PRODUCT INFORMATION & MANUAL

Mouse TNF-α Valukine™ ELISA

VAL609

For the quantitative determination of natural and recombinant mouse TNF-α concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 3 months from date of receipt

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I. BACKGROUND

Tumor necrosis factor alpha (TNF-α), also known as cachectin and TNFSF1A, is the prototypic ligand of the TNF superfamily (1). It is a pleiotropic molecule that plays a central role in inflammation, immune system development, apoptosis, and lipid metabolism (2-5). TNF-α 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 (5-11).

Mouse TNF-α is synthesized as a 26 kDa type II transmembrane protein that consists of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 179 aa extracellular domain (ECD) (12). Within the ECD mouse TNF-α shares 95% aa sequence identity with rat, and 80% aa identity with canine, equine, feline, human, rabbit, and porcine TNF-α. It is produced by a wide variety of immune, epithelial, endothelial, and tumor cells. TNF-α is assembled intracellularly to form a nonconvalently linked homotrimer which is expressed on the cell surface (13). Cell surface TNF-α can both induce the lysis of tumor cells and virus infected cells, and generate its own downstream cell signaling following ligation by soluble TNF RI (14, 15). Shedding of membrane bound TNF-α by TACE/ADAM17 releases the bioactive cytokine, a 55 kDa soluble trimer containing the TNF-α extracellular domain (16-18).

TNF-α binds the ubiquitous 55-66 kDa TNF RI (19, 20) and the hematopoietic cell restricted 78-80 kDa TNF RII (21, 22), both of which are also expressed as homotrimers (1, 23). Both type I and type II receptors bind TNF-α with comparable affinity and can promote NFκB activation (25-28). Only TNF RI, however, contains a cytoplasmic death domain which triggers the activation of apoptosis (3, 28). Soluble forms of both types of receptors are released into human serum and urine and can neutralized the biological activity of TNF-α (29-31).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. Monoclonal antibody specific for mouse TNF-α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF-α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse TNF-α is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of mouse TNF-α bound in the initial step. The color development is stopped and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernate and serum.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Diluent 1× and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Two samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision		Inter-assay Precision		
Sample	1	2	1	2	3
n	20	20	20	20	20
Mean (pg/mL)	54.6	116	405	45.0	111
Standard Deviation	1.5	4.5	12.5	4.27	7.3
CV%	2.7	3.9	3.1	9.5	6.6

B. RECOVERY

The recovery of mouse TNF-α spiked to different levels throughout the range of the assay in cell culture media was evaluated. The mouse sample recovery ranged from 88-107% with an average of 94%.

The recovery of mouse TNF-α spiked to different levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 75.3-89.3% with an average of 81.1%

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse TNF-α is typically less than 5.5 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This immunoassay is calibrated against highly purified *E. coli*-expressed recombinant mouse TNF-α produced at R&D Systems®.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of mouse TNF-α and diluted with Diluent 1× to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	97	94-103
1:4	105	99-111
1:8	106	97-114
1:16	107	104-111

F. SAMPLE VALUES

Cell Culture Supernates - Mouse thymoma cells (EL-4; 2×10^5 cells/mL) were cultured for 4 days in DMEM supplemented with 10% fetal calf serum and stimulated with 10 µg/mL LPS and 100 ng/mL recombinant mouse IL-10. An aliquot of the cell culture supernate was removed, assayed for levels of natural mouse TNF-α and measured 1220 pg/ml.

Serum - Four serum samples were evaluated for the presence of TNF-α in this assay. All samples measured ranged from 12.0 to 18.4 pg/mL with an average of 14.4 pg/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant mouse TNF-α. The following factors were prepared at 100 ng/mL and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range rmTNF-α control were assayed for interference. No significant cross-reactivity or interference was observed.

