PRODUCT INFORMATION & MANUAL

Rat TNF-α Valukine™ ELISA

VAL902

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

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

Bio-Techne China Co. Ltd
P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001
info.cn@bio-techne.com

Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version202209.2

TABLE OF CONTENTS

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).

Rat 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 156 aa extracellular domain (ECD) (12). Within the ECD, rat TNF-α shares 95% aa sequence identity with mouse, and 73% - 79% aa identity with bovine, canine, cotton rat, equine, feline, human, rhesus macaque, 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 noncovalently 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 of the TNF-α extracellular domain(16-18).

TNF-α binds the ubiquitous 55 - 60 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 (24-27). 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 neutralize the biological activity of TNF-α (29-31).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for rat 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, a biotinylated detection antibody specific for rat TNF-α are pipetted into the wells. After washing away any unbound substances, streptavidin-HRP are pipetted into the wells. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of 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 rat 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 Calibrator Diluent 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)

Three 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	3	1	2	3
Mean (pg/mL)	143.6	583.7	2993.4	148.5	597.7	3012.5
Standard Deviation	6.9	34.5	166.5	11.7	43.2	179.9
CV%	4.8%	5.9%	5.6%	7.9%	7.2%	6.0%

B. RECOVERY

The recovery of rat TNF-α spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 106.5-118.1% with an average of 111.6%.

The recovery of rat TNF-α spiked to different levels throughout the range of the assay in rat serum was evaluated. The recovery ranged from 97.4-110.8% with an average of 108.3%.

C. SENSITIVITY

The minimum detectable dose (MDD) of rat TNF-α is typically less than 3.165 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 a highly purified *E. Coli*-expressed recombinant rat 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 rat TNF-α and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	99.2	90.0-110.8
1:4	106.3	91.8-117.0
1:8	106.2	95.8-116.7
1:16	105.0	95.5-112.1

F. SAMPLE VALUES

Serum - Four rat serum samples were evaluated for the presence of rat TNF-α in this assay. All samples measured less than the lowest rat TNF-α standard, 93.8 pg/mL.

G. SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

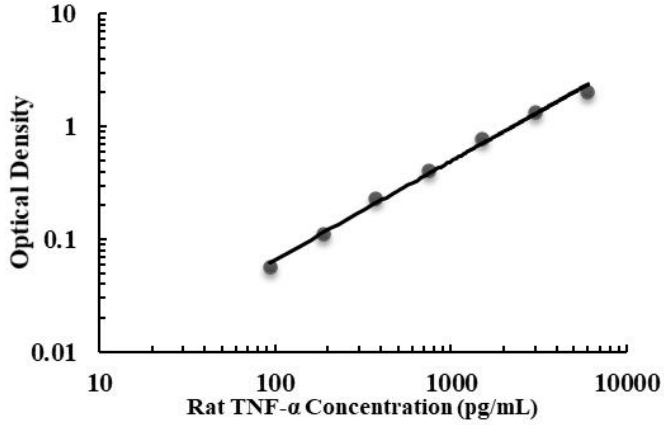
Recombinant human	Recombinant porcine
TNF-α	TNF-α
TNF-β	

A sample containing 50 ng/mL of recombinant mouse TNF-α reads as 1200 pg/mL (2.4% cross-reactivity).

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	OD	Average	Corrected
0	0.051 0.051	0.051	-
93.8	0.108 0.106	0.107	0.056
187.5	0.167 0.158	0.163	0.112
375.0	0.286 0.271	0.279	0.228
750	0.488 0.431	0.460	0.409
1500	0.832 0.807	0.820	0.769
3000	1.425 1.384	1.405	1.354
6000	2.091 2.077	2.084	2.033

