PRODUCT INFORMATION & MANUAL

Mouse/Rat TNF RI/TNFRSF1A Valukine™ ELISA

Catalog Number: VAL649

For the quantitative determination of natural and recombinant mouse or rat Tumor Necrosis Factor Receptor I (TNF RI) 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

Version 202411.1

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I. BACKGROUND

Mouse TNF RI is the prototypical member of the TNF receptor superfamily that includes molecules as diverse as NGFR/p75, CD40, CD27, Fas, and TRAIL receptors (1). TNF RI is a 55-60 kDa, 425 amino acid (aa) type I transmembrane glycoprotein that contains a 219 aa cytoplasmic segment, a 23 aa transmembrane domain, and a 183 aa extracellular region rich in cysteine residues (2). The cytoplasmic segment contains a superfamily-typical "death domain" that interacts with cytoplasmic TRADD and initiates cellular apoptosis (1). The extracellular domain of mouse TNF RI shares 70% and 89% aa identity with human and rat TNF RI, respectively (2-5). Cells known to express TNF RI include fibroblasts (6), B cells (7), keratinocytes (8), microglia, astrocytes, and oligodendroglia (9), $CD34^+$ stem cells (10), monocytes (11), dendritic cells (8, 12), cardiac muscle cells (13), megakaryocytes (14), Schwann cells (15), neutrophils (16), adipocytes (17), endothelial cells (18), NK cells (19), and macrophages (20).

TNF RI has been shown to bind TNF-α with high affinity (2). In addition, TNF RI has also been shown to bind lymphotoxin-α_3 homotrimer and the lymphotoxin-$\alpha_2\beta_1$ heterotrimer (21). Unlike TNF RII, the expression level of TNF RI appears to be stable and relatively unaffected by cytokines and activating molecules (22). Ligation of TNF to TNF RI can stimulate the production of parasite-protective nitric oxide (23) and induce the internalization of TNF RI and release of soluble TNF RII (24). Both TNF RI and TNF RII are required for induction of TNF-mediated apoptosis of multiple cell types (25, 26).

Soluble TNF RI, generated by proteolytic cleavage of the cell surface receptor (1, 27), has been shown to bind TNF with high-affinity. It has been postulated that soluble TNF receptors may inhibit circulating TNF activity (28). Alternatively, soluble TNF R can also potentiate TNF activity by stabilizing the trimeric structure of physiologic TNF-α (29).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse/rat TNF RI has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse/rat TNF RI present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse/rat TNF RI is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of mouse/rat TNF RI 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 supernates and mouse/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 RD5Y 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)	46.0	118	581	48.3	120	592
Standard Deviation	2.7	6.5	27.5	2.5	6.0	33.3
CV%	5.9	5.5	4.7	5.2	5.0	5.6

B. RECOVERY

The recovery of mouse/rat TNF RI spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Mouse cell culture supernates (n=5)	97	89-109
Mouse serum* (n=5)	96	83-104
Rat cell culture supernates (n=5)	98	92-116
Rat serum* (n=5)	96	88-104

*Samples were diluted prior to assay as directed in the Sample Preparation section.

C. SENSITIVITY

Twenty-two assays were evaluated and the minimum detectable dose (MDD) of mouse/rat TNF RI ranged from 0.5-2.4 pg/mL. The mean MDD was 1.1 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 mouse TNF RI 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/rat TNF RI and diluted with Calibrator Diluent RD5Y to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture supernates (n=4)	Mouse serum* (n=4)	Rat serum* (n=4)
1:2	Average % of Expected	99	101	103
	Range (%)	92-104	97-104	99-106
1:4	Average % of Expected	102	101	106
	Range (%)	96-109	95-105	104-111
1:8	Average % of Expected	100	102	106
	Range (%)	93-105	95-113	99-108
1:16	Average % of Expected	99	99	103
	Range (%)	93-108	89-113	96-106

* Samples were diluted prior to assay as directed in the Sample Preparation section.

F. SAMPLE VALUES

Mouse/Rat serum - Samples were evaluated for detectable levels of mouse and rat TNF RI in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Mouse serum (n=40)	891	575-1575	249
Rat serum (n=20)	332	218-980	162

Cell Culture Supernates - L-929 mouse fibroblast cells (1 x 10^5 cells/mL) were cultured in MEM containing L-glutamine and 10% equine serum for 3 days. The cell culture supernate was removed, assayed for mouse TNF RI, and measured 461 pg/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant mouse and rat TNF RI.

