as a diverse modulator of immune response and further acts as a potent pyrogen activating neutrophils, altering the properties of vascular endothelial cells, regulating metabolic activities of other tissues.

TNF- $\alpha$  also inhibits lipoprotein lipase activity resulting in cachexia. Activation of B-cells by the Epstein Barr virus can be inhibited by TNF- $\alpha$ .

TNF- $\alpha$  is also produced by epithelial, endothelial, and tumor cells mediated by the activation of lymphokines and endotoxins on the macrophage. It is reported that gamma interferon can enhance the secretion of TNF- $\alpha$ .

TNF- $\alpha$  may play a significant role in the pathogenesis of inflammatory disease of the joints and other tissues. TNF- $\alpha$  is also involved in a number of pathological conditions including asthma, Crohn's disease, rheumatoid arthritis, neuropathic pain, obesity, type 2 diabetes, septic shock, autoimmunity, and cancer.

### **Principles of the Test**

The kit is a solid sandwich enzyme-linked immunosorbent assay for detection of mouse TNF- $\alpha$ . An anti-mouse TNF- $\alpha$  monoclonal antibody has been absorbed onto the wells of the microtiter strips provided. Samples including specimens or standards were pipetted into wells. The mouse TNF- $\alpha$  in specimens or standards would be captured by the coated antibody and the free others were removed by washing. The mouse TNF- $\alpha$  biotin-conjugated antibody were added and binds to mouse TNF- $\alpha$  captured by the first antibody, which formed a sandwich. Streptavidin-HRP would be added and binds to the biotin conjugated antibody, then free Streptavidin-HRP would be removed during a wash step. After this, substrate solution would be added and catalyzed by the HRP, and a colored product is formed. The intensity of the colored product is used to calculate in proportion to the amount of mouse TNF- $\alpha$  in the original specimen.

### **Materials provided with the kits:**

reagent	96/48 Test Kit
Assay Buffer	5ml/3 ml
TNF- $\alpha$ Antibody-Coated Wells	12 strips/6 strips
Standard Diluent	10ml/5ml
TNF- $\alpha$ Standard	2/1 vial(s)
Detection Antibody	10ml/5ml
Streptavidin-HRP	10ml/5ml
Wash Buffer Concentrate 20 $\times$	30ml
TMB	10ml/5 ml
Stop Solution	5 ml/3 ml
Plate Covers	3/2

## Specimen Collection

1. Collecting specimen as following:

A. The particulate of the cell culture supernatants should be removed before use.

B. Serum was obtained from clot at room temperature.

C. Please collect plasma with EDTA.

D. Assay immediately or store samples at  $-20^{\circ}\text{C}$ . Avoid freeze-thaw cycles.

2. Antiseptic and anticoagulant should not appear in Serum samples.

3. Any particulate should be removed from samples before use.

4. Do not use grossly hemolyzed or lipemic samples.

Note: Strongly recommend that the serum and plasma samples should be diluted as doubling dilution before use.

### Precautions for use:

1. Please store the Kit at  $2\sim 8^{\circ}\text{C}$ .

2. Washing buffer concentrate may have crystals in low temperature, and you can melt it in water-bath before use.

3. Please discard the dissolved standard after 3 days for use.

4. Avoid contact of substrate solution with oxidizing agents and metal.

5. Usage of disposable pipette tips avoid microbial contamination or cross-contamination of reagents or specimens.

6. Do not mix or substitute reagents with those from other lots or other sources.

7. To ensure the adequate mixture of added reagents, please tap gently the plate after the wells were filled with liquid.

8. Incubation temperature should be  $25\sim 28^{\circ}\text{C}$ .

9. Wash step was crucial for whole assay process.

10. Duplicate wells of the same sample were recommended in assay process.

11. Avoid the foam while pour the liquid into wells.

12. For serum or plasma samples, the biotin-conjugated antibody should be incubated for at least 90 minutes.

## Reagent Preparation

1. The reagents should be warmed up to room temperature before use. The remaining reagents must be resealed and put into refrigeration again as soon as possible.