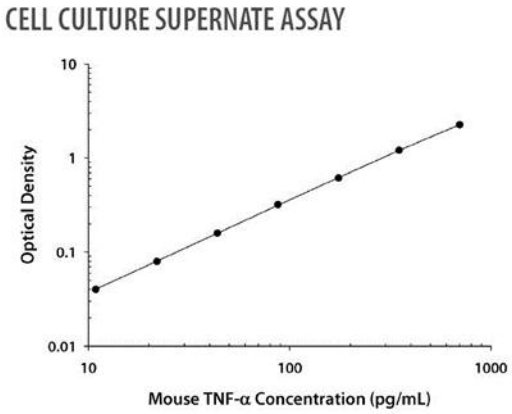
Recombinant Mouse	**Other Recombinant**
CD40	Canine TNF-α
CD40 ligand	Human TNF-α
Fas	Porcine TNF-α
Fas ligand	
LIF	
OPG	
RANK	
RANK ligand	
TRAIL	
TROY	
TNF-β	

40% cross-reactivity was observed with recombinant rat TNF-α. Recombinant mouse TNF RII ad recombinant mouse TNF RII were found to interfere in this assay at concentrations above 1.25 ng/mL and 12.5 ng/mL, respectively.

IV. EXPERIMENT

EXAMPLE STANDARD

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



(pg/mL)	O.D.	Average	Corrected
0	0.020 0.021	0.021	—
10.9	0.060 0.061	0.061	0.040
21.9	0.099 0.101	0.100	0.079
43.8	0.179 0.181	0.180	0.159
87.5	0.336 0.344	0.340	0.319
175	0.632 0.637	0.635	0.614
350	1.203 1.242	1.223	1.202
700	2.265 2.281	2.273	2.252

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse TNF-α Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody against mouse TNF-α	1 plate
Mouse TNF-α Conjugate	Solution of a polyclonal antibody against mouse TNF-α conjugated to horseradish peroxidase	1 vial
Mouse TNF-α Standard	Recombinant mouse TNF-α in a buffered protein base; lyophilized	2 vials
Calibrator Diluent (5×)	A 5× concentrated buffered protein base	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
Color Reagent A	Stabilized hydrogen peroxide	1 vial
Color Reagent B	Stabilized chromogen (tetramethylbenzidine)	1 vial
Stop Solution	Diluted hydrochloric acid solution	1 vial
Plate Covers	Adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8 °C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Diluted Wash Solution</td><td rowspan="6">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Diluent 1×</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Standard</td><td>Use fresh standard for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the oil ouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*</td></tr>
</table>

* Provided this is within the expiration date of the kit.

C. OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.

D. PRECAUTION

- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

B. SAMPLE PREPARATION

Serum samples require a 2-fold dilution. A suggested 5-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent (1×).

C. REAGENT PREPARATION

Note: *Bring all reagents to room temperature before use.*

Wash Solution - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer.

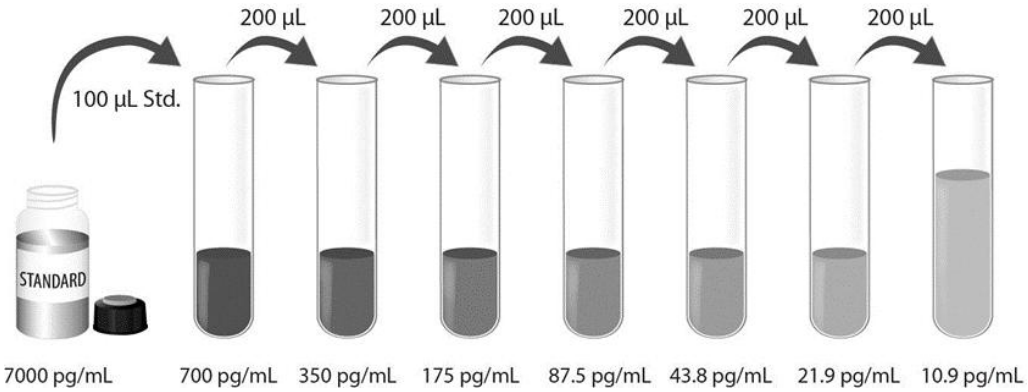
Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Diluent 1× - Add 20 mL of Calibrator Diluent 5× into 80 mL of deionized or distilled water to prepare 100 mL of Diluent 1×.

Mouse TNF-α Standard – Refer to the vial label for the reconstitution volume.* This reconstitution produces a stock solution of 7000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions**.**

*if you have any question, please contact our Technical Support.