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Rat TNF-α Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse antibody against rat TNF-α.	1 plate
Rat TNF-α Standard	Recombinant rat TNF-α in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Rat TNF-α Detection Antibody	Biotinylated TNF-α antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent (1×)	Concentrated buffered diluent used to dilute standard and samples.	2 vials
Detection Antibody Diluent (4×)	Concentrated buffered diluent used to dilute Detection Antibody.	1 vial
Streptavidin-HRP B (40×)	40× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent (10×)	A 10× concentrated buffered protein base used to dilute Detection Antibody and HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	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="9">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP B</td><td rowspan="4">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Reagent Diluent (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Calibrator Diluent (1×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody Diluent (4×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Detection Antibody Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch 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.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500±50 rpm.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

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. Samples may require dilution with Calibrator Diluent (1×).

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

B. SAMPLE PREPARATION

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

Serum samples require a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent (1×).

C. REAGENT PREPARATION

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

Wash Buffer - 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.

Detection Antibody Diluent (1×) - Use deionized or distilled water to prepare Detection Antibody Diluent (1×).

Reagent Diluent (1×) - Use deionized or distilled water to prepare Reagent Diluent (1×).

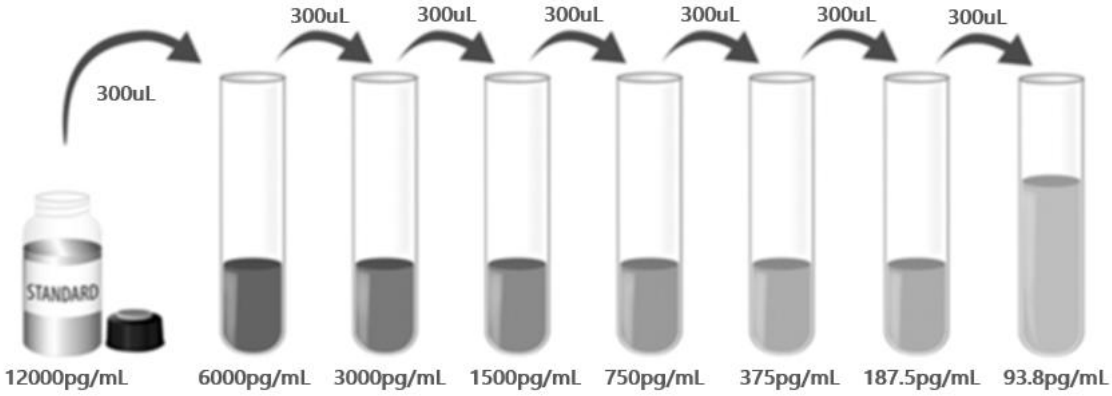
Detection Antibody - Centrifuge briefly before opening. Reconstitution Volume refer to vial label with Reagent Diluent (1×). Aliquot and store if needed. Dilute stock solution in Detection Antibody Diluent (1×) to the working concentration of 50 ng/mL. Prepare at least 15 minutes prior to use.

Streptavidin-HRP B (1×) **- Centrifuge briefly before opening.** Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Rat TNF-α Standard - Centrifuge briefly before opening. Refer to the vial label for the reconstitution volume*. This reconstitution produces a stock solution of 12000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

*if you have any question, please seek help from our Technical Support.

Pipette 300 µL of the appropriate Calibrator Diluent (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 6000 pg/mL standard serves as the high standard. The Calibrator 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.
- TMB Substrate 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 100 µL of standard, or prepared sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500±50rpm. A plate layout is provided for a record of standards and samples assayed. (Samples may require dilution. See Sample Preparation section.)
4. 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.
5. Add 100 µL of the Detection Antibody diluted in Detection Antibody Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature on a horizontal orbital microplate shaker set at 500±50rpm.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP B to each well. Cover the plate and incubate for 30 minutes at room temperature on a horizontal orbital microplate shaker set at 500±50rpm. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate to each well. Incubate for 30 minutes at room temperature on a horizontal orbital microplate shaker set at 500±50rpm. Avoid placing the plate in direct light.
10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 10 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.

12. **CALCULATION OF RESULTS**

    Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). 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.

