



PRODUCT INFORMATION & MANUAL

Human TNF- α Valukine™ ELISA

VAL105G

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

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

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

Version202210.3

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I. BACKGROUND

Tumor necrosis factor alpha (TNF- α), also known as cachectin, 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).

Human 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 177 aa extracellular domain (ECD) (12, 13). Within the ECD human TNF- α shares 97% aa sequence identity with rhesus monkey, and 71% - 92% aa identity with bovine, canine, cotton rat, equine, feline, mouse, porcine, and rat 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 (14). 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 (15, 16). Shedding of membrane bound TNF- α by TACE/ADAM17 releases the bioactive cytokine, a 55 kDa soluble trimer of the TNF- α extracellular domain (17-19).

TNF- α binds the ubiquitous 55-66 kDa TNF RI (20, 21) and the hematopoietic cell-restricted 78-80 kDa TNF RII (22, 23), both of which are also expressed as homotrimers (1, 24). Both type I and type II receptors bind TNF- α with comparable affinity and can promote NF- κ B activation (25-28). Only TNF RI, however, contains a cytoplasmic death domain which triggers the activation of apoptosis (3, 29). Soluble forms of both types of receptors are released into human serum and urine and can neutralize the biological activity of TNF- α (30-32).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human TNF- α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and an TNF- α present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human 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, TMB 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, human serum and plasma.
- ◆ 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 Reagent Diluent (1x) 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)	384.0	93.3	24.9	383.3	93.1	24.9
Standard Deviation	9.8	1.6	1.1	9.7	2.0	1.2
CV%	2.6	1.7	4.4	2.5	2.1	4.7

B. RECOVERY

The recovery of human TNF- α spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range %
Cell culture media (n=3)	92.7	85.7-94.3
Serum (n=4)	95.0	86.3-108.6
Plasma (n=3)	86.8	70.5-101.9

C. SENSITIVITY

The minimum detectable dose (MDD) of human TNF- α is 0.67 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 human TNF- α .

The NIBSC/WHO 3rd International Standard for TNF- α (12/154), which was intended as a potency standard, was evaluated in this kit. The NIBSC/WHO standard is a *E. coli*-derived recombinant human TNF- α . The dose response curve of the International Standard (12/154) parallels the Valukine standard curve. To convert sample values obtained with the Valukine Human TNF- α kit to approximate NIBSC 12/154 units, use the equation below.

NIBSC (12/154) approximate value (IU/mL) = 0.058 × Valukine Human TNF- α value (pg/mL)

E. LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human TNF- α in various matrices and diluted with Reagent Diluent (1x) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=3)	Serum (n=4)	Plasma (n=3)
1:2	Average % of Expected	93.2	94.1	100.2
	Range (%)	87.7-97.5	85.7-100.4	94.0-103.5
1:4	Average % of Expected	96.6	94.8	95.9
	Range (%)	87.5-103.5	82.7-100.5	91.6-103.5
1:8	Average % of Expected	95.8	94.5	99.1
	Range (%)	87.1-101.1	84.2-103.9	94.2-103.8
1:16	Average % of Expected	100.3	91.7	96.8
	Range (%)	82.6-109.6	85.0-109.6	92.6-99.5

F. SAMPLE VALUES

Cell Culture Supernates - Human peripheral blood mononuclear cells (PBMCs: 1×10^6 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were stimulated with 20 μ g/mL of PHA for 5 days. Aliquots of the culture supernates were removed, assayed for levels of human TNF- α , and measured 382.5 pg/mL.

Serum - Four human serum samples were evaluated for the presence of human TNF- α in this assay. All samples measured below the lowest standard, 11.7 pg/mL.

Plasma - Three human plasma samples were evaluated for the presence of human TNF- α in this assay. All samples measured ranged from 21.4 to 58.8 pg/mL with an average of 38.4 pg/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant human TNF- α .

The following factors were prepared at 50 ng/mL and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhTNF- α control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human	Other Recombinant
CD40 Ligand	mouse TNF- α
TL1A	porcine TNF- α
TNF- β	rat TNF- α
	bovine TNF- α

Recombinant human Pro-TNF- α does not interfere but cross-reacts approximately 0.4% in this assay.