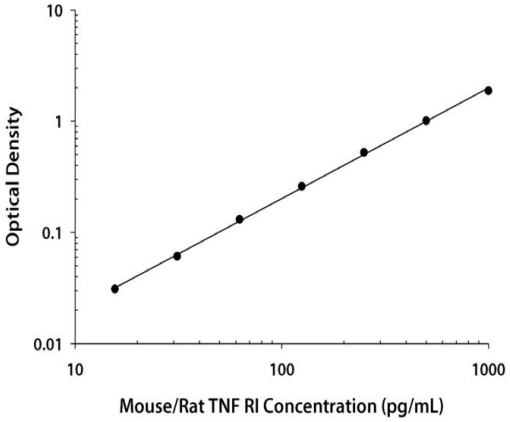
The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5Y and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse/rat TNF RI control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:			**Recombinant human:**
C10	IL-6	LIF	TNF-α
Eotaxin	IL-7	M-CSF	TNF-β
G-CSF	IL-9	MIP-1α	TNF RI
GM-CSF	IL-10	MIP-1β	TNF RII
IFN-γ	IL-10 R	MIP-2	**Other recombinants:**
IL-1α	IL-12	OSM	rat TNF-α
IL-1β	IL-13	SCF	canine TNF RI
IL-1ra	IL-17	TNF-α	
IL-2	IL-18	TNF-β	
IL-3	JE/MCP-1	TNF RII	
IL-4	KC	Tpo	
IL-5	Leptin	VEGF	

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	0.024 0.025	0.024	—
15.6	0.055 0.055	0.055	0.031
31.3	0.084 0.087	0.085	0.061
62.5	0.155 0.155	0.155	0.131
125	0.279 0.290	0.284	0.260
250	0.533 0.562	0.548	0.524
500	1.005 1.065	1.035	1.011
1000	1.828 1.972	1.900	1.876

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse/Rat TNF RI Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse/rat TNF RI.	1 plate
Mouse/Rat TNF RI Conjugate	An antibody specific for mouse/rat TNF RI conjugated to horseradish peroxidase.	1 vial
Mouse/Rat TNF RI Standard	Recombinant mouse TNF RI in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Mouse/Rat TNF RI Control	Recombinant mouse TNF RI in a buffered protein base; lyophilized. The assay value of the control should be within the range specified on the vial label.	1 vial
Assay Diluent RD1W	A buffered protein base.	1 vial
Calibrator Diluent RD5Y	A buffered protein base used to dilute standard and samples.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant.	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution.	2 vials
Stop Solution	Diluted hydrochloric 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="8">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="6">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Assay Diluent RD1W</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Calibrator Diluent RD5Y</td></tr>
<tr><td>Control</td><td rowspan="2">Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.*</td></tr>
<tr><td>Standard</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.
- Test tubes for dilution of standards and samples.

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 eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 RD5Y.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5Y.

Note: *Grossly hemolyzed or lipemic samples are not suitable for use in this assay.*

B. SAMPLE PREPARATION

Mouse serum samples recommend a 10-fold dilution into Calibrator Diluent RD5Y. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD5Y. Optimal dilutions should be determined by the end user.

Rat serum samples recommend a 4-fold dilution into Calibrator Diluent RD5Y. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD5Y. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse/Rat TNF RI Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

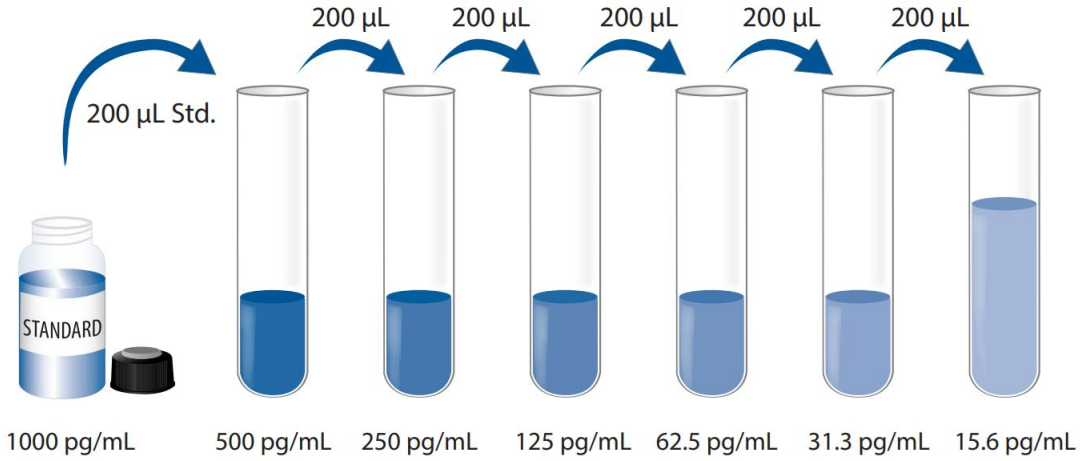
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Mouse/Rat TNF RI Standard- Refer to the vial label for the reconstitution volume* Reconstitute the Mouse/Rat TNF RI Standard with Calibrator Diluent RD5Y. Do not substitute other diluents. This reconstitution produces a stock solution of 1000