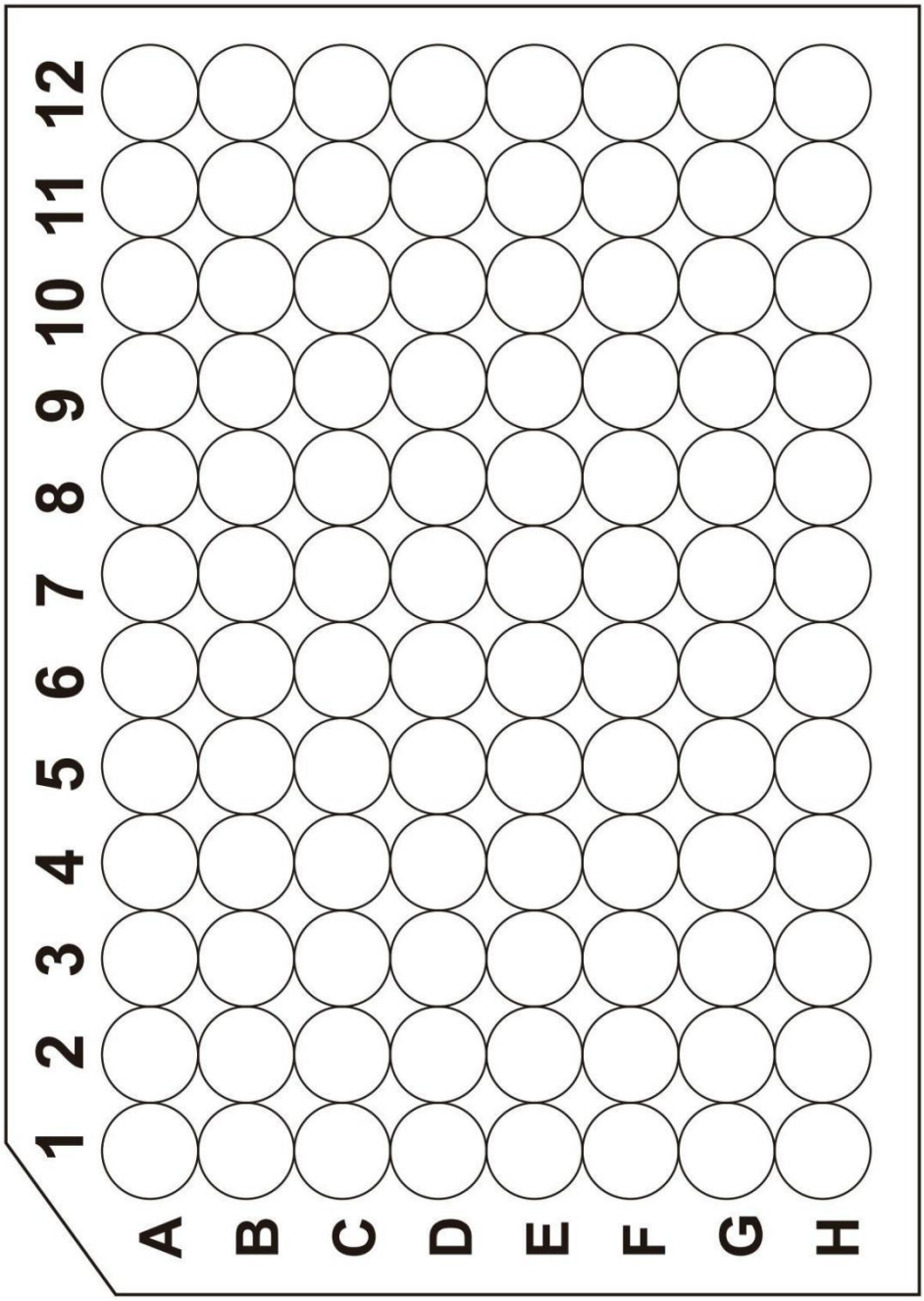
    If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Croft, M. et al. (2012) Trends Immunol. 33:144.
2. Juhasz, K. et al. (2013) Expert Rev. Clin. Immunol. 9:335.
3. Summers, de L.L and J.L. Gommerman (2012) Nat. Rev. Immunol. 12:339.
4. Chen, X. et al. (2009) Cell Biochem. Funct. 27:407.
5. Zelova, H. and J. Hosek (2013) Inflamm. Res. 62:641.
6. Berry, M. et al. (2007) Curr. Opin. Pharmacol. 7:279.
7. D'Haens, G. (2003) Curr. Pharm. Des. 9:289.
8. Feldmann, M. and R.N. Maini (2001) Annu. Rev. Immunol. 19:163.
9. Leung, L. and C.M. Cahill (2010) J. Neuroinflammation 7:27.
10. Tzanavari, T. et al. (2010) Curr. Dir. Autoimmun. 11:145.
11. Wu, Y. and B.P. Zhou (2010) Br. J. Cancer 102:639.
12. Kwon, J. et al. (1993) Gene 132:227.
13. Tang, P. et al. (1996) Biochemistry 35:8216.
14. Perez, C. et al. (1990) Cell 63:251.
15. Watts, A.D. et al. (1999) EMBO J. 18:2119.
16. Black, R.A. et al. (1997) Nature 385:729.
17. Moss, M.L. et al. (1997) Nature 385:733.
18. Gearing, A.J.H. et al. (1994) Nature 370:555.
19. Schall, T.J. et al. (1990) Cell 61:361.
20. Loetscher, H. et al. (1990) Cell 61:351.
21. Dembic, Z. et al. (1990) Cytokine 2:231.
22. Smith, C.A. et al. (1990) Science 248:1019.
23. Loetscher, H. et al. (1991) J. Biol. Chem. 266:18324.
24. Rothe, M. et al. (1995) Science 269:1424.
25. Ruby, J. et al. (1997) J. Exp. Med. 186:1591.
26. Pinckard, J.K. et al. (1997) J. Biol. Chem. 272:10784.
27. Mukhopadhyay, A. et al. (2001) J. Biol. Chem. 276:31906.
28. Hsu, H. et al. (1995) Cell 81:495.
29. Seckinger, P. et al. (1989) J. Biol. Chem. 264:11966.
30. Olsson, I. et al. (1989) Eur. J. Haematol. 42:270.
31. Engelmann, H. et al. (1990) J. Biol. Chem. 265:1531.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