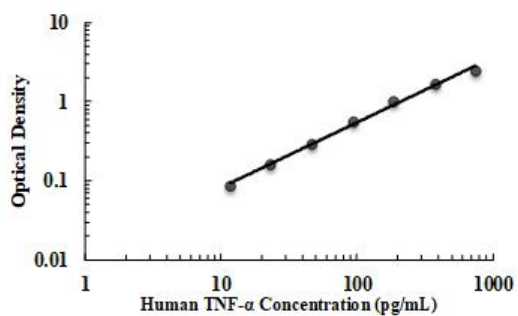
Recombinant human TNF RI does not cross-react but interferes at concentrations > 3 ng/mL in this assay.

Recombinant human TNF RII does not cross-react but interferes at concentrations > 25 ng/mL in this assay.

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	0.061	0.061	-
	0.061		
11.7	0.128	0.132	0.088
	0.136		
23.4	0.206	0.204	0.160
	0.202		
46.9	0.339	0.339	0.295
	0.339		
93.8	0.610	0.609	0.565
	0.608		
188	1.040	1.055	1.011
	1.069		
375	1.694	1.716	1.672
	1.738		
750	2.518	2.523	2.479
	2.527		

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human TNF- α Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human TNF- α .	1 plate
Human TNF- α Detection Antibody	Biotinylated human TNF- α antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Human TNF- α Standard	Recombinant human TNF- α in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Reagent Diluent Concentrate (10 \times)	A 10 \times concentrated buffered protein base used to dilute standard, samples and HRP.	1 vial
Detection Antibody Diluent Concentrate (10 \times)	Concentrated buffered diluent used to dilute Detection Antibody.	1 vial
Streptavidin-HRP A (200 \times)	200 \times Streptavidin conjugated to horseradish peroxidase.	1 vial
Wash Buffer Concentrate (25 \times)	A 25 \times 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 Covers	Adhesive strip.	3 strips

B. STORAGE

Unopened Kit	Store at 2-8 °C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Streptavidin-HRP A	May be stored for up to 1 month at 2-8 °C.*
	Diluted Wash Solution	
	TMB Substrate	
	Stop Solution	
	Standard	Prepare fresh for each assay.* Standard may be stored for up to 1 month at -20 °C.*
	Detection Antibody	Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *
	Reagent Diluent Concentrate (10×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.
	Detection Antibody Diluent Concentrate (10×)	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.
Microplate Wells	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.*	

* 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

- ◆ 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 Supernate - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples may require dilution with Reagent Diluent (1 \times).

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 $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples may require dilution with Reagent Diluent (1 \times).

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples may require dilution with Reagent Diluent (1 \times).

B. SAMPLE PREPARATION

Cell Culture Supernate, serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 100 μL of sample + 100 μL of Reagent Diluent (1 \times).

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 \times) into deionized or distilled water to prepare 500 mL of Wash Buffer.

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

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

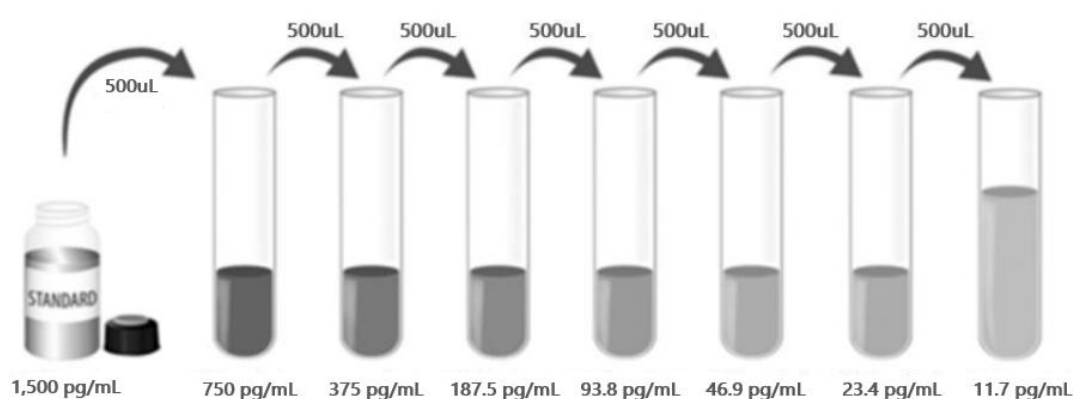
Detection Antibody - **Centrifuge briefly before opening. Reconstitution volume refer to vial label with Detection Antibody Diluent (1 \times).** Aliquot and store if needed. Dilute stock solution in Detection Antibody Diluent (1 \times) to the working concentration of 0.25 $\mu\text{g}/\text{mL}$. Prepare at least 15 minutes prior to use.