Pipette 900 µL of Diluent 1× into the 700 pg/mL tube. Pipette 200 µL of Diluent 1× into each remaining tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The standard 700 pg/mL serves as the high standard. The Diluent 1× serves as the zero standard (0 pg/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

VII.ASSAY PROCEDURE

Note: *Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.*

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Diluent 1× to each well.
4. Add 50 µL of Standard, sample, or control per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Solution (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of mouse TNF-conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light**.
9. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
11. **CALCULATION OF RESULTS.** Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the TNF-α concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

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2. Salek-Ardakani, S. and M. Croft (2010) J. Interferon Cytokine Res. 30:205.
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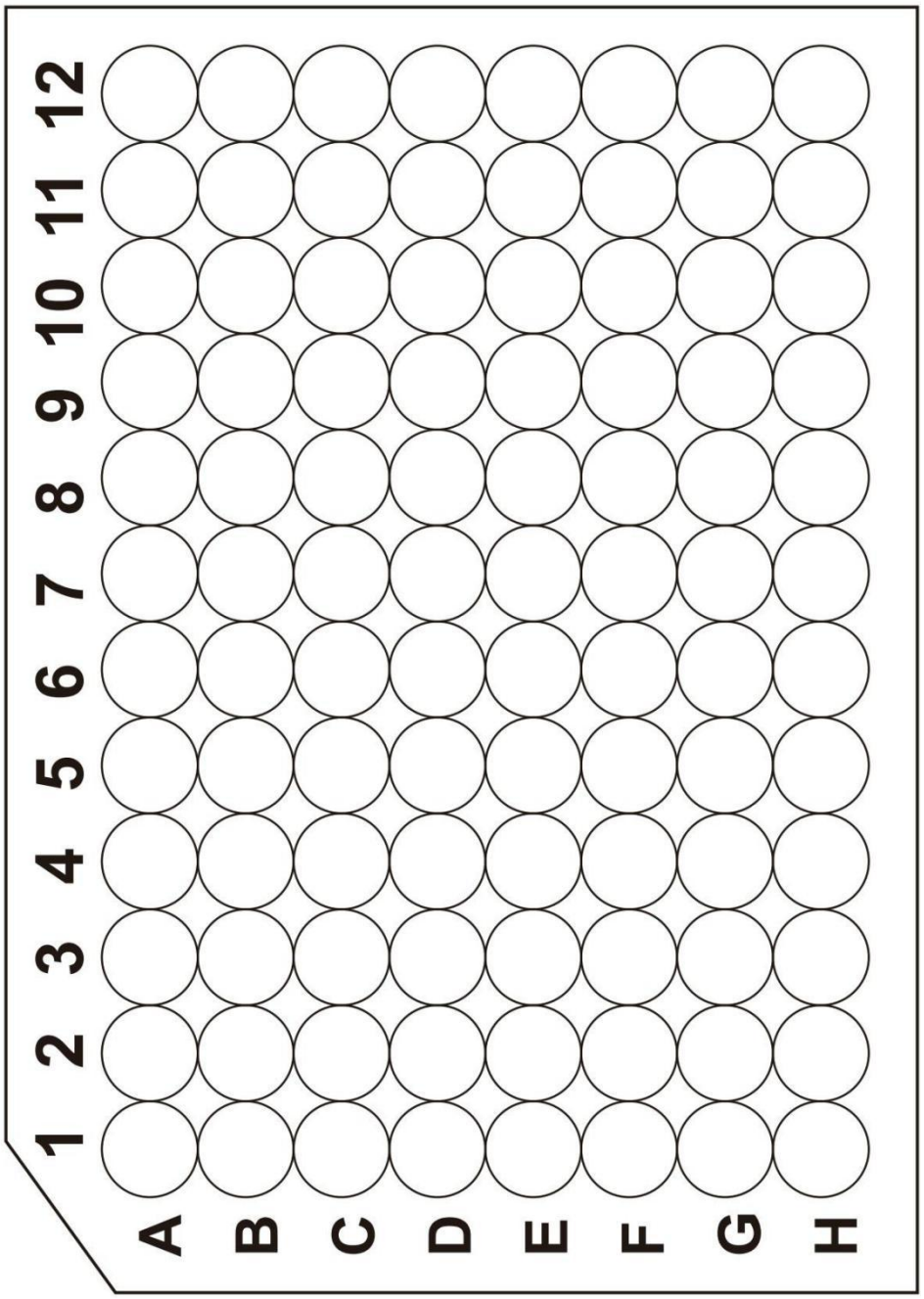
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠TNF-α Valukine™ ELISA 试剂盒