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 200 µL of Calibrator Diluent RD5Y into each tube. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse/Rat TNF RI Standard (1000 pg/mL) serves as the high standard. The Calibrator Diluent RD5Y 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

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

1. Prepare all reagents, working standards, control and samples 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 Assay Diluent RD1W to each well.
4. Add 50 µL of standard, control and prepared sample per well. Mix by gently tapping the plate frame for 1 minute. 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 four times for a total of five washes. Wash by filling each well with Wash Buffer (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/Rat TNF RI 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 TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. 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.

11. 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 log/log 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 a log/log graph. The data may be linearized by plotting the log of the mouse/rat TNF RI concentrations versus the log of the O.D. on a linear scale and the best fit line can be determined by regression analysis.

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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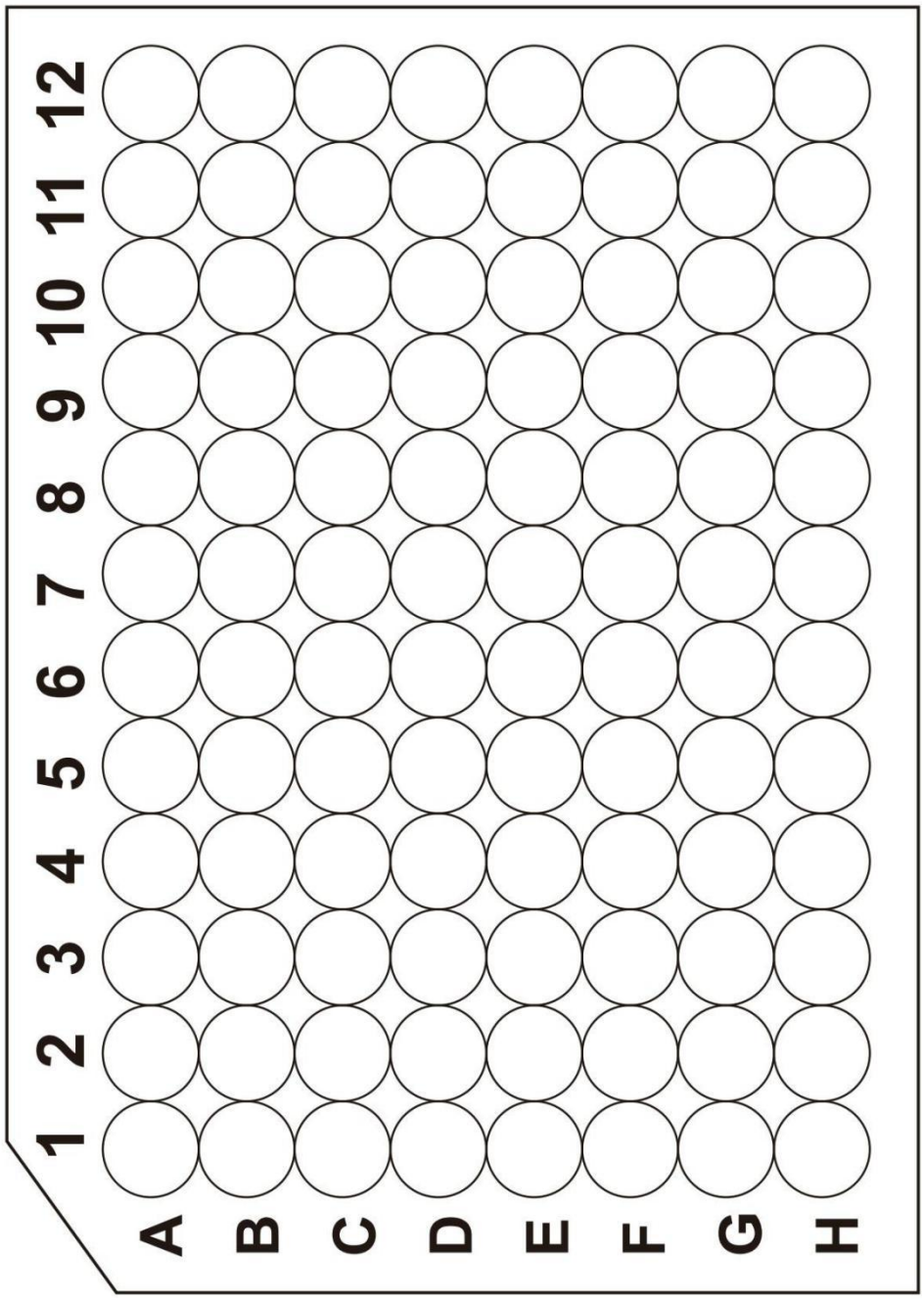
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠/大鼠 TNF RI/TNFRSF1A Valukine™ ELISA 试剂盒