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

TNF- α Standard - Centrifuge briefly before opening. Refer to the vial label for reconstitution volume*. This reconstitution produces a stock solution of 1500 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 500 μ L of Reagent Diluent (1 \times) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 750 pg/mL standard serves as the high standard. The Reagent Diluent (1 \times) 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 TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB Substrate. 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 TMB Substrate.

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 μ L of standard, or prepared sample 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. (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 μ L) 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 μ L of the Detection Antibody diluted in Detection Antibody Diluent (1x), to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μ L of the working dilution of Streptavidin-HRP A to each well. Cover the plate and incubate for 30 minutes at room temperature. **Avoid placing the plate in direct light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μ L of TMB Substrate to each well. Incubate for 20 minutes at room temperature. **Avoid placing the plate in direct light.**
10. Add 50 μ L 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.
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. 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.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

1									
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	A	B	C	D	E	F	G	H	



产品信息及操作手册

人 TNF- α Valukine™ ELISA 试剂盒

目录号: VAL105G

适用于定量检测天然和重组人肿瘤坏死因子- α (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

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I. 背景

肿瘤坏死因子 α (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、细胞凋亡和类脂代谢中起中心作用(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)。

人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177 aa的胞外域(ECD)组成(12, 13)。在ECD区，人TNF- α 和猕猴有97%氨基酸序列同源性，与牛、犬、棉鼠、马、猫、小鼠、猪、大鼠有71-92%的氨基酸序列同源性。它可以由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 在细胞内组装并在细胞表面表达形成非共价连接的同源三聚体(14)。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到信号(15, 16)。TACE/ADAM17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，它是由TNF- α 胞外可溶性结构构成的三聚体，分子量为55 kDa(17-19)。

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(20, 21)；TNF RII分子量为78-80 kDa，仅限于造血细胞表达(22, 23)。两者都是以同源三聚体形式表达(1, 24)；TNF- α 和TNF RI(20, 21)和TNF RII结合的亲和力相似，可促进NF- κ B的激活(25-28)。然而，仅有TNF RI具有细胞死亡结构域，能引发细胞凋亡(3, 29)。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(30-32)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人TNF- α 抗体包被于微孔板上，样品和标准品中的人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人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（显色剂）。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、人血清和人血浆样本；
- ◆ 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，应将样本用试剂稀释液（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，在不同板间分别检测20次，以确定板间精确度。

样本	板内精确度			板间精确度		
	1	2	3	1	2	3
平均值 (pg/mL)	384.0	93.3	24.9	383.3	93.1	24.9
标准差	9.8	1.6	1.1	9.7	2.0	1.2
CV%	2.6	1.7	4.4	2.5	2.1	4.7

B. 回收率

在不同类型样本中掺入检测范围内不同水平的人TNF- α ，测定其回收率。

样本类型	平均回收率%	范围%
细胞培养上清 (n=3)	92.7	85.7-94.3
血清 (n=4)	95.0	86.3-108.6
血浆 (n=3)	86.8	70.5-101.9

C. 灵敏度

人TNF- α 的最低可测剂量（MDD）一般小于 0.67 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对应浓度。

D. 校正

此ELISA试剂盒经由大肠杆菌表达的高纯度重组人TNF- α 蛋白所校正。

NIBSC/WHO TNF- α 第3代国际标准品(12/154)作为效价标准，在本试剂盒中进行了评估。

NIBSC/WHO标准品是大肠杆菌来源的重组人TNF- α 。国际标准品(12/154)的剂量反应曲线与Valukine 标准曲线平行。若要将使用Valukine Human TNF- α kit获得的样本值转换为NIBSC 12/154的近似单位, 请使用以下公式:

$$\text{NIBSC (12/154) approximate value (IU/mL)} = 0.058 \times \text{Valukine Human TNF-}\alpha \text{ value (pg/mL)}$$

