PRODUCT INFORMATION & MANUAL

Human TNF RI/TNFRSF1A Valukine™ ELISA

Catalog Number: VAL212

For the quantitative determination of natural and recombinant human Tumor Necrosis Factor Receptor I (TNF RI)/TNFRSF1A 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

Version 202411.1

TABLE OF CONTENTS

I. BACKGROUND

Tumor necrosis factors (TNFs) are pleiotropic cytokines that are considered primary modifiers of the inflammatory and immune reactions of animals produced in response to injury or infection. Two forms of TNF, designated TNF-α (or cachectin) and TNF-β (or lymphotoxin), have been described that share 30% sequence similarity and compete for binding to the same receptors. TNFs play a necessary and beneficial role as mediators of host resistance to infections and tumor formation. However, over-production or inappropriate expression of these factors can lead to a variety of pathological conditions, including wasting, systemic toxicity, and septic shock. For reviews of the literature relating to these factors, see references 1 and 2.

The actions of TNFs are produced subsequent to binding of the factors to cell surface receptors. Two distinct TNF receptors have been identified and cloned. Virtually all cell types studied show the presence of one or both of these receptor types. One receptor type, termed TNF RII (Type A, Type a, 75 kDa or utr antigen), shows an apparent molecular weight of 75 kDa. The gene for this receptor encodes a presumptive transmembrane protein of 439 amino acid (aa) residues (3, 19). The other receptor type, termed TNF RI (Type B, Type b, 55 kDa or htr antigen), shows an apparent molecular weight of 55 kDa. The gene for this protein encodes a transmembrane protein of 426 aa residues (4, 5, 19). Both receptor types show high affinity binding of either TNF-α or TNF-β. The two receptor types are immunologically distinct but their extracellular domains show similarities in the pattern of cysteine residue locations in four domains (3). The intracellular domains of the two receptor types are apparently unrelated, suggesting the possibility that the two receptor types employ different signal transduction pathways.

Several groups have identified soluble TNF binding proteins in human serum and urine (6-8) that can neutralize the biological activities of TNF-α and TNF-β. Two types have been identified and designated sTNF RI (or TNF BPI) and sTNF RII (or TNF BPII). These soluble forms have now been shown to represent truncated forms of the two types of TNF receptors discussed above. The soluble receptor forms apparently arise as a result of shedding of the extracellular domains of the receptors, and concentrations of about 1-2 ng/mL are found in the serum and urine of healthy subjects (9, 10). The levels of the soluble receptors vary from individual to individual but are stable over time for given individuals (9).

Elevated levels of TNF receptors have been found in the amniotic fluid and urine of pregnant women (11), in serum or plasma in association with pathological conditions such as endotoxinemia (12, 13), meningococcemia (14), and HIV infection (15), and in plasma and ascites of patients in association with infections and malignancies (16). The mechanisms involved in the induction of shedding of the TNF receptors are not well understood. There are reports of correlations between increased TNF levels and soluble receptor levels, suggesting generally that stimuli that cause TNF levels to rise also induce shedding of TNF receptors(12-14, 17). There is also evidence, however, that suggests the shedding of the two types of soluble receptors is independently regulated (13).

The physiological role of the soluble TNF receptors is not known. It is known that both types of soluble receptors can bind to TNF in vitro and inhibit its biological activity by competing with cell surface receptors for TNF binding. Consequently it has been suggested that shedding of soluble receptors in response to TNF release could serve as a mechanism for binding and inhibiting the TNF not immediately bound to surface receptors, thus protecting other cells from the effects of TNF and localizing the inflammatory response (12, 17). It is also possible that shedding of receptors represents a mechanism for desensitizing the cells that shed the receptors from the effects of TNF (17). On the other hand, it has been reported that at low concentrations of TNF, binding to soluble receptors can stabilize TNF and augment some of its activities (18). Thus it is possible that under some conditions the pool of TNF bound to soluble receptors could represent a reservoir for the stabilization and controlled release of TNF.

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human TNF RI has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human TNF RI present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human 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 human 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, human serum, human plasma and human urine.
- 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 RD5-5 or Calibrator Diluent RD6O 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.