大鼠 TNF-α Valukine™ ELISA 试剂盒

目录号：VAL902

适用于定量检测天然和重组大鼠 TNF-α 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

info.cn@bio-techne.com

有效期详见试剂盒包装标签

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α (TNF-α)，也被称为cachectin和TNFSF1A，是TNF超家族的原型配体(1)。它是一种多效分子，在炎症、免疫系统发育、凋亡和脂质代谢中起核心作用(2-5)。TNF-α也参与许多病理状态，包括哮喘、克罗恩病、类风湿关节炎、神经病理性疼痛、肥胖、2型糖尿病、感染性休克、自身免疫和癌症(5-11)。

大鼠TNF-α生成为26 kDa II型跨膜蛋白，由35个氨基酸(aa)的胞质结构域、21个aa跨膜片段和156个aa胞外结构域(ECD)组成(12)。在ECD中，大鼠TNF-α与小鼠的同源性为95%，与牛、犬、棉鼠、马、猫、人、恒河猴和猪TNF-α的同源性为73%-79%。它是由多种免疫细胞、上皮细胞、内皮细胞和肿瘤细胞产生的。TNF-α在细胞内组装形成非共价连接的同源三聚体，并在细胞表面表达 (13)。细胞表面TNF-α可诱导肿瘤细胞裂解和病毒感染细胞裂解，并通过可溶性TNF- RI连接产生其自身的下游细胞信号(14, 15)。通过TACE/ADAM17脱落膜结合的TNF-α释放生物活性细胞因子，一个55 kDa可溶性的TNF-α胞外域三聚体(16-18)。