目录号：VAL649

适用于定量检测天然和重组小鼠或大鼠肿瘤坏死因子受体 I (TNF RI) 的浓度

科研专用，不可用于临床诊断

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版本号 202411.1

目录

I. 背景

小鼠 TNF RI 是 TNF 受体超家族的典型成员，该家族包括 NGFR/p75、CD40、CD27、Fas 和 TRAIL 受体等多种分子（1）。TNF RI 是一种 55-60 kDa、425 氨基酸（amino acid, aa）的 I 型跨膜糖蛋白，包含 219 aa 的胞质段、23 aa 的跨膜结构域和 183 aa 的富含半胱氨酸残基的胞外区（2）。细胞质区段包含一个超家族典型的“死亡结构域”，它与细胞质 TRADD 相互作用，启动细胞凋亡（1）。小鼠 TNF RI 的胞外结构域与人类和大鼠 TNF RI 分别有 70%和 89%的 aa 一致性（2-5）。已知能表达 TNF RI 的细胞包括成纤维细胞（6）、B 细胞（7）、角质形成细胞（8）、小胶质细胞、星形胶质细胞和少突胶质细胞（9）、$CD34^+$干细胞（10）、单核细胞（11）、树突状细胞（8, 12）、心肌细胞（13）、大鼠细胞（14）、小鼠细胞（15）和大鼠细胞（16）、树突状细胞（8, 12）、心肌细胞（13）、巨核细胞（14）、雪旺细胞（15）、中性粒细胞（16）、脂肪细胞（17）、内皮细胞（18）、NK 细胞（19）和巨噬细胞（20）。

研究显示，TNF RI 与 TNF-α的结合亲和力很强（2）。此外，TNF RI 被证明还能与淋巴毒素-α_3 同源三聚体和淋巴毒素-$\alpha_2\beta_1$ 异源三聚体结合（21）。与 TNF RII 不同，TNF RI 的表达水平似乎比较稳定，相对不受细胞因子和活化分子的影响（22）。TNF 与 TNF RI 连接可刺激寄生虫保护性一氧化氮的产生（23），并诱导 TNF RI 内化和可溶性 TNF RII 的释放（24）。TNF RI 和 TNF RII 都是诱导 TNF 介导的多种细胞凋亡所必需的（25, 26）。

可溶性 TNF RI 由细胞表面受体蛋白水解裂解产生（1, 27），已被证明可与 TNF 高亲和力结合。据推测，可溶性 TNF 受体可抑制循环 TNF 活性（28）。另外，可溶性 TNF R 还可通过稳定生理性 TNF-α的三聚体结构而增强 TNF 的活性（29）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠/大鼠TNF RI抗体包被于微孔板上。样品，质控品和标准品中的小鼠/大鼠TNF RI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/大鼠TNF RI检测抗体进行孵育。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小鼠/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RD5Y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、试剂盒的效期等。

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。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	46.0	118	581	48.3	120	592
标准差	2.7	6.5	27.5	2.5	6.0	33.3
CV%	5.9	5.5	4.7	5.2	5.0	5.6

B. 回收率

在不同类别样本中掺入检测范围内不同水平的小鼠/大鼠TNF RI，测定其回收率。

样本类型	平均回收率%	范围 (%)
小鼠细胞培养上清 (n=5)	97	89-109
小鼠血清* (n=5)	96	83-104
大鼠细胞培养上清 (n=5)	98	92-116
大鼠血清*(n=5)	96	88-104