E. 线性

在不同类型样本中掺入高浓度的人TNF- α , 然后用试剂稀释液(1 \times)将样本稀释到检测范围内, 测定其线性。

稀释倍数		细胞培养上清 (n=3)	血清 (n=4)	血浆 (n=3)
1:2	平均值/期待值 (%)	93.2	94.1	100.2
	范围 (%)	87.7-97.5	85.7-100.4	94.0-103.5
1:4	平均值/期待值 (%)	96.6	94.8	95.9
	范围 (%)	87.5-103.5	82.7-100.5	91.6-103.5
1:8	平均值/期待值 (%)	95.8	94.5	99.1
	范围 (%)	87.1-101.1	84.2-103.9	94.2-103.8
1:16	平均值/期待值 (%)	100.3	91.7	96.8
	范围 (%)	82.6-109.6	85.0-109.6	92.6-99.5

F. 样本预值

细胞培养上清 - 人外周血单核细胞 (PBMCs: 1×10^6 cells/mL) 培养于含有10%胎牛血清的RPMI 1640培养基中, 加100 U/mL青霉素和100 μ g/mL硫酸链霉素。细胞用20 μ g/mL的PHA刺激5天。得到的细胞培养上清分装检测人TNF- α 含量, 检测平均值为382.5 pg/mL。

血清样本 - 使用本试剂盒检测了4份人血清样本中TNF- α 的水平。4份样本的检测值均低于最低标准品, 11.7 pg/mL。

血浆样本 - 使用本试剂盒检测了3份人血浆样本中TNF- α 的水平。3份样本的检测值在21.4 -58.8 pg/mL之间, 平均值为 38.4 pg/mL。

G. 特异性

此ELISA法可检测天然及重组人TNF- α 蛋白。将以下因子用试剂稀释液（1 \times ）配制成 50 ng/mL的浓度来检测与人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人TNF- α 对照品中，来检测对人TNF- α 的干扰。没有观察到明显的交叉反应或干扰。

Recombinant human	Other Recombinant
CD40 Ligand	mouse TNF- α
TL1A	porcine TNF- α
TNF- β	rat TNF- α
	bovine TNF- α

重组人 Pro-TNF- α 并不干扰实验结果，但是测试中观察到约0.4%的交叉反应。

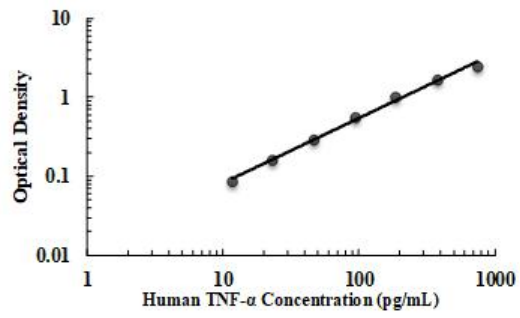
重组人TNF RI在本实验中不发生交叉反应，但浓度为> 3 ng/mL时会对实验产生干扰。

重组人TNF RII在本实验中不发生交叉反应，但浓度为> 25 ng/mL时会对实验产生干扰。

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	OD	Average	Corrected
0	0.061	0.061	-
	0.061		
11.7	0.128	0.132	0.088
	0.136		
23.4	0.206	0.204	0.160
	0.202		
46.9	0.339	0.339	0.295
	0.339		
93.8	0.610	0.609	0.565
	0.608		
188	1.040	1.055	1.011
	1.069		
375	1.694	1.716	1.672
	1.738		
750	2.518	2.523	2.479
	2.527		

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human TNF- α Microplate	包被小鼠抗人 TNF- α 抗体的 96 孔聚苯乙烯板, 8 孔 $\times 12$ 条	1 块板
Human TNF- α Detection Antibody	生物素化的人 TNF- α 检测抗体, 冻干粉, 参考瓶身标 签进行重溶	1 瓶
Human TNF- α Standard	标准品 (冻干粉), 参考瓶身标签进行重溶	2 瓶
Reagent Diluent Concentrate (10 \times)	浓缩的试剂稀释液 (10 \times) 用于稀释标准品、样本和 HRP	1 瓶
Detection Antibody Diluent Concentrate (10 \times)	浓缩的检测抗体稀释液 (10 \times)	1 瓶
Streptavidin-HRP A (200 \times)	200 \times 浓缩的链霉亲和素标记的 HRP A	1 瓶
Wash Buffer Concentrate (25 \times)	浓缩洗涤缓冲液 (25 \times)	1 瓶
TMB Substrate	TMB 底物溶液	1 瓶
Stop Solution	终止液	1 瓶
Plate Covers	封板膜	3 张