TNF-α结合普遍存在的55–60 kDa的TNF RI (19, 20)和造血细胞限制性的78- 80 kDa的TNF RII (21, 22)，两者也以同源三聚体的形式表达(1, 23)。I型和II型受体与TNF-α具有相似的亲和力，并能促进NFκB活化(24-27)。然而，只有TNF RI包含一个细胞质死亡结构域，该结构域可触发凋亡的激活(3, 28)。这两种受体的可溶性形式被释放到人血清和尿液中，并能中和TNF-α的生物活性(29-31)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗大鼠TNF-α抗体包被于微孔板上，样品和标准品中的大鼠TNF-α会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗大鼠TNF-α检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶（streptavidin-HRP）孵育。洗涤去除未结合的试剂后，加入TMB底物溶液。溶液颜色与结合的目标蛋白成正比；加入终止液；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大鼠血清样本；
- 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，应将样本用标准品稀释液（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，在不同板中分别检测20次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	143.6	583.7	2993.4	148.5	597.7	3012.5
标准差	6.9	34.5	166.5	11.7	43.2	179.9
CV%	4.8%	5.9%	5.6%	7.9%	7.2%	6.0%

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大鼠 TNF-α，测定其回收率。回收率范围在 106.5-118.1%，平均回收率在 111.6%。

在大鼠血清样本中掺入检测范围内不同水平的大鼠TNF-α，测定其回收率。回收率范围在97.4-110.8%，平均回收率在108.3%。

C. 灵敏度

大鼠TNF-α的最低可测剂量（MDD）一般小于3.165 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试剂盒经由R&D Systems生产的大肠杆菌表达的高纯度重组大鼠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大鼠TNF-α，然后用标准品稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数	平均值(%)	范围 (%)
1:2	99.2	90.0-110.8
1:4	106.3	91.8-117.0
1:8	106.2	95.8-116.7
1:16	105.0	95.5-112.1

F. 样本预值

血清样本 - 使用本试剂盒检测了4份大鼠血清样本中TNF-α的水平。4份样本的检测值均低于大鼠TNF-α最低标准品93.8 pg/mL。

G. 特异性

将以下因子配置成50ng/mL的浓度来检测没有观察到明显的交叉反应或干扰。

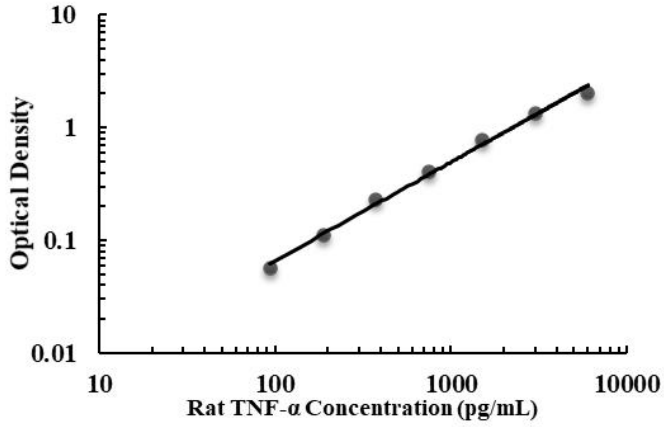
Recombinant human	Recombinant porcine
TNF-α	TNF-α
TNF-β	

含50ng /mL重组小鼠TNF-α的样品检测值为1200 pg/mL (2.4%的交叉反应性)。

IV. 实验

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	OD	Average	Corrected
0	0.051 0.051	0.051	-
93.8	0.108 0.106	0.107	0.056
187.5	0.167 0.158	0.163	0.112
375.0	0.286 0.271	0.279	0.228
750	0.488 0.431	0.460	0.409
1500	0.832 0.807	0.820	0.769
3000	1.425 1.384	1.405	1.354
6000	2.091 2.077	2.084	2.033