*样品在检测前按照样品制备部分的指示进行稀释

C. 灵敏度

22次检测结果表明，小鼠/大鼠TNF RI的最低可测剂量（MDD）范围为0.5-2.4 pg/mL。平均MDD为1.1 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

该免疫测定法以R&D Systems生产的高纯度的大肠杆菌表达的重组小鼠TNF RI校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠/大鼠TNF RI，然后用标准品稀释液RD5Y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=4)	小鼠血清* (n=4)	大鼠血清* (n=4)
1:2	平均值/期待值（%）	99	101	103
	范围（%）	92-104	97-104	99-106
1:4	平均值/期待值（%）	102	101	106
	范围（%）	96-109	95-105	104-111
1:8	平均值/期待值（%）	100	102	106
	范围（%）	93-105	95-113	99-108
1:16	平均值/期待值（%）	99	99	103
	范围（%）	93-108	89-113	96-106

*样品在检测前按照样品制备部分的指示进行稀释

F. 样本预值

小鼠/大鼠血清样本 - 在此测定中对样品进行小鼠/大鼠TNF RI的可检测水平的评估。

样本类型	平均值 (pg/mL)	范围(pg/mL)	标准差(pg/mL)
小鼠血清样本 (n=40)	891	575-1575	249
大鼠血清样本 (n=20)	332	218-980	162

细胞培养上清样本：

将 L-929 小鼠成纤维细胞（1 x 10^5 cells/mL）培养在含L-谷氨酰胺和10%马血清的MEM培养基中，培养3 天。取出等分的细胞培养上清，检测小鼠 TNF RI，结果为 461 pg/mL。

G. 特异性

此ELISA法可检测天然及重组小鼠/大鼠TNF RI。

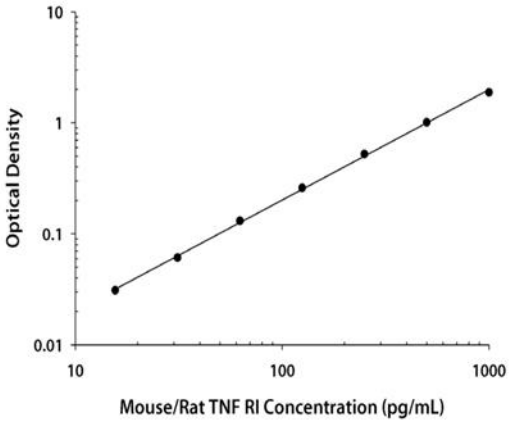
将以下因子用标准品稀释液RD5Y配制成50 ng/mL的浓度来检测与小鼠/大鼠TNF RI的交叉反应。将50 ng/mL的干扰因子掺入中间范围的小鼠/大鼠TNF RI对照品中，来检测对小鼠/大鼠TNF RI的干扰。没有观察到明显的交叉反应或干扰。

Recombinant mouse:			**Recombinant human:**
C10	IL-6	LIF	TNF-α
Eotaxin	IL-7	M-CSF	TNF-β
G-CSF	IL-9	MIP-1α	TNF RI
GM-CSF	IL-10	MIP-1β	TNF RII
IFN-γ	IL-10 R	MIP-2	**Other recombinants:**
IL-1α	IL-12	OSM	rat TNF-α
IL-1β	IL-13	SCF	canine TNF RI
IL-1ra	IL-17	TNF-α	
IL-2	IL-18	TNF-β	
IL-3	JE/MCP-1	TNF RII	
IL-4	KC	Tpo	
IL-5	Leptin	VEGF	

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)	O.D.	Average	Corrected
0	0.024 0.025	0.024	—
15.6	0.055 0.055	0.055	0.031
31.3	0.084 0.087	0.085	0.061
62.5	0.155 0.155	0.155	0.131
125	0.279 0.290	0.284	0.260
250	0.533 0.562	0.548	0.524
500	1.005 1.065	1.035	1.011
1000	1.828 1.972	1.900	1.876

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse/Rat TNF RI Microplate	包被抗小鼠/大鼠TNF RI抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Mouse/Rat TNF RI Conjugate	酶标检测抗小鼠/大鼠TNF RI抗体	1瓶
Mouse/Rat TNF RI Standard	小鼠TNF RI标准品（冻干），参考瓶身标签进行重溶	1瓶
Mouse/Rat TNF RI Control	小鼠TNF RI质控品（冻干），质控品的测定值应在标签上规定的范围内	1瓶
Assay Diluent RD1W	检测液	1瓶
Calibrator Diluent RD5Y	标准品稀释液用于稀释标准品和样本	1瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2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="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="6">2-8℃储存，最多30天*</td></tr>
<tr><td>检测液RD1W</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品稀释液RD5Y</td></tr>
<tr><td>质控品</td><td rowspan="2">分装并≤ -20 °C 储存，最多 30 天*</td></tr>
<tr><td>标准品</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
- 用于稀释标准品和样品的管子