目录号：VAL609

适用于定量检测天然和重组小鼠TNF-α的含量

科研专用，不可用于临床诊断

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Novus 试剂盒确保在你收货日期 3 个月内有效

目录

I. 背景

肿瘤坏死因子alpha (TNF-α，又称为cachectin和 TNFSF1A)，是肿瘤坏死因子超家族的原型配体（1）。它是一个多效因子，在炎症反应、免疫系统发育、细胞凋亡和类脂物代谢中起到的一个中心作用（2-5）。TNF-α也参与到许多病理过程，包括哮喘、克罗恩病、类风湿关节炎、神经性疼痛、肥胖症、II 型糖尿病、感染性休克、自身免疫和癌症（5-11）。

小鼠 TNF-α是一个 26 kDa 的 II 型跨膜蛋白，由一个 35 个氨基酸（aa）胞内域、一个21 aa 跨膜段和一个 179 aa 的胞外域（ECD）组成（12）。在ECD 区，小鼠TNF-α 和大鼠有 95%氨基酸序列同源性，与犬、马、猫，人、兔和猪有 80%的氨基酸序列同源性。它可以有多种不同细胞，如免疫细胞、上皮细胞、内皮细胞、肿瘤细胞表达产生。TNF-α在细胞内装配组成非共价键相连的同源三聚体，然后在细胞表面表达（13）。细胞表面的TNF-α能诱导肿瘤细胞和病毒感染细胞的裂解，并与可溶性TNF RI 结合，产生下游细胞的跨膜转运信号（14, 15）。TACE/ADMA17 可引起含有TNF-α细胞膜的脱落，从而释放具有活性的TNF-α细胞因子，它由TNF-α胞外可溶性结构构成的三聚体， 分子量为 55 kDa (16-18)。

TNF-α有两个受体：TNF RI 和 TNF RII。 TNF RI 分子量为 55-60 kDa，表达广泛(19, 20)；TNF RII 分子量为 78-80 kDa，仅限于造血细胞表达(21, 22)。两者都是以同源三聚体形式表达（1, 23）；TNF-α与 TNF RI 和 TNF RII 结合的亲和力相似，可促进NKκβ的激活（25-28）。然而，仅有 TNF RI 具有胞内死亡结构域，能引发细胞凋亡（3, 28）。这两种可溶性受体都被释放到人的血清和尿液中，并能中和TNF-α的活性（30-31）。

II. 概述

A. 检测原理

本实验采用双抗体夹心 ELISA 法。抗小鼠TNF-α单抗包被于微孔板上，样品和标准品中的TNF-α会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗小鼠TNF-α多抗，与结合在微孔板上的 TNF-α结合而形成免疫复合物， 游离的成分被洗去；加入底物溶液（显色剂），溶液颜色逐渐变成蓝色，加入终止液溶液变黄并且停止变化。用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清样本和小鼠血清；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用稀释剂（1×）稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的储存等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测 20 次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板间分别检测 10 次，以确定板间精确度。

	板内精确度		板间精确度		
样本	1	2	1	2	3
平均值(pg/mL)	54.6	116	405	45	111
标准差	1.5	4.5	12.5	4.27	7.3
CV%	2.7	3.9	3.1	9.5	6.6

B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的小鼠TNF-α，测定其回收率。小鼠样本回收率范围 在88-107%，平均回收率在 94%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠TNF-α，测定其回收率。回收率范围在75.3-89.3%，平均回收率在81.1%。

C. 灵敏度

小鼠TNF-α的最低可测值一般小于 5.5 pg/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

此 ELISA 试剂盒经R&D System®生产的大肠杆菌表达的高纯度重组小鼠TNF-α蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠 TNF-α，然后用稀释剂（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数	平均值/期待值（%）	范围 (%)
1:2	97	94-103
1:4	105	99-111
1:8	106	97-114
1:16	107	104-111

F. 样本预值

细胞培养上清液 - 取小鼠胸腺瘤细胞（EL-4, 2×10^5 细胞/mL）培养于含有 10%胎牛血清的 DMEM 培养基中，另加 10 µg/mL LPS 和 100 ng/mL 重组小鼠IL-10 刺激细胞，培养 4 天。取细胞培养上清液测定天然小鼠TNF-α含量，结果为 1220 pg/mL。