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Rat TNF-α Microplate	包被小鼠抗大鼠 TNF-α抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Rat TNF-α Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Rat TNF-α Detection antibody	生物素化的 TNF-α检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Streptavidin-HRP B (40×)	40×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent (10×)	浓缩的试剂稀释液（10×）	1 瓶
Calibrator Diluent (1×)	浓缩的标准品稀释液（1×）	2 瓶
Detection Antibody Diluent (4×)	浓缩的检测抗体稀释液（4×）	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
TMB Substrate	TMB 底物溶液	1 瓶
Stop Solution	终止液	1 瓶
Plate Sealers	封板膜	3 张

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="9">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP B</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</td></tr>
<tr><td>标准品稀释液（1×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×标准品稀释液</td></tr>
<tr><td>检测抗体稀释液（4×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×检测抗体稀释液</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液</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，最多 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450nm检测波长的吸收值及540nm或570nm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
- 500mL量筒
- 振荡器（500±50 rpm）

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。使用后请彻底洗手。

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。样本可能需要用标准品稀释液（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

细胞上清样本需要用标准品稀释液（1×）2倍稀释后进行检测，例如：100μL样本+100μL标准品稀释液（1×）。

血清样本需要用标准品稀释液（1×）4倍稀释后进行检测，例如：50μL样本+150μL标准品稀释液（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20mL浓缩洗涤液用去离子水或蒸馏水稀释配置成 500mL工作浓度的洗涤液。

检测抗体稀释液（1×）：使用去离子水或蒸馏水稀释配置成检测抗体稀释液（1×）。

试剂稀释液（1×）：使用去离子水或蒸馏水稀释配置成试剂稀释液（1×）。

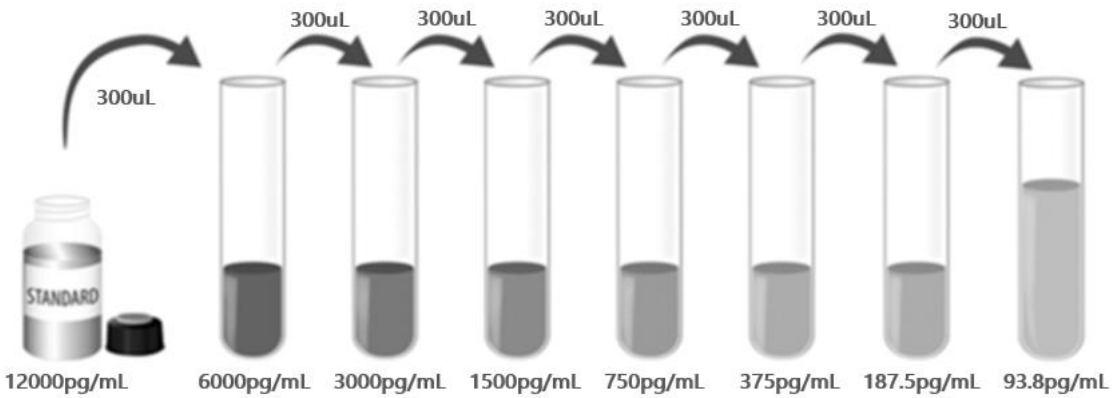
检测抗体：开盖前请瞬时离心。参考检测抗体瓶标签指示，用试剂稀释液（1×）将冻干粉进行重溶。再用检测抗体稀释液（1×）稀释至工作浓度50ng/mL，至少在使用前15分钟准备。

链霉亲和素- HRP B：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP B（40×）稀释至工作浓度链霉亲和素- HRP B（1×）。

TNF-α标准品：开盖前请瞬时离心。冻干标准品的重溶体积请参考瓶身标签，得到浓度为12000pg/mL标准品母液。轻轻震摇至少15分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