CELL CULTURE SUPERNATE/URINE ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	41.5	122	231	54.0	251	350
Standard Deviation	2.14	5.38	11.0	2.7	9.7	23.3
CV%	5.2	4.4	4.8	5.0	3.9	6.7

SERUM/PLASMA ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	69.0	198	355	54.8	252	356
Standard Deviation	3.24	7.17	17.8	4.8	9.3	20.6
CV%	4.7	3.6	5.0	8.8	3.7	5.8

B. RECOVERY

The recovery of human TNF RI spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture media (n=8)	85	80-92
Human serum (n=8)	90	77-103
Human EDTA plasma (n=8)	86	70-97
Human heparin plasma (n=8)	93	79-103
Human urine (n=8)	85	71-109

C. SENSITIVITY

Twelve assays were evaluated and the minimum detectable dose (MDD) of human TNF RI ranged from 0.43-1.20 pg/mL. The mean MDD was 0.77 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 human 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 human TNF RI and diluted with Calibrator Diluent RD5-5/Calibrator Diluent RD6O to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture samples (n=8)	Human serum* (n=13)	Human EDTA plasma* (n=13)	Human heparin plasma* (n=13)	Human citrate plasma* (n=8)	Human urine* (n=13)
1:2	Average % of Expected	106	100	98	97	90	106
	Range (%)	99-115	93-107	92-108	82-112	85-98	97-114
1:4	Average % of Expected	101	101	100	96	89	105
	Range (%)	93-107	94-109	90-110	82-106	81-98	94-122
1:8	Average % of Expected	99	99	100	93	91	104
	Range (%)	85-119	88-106	90-115	81-101	82-99	97-120
1:16	Average % of Expected	99	98	99	88	87	88
	Range (%)	77-114	85-106	86-121	82-97	81-94	81-118

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

F. SAMPLE VALUES

Human Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human TNF RI in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Human serum (n=40)	1198	749-1966	256
Human EDTA plasma (n=40)	914	484-1407	208
Human heparin plasma (n=40)	1015	512-1739	245
Human citrate plasma (n=40)	856	488-1598	210
Human urine* (n=33)	1029	173-4030	832

*Values are actual and not normalized for creatinine content.

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were stimulated with 10 µg/mL PHA, 10 µg/mL PHA + 10 ng/mL recombinant human (rh) IL-2, 50 ng/mL PMA, or 50 ng/mL LPS. Aliquots of the cell culture supernates were removed on days 1, 3, and 5 and assayed for levels of human TNF RI.

Stimulant	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
PHA	24	76	141
PHA + rhIL-2	26	71	143
PMA	16	27	67
LPS	17	36	49

G. SPECIFICITY

This assay recognizes natural and recombinant human TNF RI.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-5/Calibrator Diluent RD6O and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human TNF RI control were also assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human：		**Recombinant mouse:**
ANG	IL-10	EGF
β-ECGF	IL-11	IL-1β
EGF	LIF	IL-3
FGF acidic	M-CSF	IL-4
FGF basic	MCP-1	IL-5
G-CSF	MIP-1α	IL-7
GROα	MIP-1β	IL-9
IFN-γ	OPG	MIP-1α
IGF-I	OSM	MIP-1β
IGF-II	PDGF-AA	SCF
IL-1β	PDGF-AB	TNF RI
IL-1ra	PDGF-BB	TNF RII
IL-2	RANTES	**Other recombinants:**
IL-3	SLPI	amphibian TGF-β5
IL-4	TGF-α	chicken TGF-β3
IL-5	TGF-β1	**Natural proteins:**
IL-6	TGF-β3	bovine FGF acidic
IL-6 R	TNF-β	bovine FGF basic

IL-8	TNF RII	human PDGF
IL-9	TRANCE	human TGF-β1
		porcine TGF-β1
		porcine TGF-β1.2
		porcine TGF-β2