血清样本 - 使用本试剂盒检测了4份小鼠血清样本中TNF-α的水平。4份样本的检测值在12.0-18.4 pg/mL之间，平均值为14.4 pg/mL。

G. 特异性

此ELISA 法可检测天然及重组小鼠TNF-α蛋白。将以下因子用稀释剂（1×）配置成 50ng/mL 的浓度来检测与小鼠 TNF-α的交叉反应。将 50 ng/mL 的干扰因子掺入中间范围的重组小鼠 TNF-α标准中，使其浓度到达 50 ng/mL，以此来检测对小鼠 TNF-α的干扰。没有观察到明显的交叉反应或干扰。

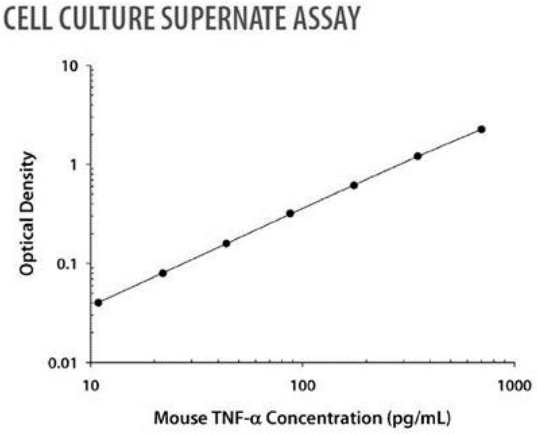
重组小鼠蛋白	其他重组蛋白
CD40	犬 TNF-α
CD40 ligand	人 TNF-α
Fas	猪 TNF-α
Fas ligand	
LIF	
OPG	
RANK	
RANK ligand	
TRAIL	
TROY	
TNF-β	

与重组大鼠TNF-α有40%的交叉反应。重组小鼠 TNF RI 和TNF RII 在浓度分别大于 1.25ng/mL 和 12.5 ng/mL 时，会干扰TNF-α的检测。

IV. 实验标准

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



(pg/mL)	O.D.	Average	Corrected
0	0.020 0.021	0.021	—
10.9	0.060 0.061	0.061	0.040
21.9	0.099 0.101	0.100	0.079
43.8	0.179 0.181	0.180	0.159
87.5	0.336 0.344	0.340	0.319
175	0.632 0.637	0.635	0.614
350	1.203 1.242	1.223	1.202
700	2.265 2.281	2.273	2.252

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
TNF-α Microplate	包被抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
TNF-α Conjugate	酶标检测TNF-α抗体	1 瓶
TNF-α Standard	标准品（冻干）	2 瓶
Calibrator Diluent （5×）	浓缩稀释剂（5×）	1 瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25×）	1 瓶
Color Reagent A	显色液A	1 瓶
Color Reagent B	显色液B	1 瓶
Stop Solution	终止液	1 瓶
Plate Covers	封板胶纸	3 张

* 本试剂盒包含足够的试剂以用于一块 96 孔微孔板的ELISA 实验。

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>洗涤缓冲液（1×）</td><td rowspan="6">2-8℃储存，30 天*。</td></tr>
<tr><td>终止液</td></tr>
<tr><td>稀释剂 1×</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>显色剂 A</td></tr>
<tr><td>显色剂 B</td></tr>
<tr><td>标准品</td><td>每次使用前新鲜配制；即用即弃。</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8℃储存，30 天*。</td></tr>
</table>

*必须在试剂盒有效期内。

C. 实验所需自备试验器材

- 酶标仪（可测量450 nm 检测波长的吸收值及540 nm 或570 nm 校正波长的吸收值）；
- 高精度加液器及一次性吸头；
- 蒸馏水或去离子水；
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机；
- 500 mL 量筒；

D. 注意事项

- 试剂盒中的终止液是酸性溶液，使用时请做好眼镜、手、面部及衣服的防护。

VI. 实验前准备

A. 样品收集及储存

细胞培养上清液：颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于-20℃冰箱内，避免反复冻融。样本可能需要用稀释剂（1×） 稀释。

血清样本: 用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后1000 x g离心15分钟。吸取血清样本之后即刻用于检测，或者分装，-20℃贮存备用。避免反复冻融。

B. 样本准备工作

血清样本需要用稀释剂（1×）2倍稀释后进行检测，即100 µL血清+100 µL稀释剂（1×）。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