2. Dilute 1ml of wash buffer concentrate into 19ml deionized or distilled water to work.

3. Add 0.5ml standard diluent to bottle and wait 15 minutes for complete dissolution. And in turn add the half concentration diluent by standard diluent.

### Wash step:

Automated microplate washer or operating by pipette: Each well should be poured into 300ul wash buffer and soaked for 15 or 30 seconds, then be aspirated, five times process were repeated. After the last wash, remove remaining wash buffer by aspirating. Invert the plate and blot it against clean paper towels.

## Materials Required But Not Provided

1. pipettes and pipette tips

2. Microwell strip reader capable of reading at 450 nm (540 nm as optional reference wave length)

3. automated microplate washer

4. Glass-distilled or deionized water

## Assay procedure

- 1.The needed strips were putted into the frame, the remains were returned into foil pouch and resealed.
- 2.Blank well were recommended, which only color reagent and stop solution be added. It is suggested that each testing with gradient density of standard for standard curve.
- 3.Add 100ul of standard or sample.Cover with the Plate Covers provided.Incubate for 2 hours at room temperature. If assay the serum sample,you should add 50  $\mu$  l assay buffer with 50  $\mu$  l sample into the wells,if the protein concentration is higher than the range of the Kit, add the same quantity assay buffer with the sample, the deficiency should be complemented with sample diluent to 100  $\mu$  l per well.
- 4.Five times wash process were repeated.
- 5.Add 100ul of detetion antibody. Cover with the Plate Covers provided.Incubate for 1 hour at room temperature.
- 6.Five times wash process were repeated.
- 7.Add 100ul of Streptavidin-HRP. Cover with the Plate Covers provided. Lucifugal incubation for 20 minutes at room temperature.
- 8.Five times wash process were repeated.
- 9.Add 100ul of TMB, Lucifugal incubation for 20 minutes at room temperature.
- 10.Add 50ul of stop solution to each well, determine the optical density of each well within 10 minutes.

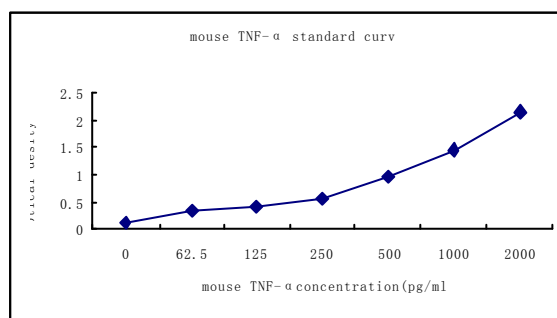
### Calculation of Results

- 1.Duplicates should be within 20 per cent of the mean. Average absorbance values for each set of duplicate samples were used as detection results.
- 2.The blank absorbance values of subtract should be deducted.
- 3.Drawing a best fit curve through the points of graph. Draw the standard curve by plotting assayed OD value (on the Y axis) vs. concentration (on the X axis). The sample concentration was obtained based on its OD value founding in the standard concentration curve.
- 4.If the values obtained are not within the expected range of the standard, Samples should be dilute and assay again.

### Typical Data and Standard Curve

concentration(pg/ml)	Typical data 1	Typical data 2	Average
0	0.1123	0.1279	0.1201
62.5	0.3321	0.3764	0.35425
125	0.4143	0.4545	0.4344
250	0.5423	0.5761	0.5592
500	0.9524	0.9803	0.96635
1000	1.4201	1.5021	1.4611
2000	2.1121	2.2231	2.1676

### Mouse TNF- $\alpha$ standard curve



### Sensitivity, Specificity, Repeatability

**Sensitivity:** repeated assays were evaluated and the minimum detectable dose was 4.9pg/ml.

**Specificity :** No significant cross-reactivity or interference with Human TNF- $\alpha$ , sTNF RI, sTNF RII.

**Repeatability:** The coefficient of variation between wells or plates is less than 10 per cent.

**REFERENCES:**

Vet Immunol Immunopathol. 1995 Aug;47(3-4):187-201.

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