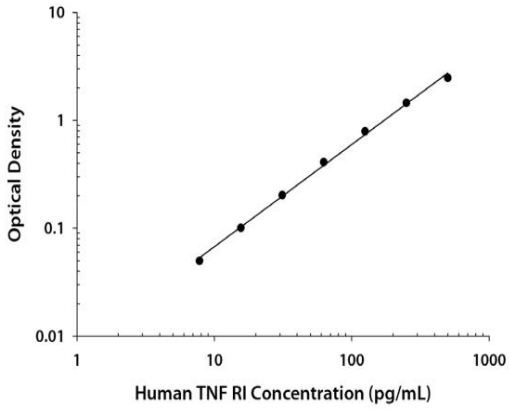
Although recombinant human TNF-α, recombinant mouse TNF-α, recombinant rat TNF-α, and recombinant porcine TNF-α did not show any significant cross-reactivity with human TNF RI in this immunoassay, these factors did show a low level of interference. When these factors were added to a mid level human TNF RI control at a concentration of 5.0 ng/mL, the observed value obtained in the immunoassay was decreased by 10%.

IV. EXPERIMENT

EXAMPLE STANDARD

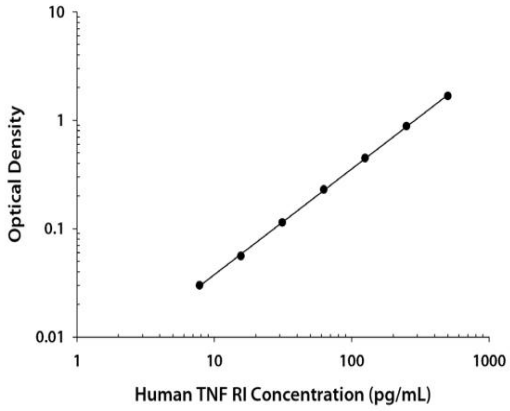
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.033 0.034	0.033	—
7.8	0.082 0.084	0.083	0.050
15.6	0.132 0.137	0.134	0.101
31.3	0.234 0.238	0.236	0.203
62.5	0.433 0.453	0.443	0.410
125	0.804 0.847	0.826	0.793
250	1.340 1.630	1.485	1.452
500	2.471 2.545	2.508	2.475

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.026 0.026	0.026	—
7.8	0.054 0.057	0.056	0.030
15.6	0.080 0.085	0.082	0.056
31.3	0.138 0.143	0.140	0.114
62.5	0.245 0.266	0.256	0.230
125	0.462 0.486	0.474	0.448
250	0.880 0.935	0.908	0.882
500	1.698 1.701	1.700	1.674

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human TNF RI Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human TNF RI	1 plate
Human TNF RI Conjugate	Solution of antibody against human TNF RI conjugated to horseradish peroxidase	1 vial
Human TNF RI Standard	Recombinant human TNF RI in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	1 vial
Assay Diluent HD1-7	A buffered protein base	1 vial
Calibrator Diluent RD5-5	A buffered protein base used to dilute standard and samples (For cell culture supernate/urine samples)	2 vials
Calibrator Diluent RD6O	Animal serum used to dilute standard and samples (For serum/plasma samples)	2 vials
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	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="8">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="7">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Assay Diluent HD1-7</td></tr>
<tr><td>Calibrator Diluent RD5-5</td></tr>
<tr><td>Calibrator Diluent RD6O</td></tr>
<tr><td>TMB Substrate</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 of this kit contain sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.
- 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 RD5-5.

Caution：*Human serum used in the preparation of cell culture media may contain high levels of TNF RI. Because of the low species cross-reactivity of this kit, human TNF RI levels in culture media containing 10% bovine or fetal bovine serum can be assayed without interference*.

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 × 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 RD6O.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD6O.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-5.

B. SAMPLE PREPARATION

Human serum and plasma samples recommend a 10-fold dilution. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD6O. Optimal dilutions should be determined by the end user.