B. 试剂盒储存

未开封试剂盒	2-8℃储存；请在试剂盒有效期内使用	
已打开，稀释 或重溶的试剂	链霉亲和素-HRP A	2-8℃储存，最多30天*
	洗涤缓冲液 (1×)	
	TMB底物溶液	
	终止液	
	标准品	使用时新鲜配制* 标准品-20℃储存，最多30天*
	检测抗体	分装， -20℃储存，最多30天*
	试剂稀释液 (10×)	2-8℃储存，最多30天* 请每次使用新鲜配制的1×试剂稀释液
	检测抗体稀释液 (10×)	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
包被的微孔板条	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*	

*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。样本可能需要用试剂稀释液（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。试剂稀释液（1×）稀释。

血浆样本：使用EDTA、肝素钠或枸橼酸钠作为抗凝剂收集血浆。然后1000 x g离心15分钟，需在30分钟内收集血浆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。试剂稀释液（1×）稀释。

B. 样本准备工作

细胞培养上清、血清和血浆样本需要用试剂稀释液（1×）2倍稀释后进行检测，即100 μL 样本+100 μL试剂稀释液（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20 mL浓缩洗涤液用蒸馏水或去离子水稀释配制成为500 mL工作浓度的洗涤液。

试剂稀释液（1×）：使用蒸馏水或去离子水稀释配制成试剂稀释液（1×）。

检测抗体稀释液（1×）：使用蒸馏水或去离子水稀释配制成检测抗体稀释液（1×）。

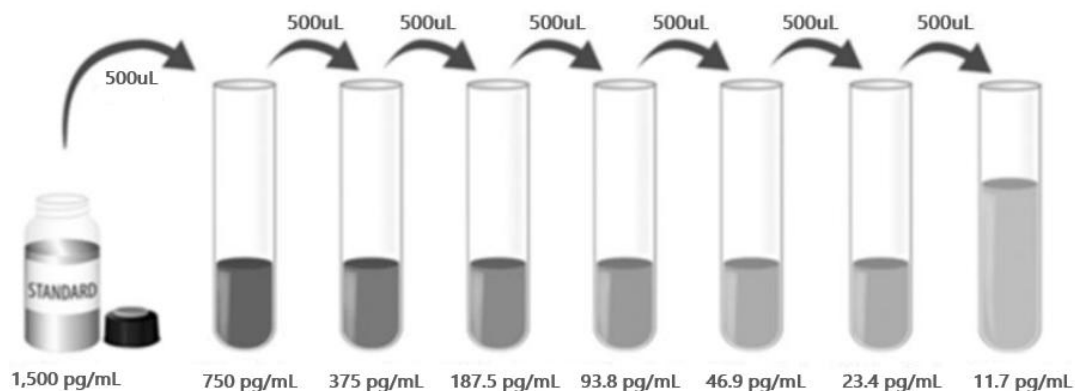
检测抗体：开盖前请瞬时离心。参考检测抗体瓶标签指示，用检测抗体稀释液（1×）将冻干粉进行重溶。再用检测抗体稀释液（1×）稀释至工作浓度 0.25 ug/mL，至少在使用前15 分钟准备。

链霉亲和素- HRP A：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP A（200×）稀释至工作浓度链霉亲和素- HRP A（1×）。

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1500 pg/mL标准品母液。轻轻震摇至少15分钟，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500 μL 试剂稀释液（1 \times ）。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。750 pg/mL 的标准品可用作标准曲线最高点，试剂稀释液（1 \times ）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，配制不同浓度标准品、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100 μL 。用封板膜封住反应孔，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；（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；
5. 在每个微孔内加入100 μL 配制好的检测抗体。用封板膜封住反应孔，室温孵育2小时；
6. 重复第4步洗板操作；
7. 在每个微孔内加入100 μL 稀释好的链霉亲和素-HRP A工作液。用封板膜封住反应孔，室温孵育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100 μL TMB底物溶液，室温孵育20分钟。**注意避光**；
10. 在每个微孔内加入50 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；
11. 加入终止液后10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；
12. **计算结果**：将每个标准品和样品的校正吸光度值($\text{OD}_{450}-\text{OD}_{540}/\text{OD}_{570}$)，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。通过样本的OD值，可从标准曲线上得到样本中分析物的浓度。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

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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