洗涤液：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀， 待结晶完全溶解后再配制洗涤液。可将 20 mL 浓缩洗涤液用蒸馏水或去离子水稀释配置成500 mL 工作浓度的洗涤液。未用完的放回 4℃。

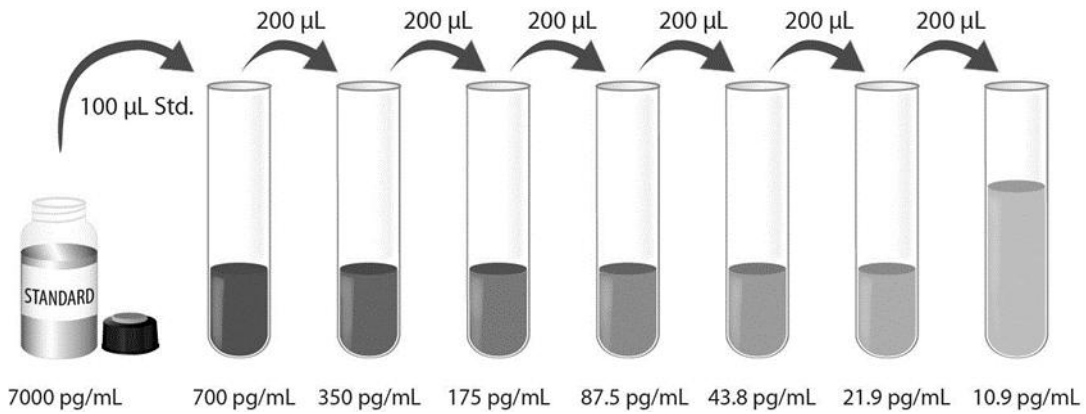
显色剂：按当次试验所需要用量将显色剂 A和显色剂B等体积混合，避光；在使用前15分钟内准备，仅供当日使用；每孔需 100 µL。

稀释剂（1×）：可将 20mL 浓缩稀释剂用 80mL 蒸馏水或去离子水稀释配置成 100 mL工作浓度的稀释剂。

标准品：参照标准品瓶身注明的方式重溶冻干标准品。得到浓度为 7000 pg/mL 标准品母液。轻轻震摇至少 5 分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

在 700 pg/mL 的稀释管中加入 900 µL 稀释剂（1×），其余每个稀释管中加入 200 µL 稀释剂（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。没有稀释的标准品母液可用作标准曲线最高点（700 pg/mL），稀释剂（1×）可用作标准曲线零点（0 pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配置不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15 分钟内完成一块板的上样；
- 每次孵育时，正确使用封板胶纸可保证结果的准确性；
- 混合后的显色底物在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同显色底物上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀请充分混合。

VII.操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 在每个微孔中加入 50 µL 稀释剂（1×）；
4. 分别将不同浓度标准品，实验样本或者质控品加入相应孔中，每孔 50 µL。用封板胶纸 封住反应孔，室温孵育 2 小时。说明书提供了一张 96 孔模板图，可用于记录标准品和试验样本的板内位置；
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 µL， 然后将板内洗涤液吸去。重复操作 4 次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
6. 在每个微孔内加入 100 µL 酶标检测抗体。用封板胶纸封住反应孔，室温孵育 2 小时；
7. 重复第 5 步洗板操作；
8. 在每个微孔内加入 100 µL 显色底物，室温孵育 30 分钟。**注意避光**；
9. 在每个微孔内加入 100 µL 终止液，孔内溶液颜色会从蓝色变为黄色。如果溶液颜色变为绿色或者颜色变化不一致，请轻拍微孔板，使溶液混合均匀；
10. 加入终止液后 30 分钟内，使用酶标仪测量 450 nm 的吸光度值，设定 540 nm 或570 nm 作为校正波长。如果没有使用双波长校正，结果准确度可能会受影响；
11. **计算结果：**将每个标准品和样品的校正吸光度值(OD450-OD540/OD570)、复孔读数取平均值，然后减去平均零标准品 OD 值。使用计算机软件作四参数逻辑(4-PL)曲线拟合创建标准曲线。另一种方法是，可以通过绘制标准品浓度做对数与相应 OD 值对数生成曲线，并通过回归分析确定最佳拟合线。这个过程可生成一个足够使用但不太精确的数据拟合。若样本经过稀释，计算浓度时应乘以稀释倍数。

VIII. 参考文献

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96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置