Human urine samples recommend a 10-fold dilution. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD5-5. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

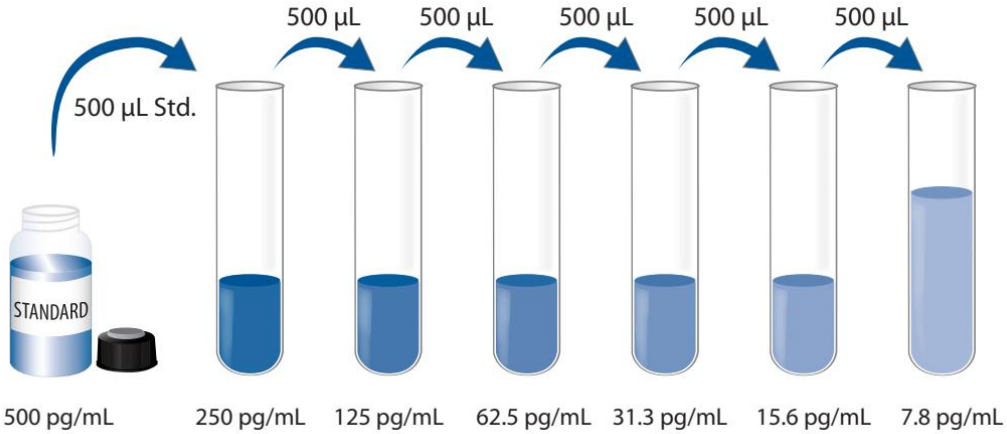
Bring all reagents to room temperature before use.

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×).

Human TNF RI Standard - Refer to the vial label for the reconstitution volume* Reconstitute the Human TNF RI Standard with Calibrator Diluent RD5-5 (*for cell culture supernate/urine samples*) or Calibrator Diluent RD6O (*for serum/plasma samples*). This reconstitution produces a stock solution of 500 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 µL of the Calibrator Diluent RD5-5 (for cell culture supernate/urine samples) or Calibrator Diluent RD6O (for serum/plasma samples) into the each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human TNF-RI Standard (500 pg/mL) serves as the high standard. The appropriate Calibrator Diluent RD5-5 (for cell culture supernate/urine samples) or Calibrator Diluent RD6O (for serum/plasma samples) 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 other reagents and samples to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.

1. Prepare all reagents, working standards 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 HD1-7 to each well.
4. Add 200 µL of standard and 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.
5. Aspirate each well and wash, repeating the process two times for a total of three 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 200 µL of Human 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 200 µL of TMB Substrate to each well. **Incubate for 20 minutes at room temperature. Protect from light**.
9. Add 50 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 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 human 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