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。

VI. 实验前准备

A. 样本收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样本稳定性尚未评估。

细胞培养上清：颗粒物应通过离心去除；立即检测样本或分装，≤ -20 °C储存备用，避免反复冻融。样本可能需要用标准品稀释液RD5Y稀释。

血清样本：血液样本在室温下凝集2小时，然后在2000 × g下离心20分钟。吸取血清样本之后即刻用于检测，或者分装，≤ -20°C储存备用。避免反复冻融。样本可能需要用标准品稀释液RD5Y稀释。

注意：*本试剂盒不适用于溶血或血脂过高样本。*

B. 样本准备工作

小鼠血清样本建议用标准品稀释液RD5Y 10倍稀释后进行检测，即20 µL样品+180 µL标准品稀释液RD5Y。最佳稀释度应由最终用户确定。

大鼠血清样本建议用标准品稀释液RD5Y 4倍稀释后进行检测，即50 µL样品+150 µL标准品稀释液RD5Y。最佳稀释度应由最终用户确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

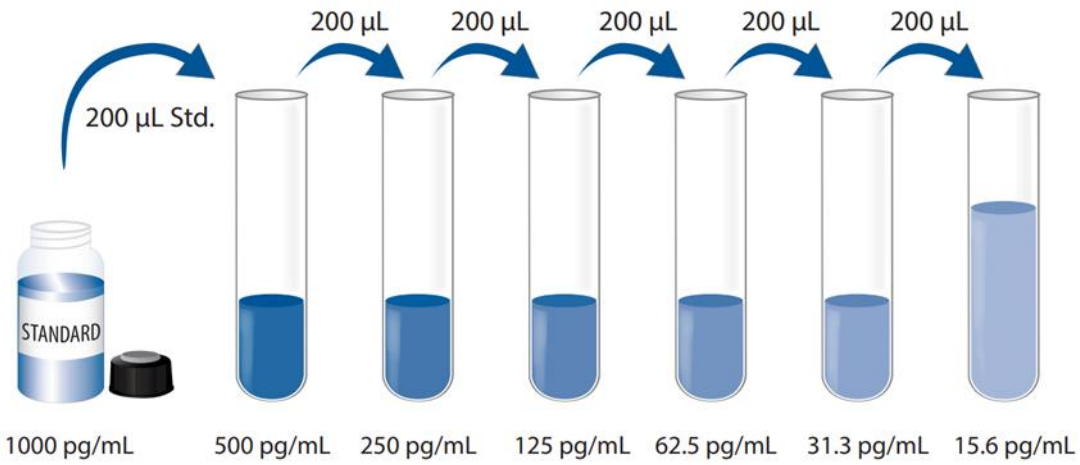
小鼠/大鼠TNF RI质控品：使用1.0 mL去离子水或蒸馏水重溶质控品。混合均匀，测定时不稀释质控品。

洗涤液（1×）：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（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

小鼠/大鼠TNF RI标准品：重溶体积请参考瓶身标签*，用标准品稀释液RD5Y重溶小鼠/大鼠TNF RI标准品，请勿使用其他稀释液。得到浓度为1000 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。

向每一稀释管中加入200 µL标准品稀释液RD5Y。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。未稀释的小鼠/大鼠TNF RI标准品可用作标准曲线最高点（1000 pg/mL），标准品稀释液RD5Y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，正确使用封板膜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，质控品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，标准品，质控品和样本；
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. 每孔加入50 µL检测液RD1W。
4. 分别将不同浓度标准品，质控品和实验样本加入相应孔中，每孔50 µL。轻轻拍打微孔板1分钟，后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；
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，共洗5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小鼠/大鼠TNF RI酶标检测抗体。用封板膜封住反应孔，**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 TMB底物溶液，**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，请轻拍微孔板，使溶液混合均匀；
10. 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。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确；
11. **计算结果：**将每个标准品，质控品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作log/log曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过log/log图上的点绘制最佳拟合曲线。数据可以通过绘制小鼠/大鼠TNF RI浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。

    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