向各稀释管中加入300μL标准品稀释液（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。6000 pg/mL管作标准曲线最高点，标准品稀释液（1×）可用作标准品零点（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；
- TMB底物溶液在上板前应为无色，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TMB底物溶液上板顺序一致；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. 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100μL。用封板膜封住反应孔，室温500±50rpm水平振荡孵育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；（样本需要稀释，详情参见样本制备部分。）
4. 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400μL，然后将板内洗涤液吸去。重复操作3次，共洗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；
5. 在每个微孔内加入100μL配置好的检测抗体。用封板胶纸封住反应孔，室温500±50rpm水平振荡孵育2小时；
6. 重复第4步洗板操作；
7. 在每个微孔内加入100μL稀释好的链霉亲和素- HRP B工作液。用封板膜封住反应孔，室温500±50rpm水平振荡孵育30分钟，**注意避光；**
8. 重复第4步洗板操作；
9. 在每个微孔内加入100μL TMB底物溶液，室温500±50rpm水平振荡孵育30分钟，**注意避光；**
10. 在每个微孔内加入50μL终止液，请轻拍微孔板，使溶液混合均匀。孔内溶液颜色会从蓝色变为黄色；
11. 加入终止液后10分钟内，使用酶标仪测量450nm的吸光度值，设定540nm或570nm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；
12. **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。通过样本的OD值，可从标准曲线上得到样本中大鼠TNF-α的浓度。如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

1. Croft, M. et al. (2012) Trends Immunol. 33:144.
2. Juhasz, K. et al. (2013) Expert Rev. Clin. Immunol. 9:335.
3. Summers, de L.L and J.L. Gommerman (2012) Nat. Rev. Immunol. 12:339.
4. Chen, X. et al. (2009) Cell Biochem. Funct. 27:407.
5. Zelova, H. and J. Hosek (2013) Inflamm. Res. 62:641.
6. Berry, M. et al. (2007) Curr. Opin. Pharmacol. 7:279.
7. D'Haens, G. (2003) Curr. Pharm. Des. 9:289.
8. Feldmann, M. and R.N. Maini (2001) Annu. Rev. Immunol. 19:163.
9. Leung, L. and C.M. Cahill (2010) J. Neuroinflammation 7:27.
10. Tzanavari, T. et al. (2010) Curr. Dir. Autoimmun. 11:145.
11. Wu, Y. and B.P. Zhou (2010) Br. J. Cancer 102:639.
12. Kwon, J. et al. (1993) Gene 132:227.
13. Tang, P. et al. (1996) Biochemistry 35:8216.
14. Perez, C. et al. (1990) Cell 63:251.
15. Watts, A.D. et al. (1999) EMBO J. 18:2119.
16. Black, R.A. et al. (1997) Nature 385:729.
17. Moss, M.L. et al. (1997) Nature 385:733.
18. Gearing, A.J.H. et al. (1994) Nature 370:555.
19. Schall, T.J. et al. (1990) Cell 61:361.
20. Loetscher, H. et al. (1990) Cell 61:351.
21. Dembic, Z. et al. (1990) Cytokine 2:231.
22. Smith, C.A. et al. (1990) Science 248:1019.
23. Loetscher, H. et al. (1991) J. Biol. Chem. 266:18324.
24. Rothe, M. et al. (1995) Science 269:1424.
25. Ruby, J. et al. (1997) J. Exp. Med. 186:1591.
26. Pinckard, J.K. et al. (1997) J. Biol. Chem. 272:10784.
27. Mukhopadhyay, A. et al. (2001) J. Biol. Chem. 276:31906.
28. Hsu, H. et al. (1995) Cell 81:495.
29. Seckinger, P. et al. (1989) J. Biol. Chem. 264:11966.
30. Olsson, I. et al. (1989) Eur. J. Haematol. 42:270.
31. Engelmann, H. et al. (1990) J. Biol. Chem. 265:1531.

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