1. Beutler, B. and A. Cerami (1988) Annu. Rev. Biochem. 57:505.

2. Vilcek, J. and T.H. Lee (1991) J. Biol. Chem. 266:7313.

3. Dembic, Z. *et al.* (1990) Cytokine 2:231.

4. Schall, T.J. *et al.* (1990) Cell 61:361.

5. Loetscher, H. *et al.* (1990) Cell 61:351.

6. Seckinger, P. *et al.* (1989) J. Biol. Chem. 264:11966.

7. Olsson, I. *et al.* (1989) Eur. J. Haematol. 42:270.

8. Engelmann, H. *et al.* (1990) J. Biol. Chem. 265:1531.

9. Aderka, D. *et al.* (1992) Lymphokine and Cyokine Res. 3:157.

10. Chouaib, S. *et al.* (1991) Immunology Today 12:141.

11. Austgulen, R. *et al.* (1992) J. Reproductive Immunol. 22:105.

12. Van Zee, K. *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:4845.

13. Spinas, G. *et al.* (1992) J. Clin. Invest. 90:533.

14. Girardin, E. *et al.* (1992) Immunology 76:20.

15. Kalinkovich, A. *et al.* (1992) Clin. Exp. Immunol. 89:351.

16. Andus, T. *et al.* (1992) Hepatology 16:749.

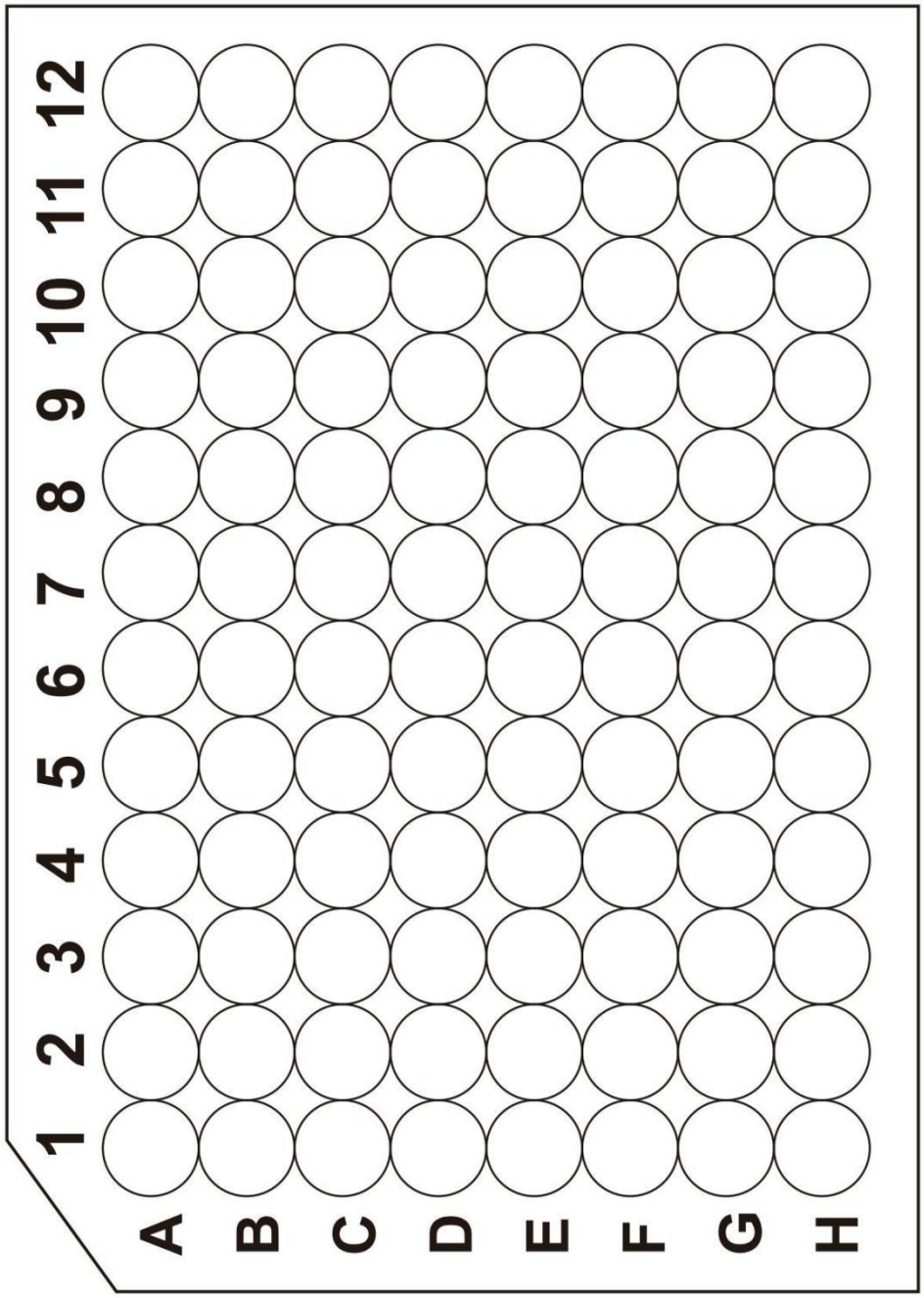
17. Porteu, F. and C. Nathan (1990) J. Exp. Med. 172:599.

18. Aderka, D. *et al.* (1992) J. Exp. Med. 175:323.

19. Tartaglia, L.A. and D.V. Goeddel (1992) Immunology Today 13:151.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 TNF RI/TNFRSF1A Valukine™ ELISA 试剂盒

目录号：VAL212

适用于定量检测天然和重组人肿瘤坏死因子受体 I (TNF RI)/TNFRSF1A 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

版本号 202411.1

目录

I. 背景

肿瘤坏死因子（Tumor necrosis factors，TNFs）是多效性细胞因子，被认为是动物在受伤或感染时产生的炎症和免疫反应的主要调节因子。目前已描述了两种形式 TNF，分别称为 TNF-α（或恶病质）和 TNF-β（或淋巴毒素），它们的序列有 30%的相似性，并会竞争性地与相同的受体结合。TNFs 作为宿主抵抗感染和肿瘤形成的介质，发挥着必要而有益的作用。然而，这些因子的过度产生或不适当表达可导致多种病理状况，包括消瘦、全身毒性和感染性休克。有关这些因子的文献综述，请参阅参考文献 1 和 2。

TNF 的作用是在因子与细胞表面受体结合后产生的。已经鉴定并克隆了两种不同的 TNF 受体。几乎所有研究过的细胞类型都显示存在其中一种或两种受体类型。其中一种受体被称为 TNF RII（A 型、a 型、75 kDa 或 utr 抗原），表面分子量为 75 kDa。这种受体的基因编码一种由 439 个氨基酸（amino acid，aa）残基组成的假定跨膜蛋白（3, 19）。另一种受体类型被称为 TNF RI（B 型、b 型、55 kDa 或 htr 抗原），其表观分子量为 55 kDa。该蛋白的基因编码一种有 426 aa 残基的跨膜蛋白（4, 5, 19）。两种受体类型都能与 TNF-α或 TNF-β产生高亲和力结合。这两种受体类型在免疫学上截然不同，但它们的胞外结构域在四个结构域的半胱氨酸残基位置上显示出相似性（3）。两类受体的胞内结构域显然无关，这表明两类受体可能采用了不同的信号转导途径。

多个研究小组已在人体血清和尿液中鉴定出可溶性 TNF 结合蛋白，这些蛋白可以中和 TNF-α和 TNF-β生物活性（6-8）。目前已鉴定出两种类型，并命名为 sTNF RI（或 TNF BPI）和 sTNF RII（或 TNF BPII）。现在已经证明，这些可溶性形式代表了上述两种 TNF 受体的截短形式。可溶性受体形式显然是由于受体的细胞外结构域脱落而产生的，在健康受试者的血清和尿液中发现的浓度约为 1-2 ng/mL（9, 10）。可溶性受体的水平因人而异，但对特定个体而言，其水平在一段时间内是稳定的（9）。

在孕妇的羊水和尿液中（11），在与内毒素血症（12, 13）、脑膜炎球菌血症（14）和 HIV 感染（15）等病理情况相关的血清或血浆中，以及在与感染和恶性肿瘤相关的患者血浆和腹水中（16），都发现了 TNF 受体水平的升高。诱导 TNF 受体脱落的机制尚不清楚。有报告称，TNF 水平升高与可溶性受体水平之间存在相关性，通常表明导致 TNF 水平升高的刺激也会诱导 TNF 受体脱落（12-14, 17）。然而，也有证据表明，这两种类型的可溶性受体的脱落是独立调节的（13）。

可溶性 TNF 受体的生理作用尚不清楚。已知两种类型的可溶性受体都能在体外与 TNF 结合，并通过与细胞表面受体竞争 TNF 结合来抑制其生物活性。因此，有人提出，TNF 释放时脱落的可溶性受体可作为一种机制，结合并抑制未立即与表面受体结合的 TNF，从而保护其他细胞免受 TNF 的影响，并使炎症反应局部化（12, 17）。受体脱落也可能是使脱落受体的细胞对 TNF 的作用脱敏的一种机制（17）。另一方面，据报道，在 TNF 浓度较低时，与可溶性受体结合可稳定 TNF 并增强其某些活性（18）。因此，在某些条件下，与可溶性受体结合的 TNF 池可能是稳定和控制释放 TNF 的储存库。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人TNF RI抗体包被于微孔板上，样品和标准品中的人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人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，人血清样本、人血浆样本和人尿液样本；
- 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RD5-5或标准品稀释液RD6O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。

细胞培养上清试验

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	41.5	122	231	54.0	251	350
标准差	2.14	5.38	11.0	2.7	9.7	23.3
CV%	5.2	4.4	4.8	5.0	3.9	6.7

血清/血浆试验

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	69.0	198	355	54.8	252	356
标准差	3.24	7.17	17.8	4.8	9.3	20.6
CV%	4.7	3.6	5.0	8.8	3.7	5.8

B. 回收率

不同类型样本中掺入检测范围内不同水平的人TNF RI，测定其回收率。

样本类型	平均回收率 (%)	范围 (%)
细胞培养基 (n=8)	85	80-92
人血清样本 (n=8)	90	77-103
人 EDTA 血浆样本 (n=8)	86	70-97
人肝素血浆样本 (n=8)	93	79-103
人尿液样本 (n=8)	85	71-109

C. 灵敏度

12次试验检测评估表明，人TNF RI 的最低检测剂量 (MDD)范围 为 0.43-1.20 pg/mL。平均 MDD 为 0.77 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人TNF RI校正。

E. 线性

不同的样本中含有或掺入高浓度的人TNF RI，然后用标准品稀释液RD5-5/标准品稀释液RD6O将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养样本 (n=8)	人血清* (n=13)	人EDTA血浆* (n=13)	人肝素血浆* (n=13)	人枸橼酸钠血浆* (n=8)	人尿液*（n=13）
1:2	平均值/期待值 (%)	106	100	98	97	90	106
	范围 (%)	99-115	93-107	92-108	82-112	85-98	97-114
1:4	平均值/期待值 (%)	101	101	100	96	89	105
	范围 (%)	93-107	94-109	90-110	82-106	81-98	94-122
1:8	平均值/期待值 (%)	99	99	100	93	91	104
	范围 (%)	85-119	88-106	90-115	81-101	82-99	97-120
1:16	平均值/期待值 (%)	99	98	99	88	87	88
	范围 (%)	77-114	85-106	86-121	82-97	81-94	81-118

*根据样品制备部分的指示，在检测前稀释样品。

F. 样本预值

人血清/血浆/尿液-使用此试剂盒评估表面健康志愿者血清、血浆和尿液样本中人TNF RI的存在情况。本研究中使用的供体没有病史。

样品类型	平均值 (pg/mL)	范围 (pg/mL)	标准差 (pg/mL)
人血清 (n=40)	1198	749-1966	256
人EDTA 血浆 (n=40)	914	484-1407	208
人肝素血浆 (n=40)	1015	512-1739	245
人枸橼酸钠血浆 (n=40)	856	488-1598	210
人尿液* (n=33)	1029	173-4030	832

*数值为实际值，未按肌酐含量进行归一化处理

细胞培养上清：

人外周血单核细胞(1 x 10^6 cells/mL)细胞培养在含10% 胎牛血清、50 μM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和 100 μg/mL 硫酸链霉素的RPMI培养基中。用 10 μg/mL PHA、10 μg/mL PHA + 10 ng/mL 重组人(rh)IL-2、50 ng/mL PMA 或 50 ng/mL LPS 刺激细胞。在第 1、3 和 5 天取出等量的细胞培养上清，检测人 TNF RI 的水平。

刺激类型	**1天 (pg/mL)**	**3天(pg/mL)**	**5天 (pg/mL)**
PHA	24	76	141
PHA + rhIL-2	26	71	143
PMA	16	27	67
LPS	17	36	49

G. 特异性

此ELISA法可检测天然及重组人TNF RI。

将以下因子用标准品稀释液RD5-5/标准品稀释液RD6O配制成50 ng/mL的浓度来检测与人TNF RI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 RI对照品中，来检测对人TNF RI的干扰。没有观察到明显的交叉反应或干扰。

Recombinant human：		**Recombinant mouse:**
ANG	IL-10	EGF
β-ECGF	IL-11	IL-1β
EGF	LIF	IL-3
FGF acidic	M-CSF	IL-4
FGF basic	MCP-1	IL-5
G-CSF	MIP-1α	IL-7
GROα	MIP-1β	IL-9
IFN-γ	OPG	MIP-1α
IGF-I	OSM	MIP-1β
IGF-II	PDGF-AA	SCF
IL-1β	PDGF-AB	TNF RI
IL-1ra	PDGF-BB	TNF RII
IL-2	RANTES	**Other recombinants:**
IL-3	SLPI	amphibian TGF-β5
IL-4	TGF-α	chicken TGF-β3
IL-5	TGF-β1	**Natural proteins:**
IL-6	TGF-β3	bovine FGF acidic
IL-6 R	TNF-β	bovine FGF basic
IL-8	TNF RII	human PDGF
IL-9	TRANCE	human TGF-β1
		porcine TGF-β1
		porcine TGF-β1.2
		porcine TGF-β2

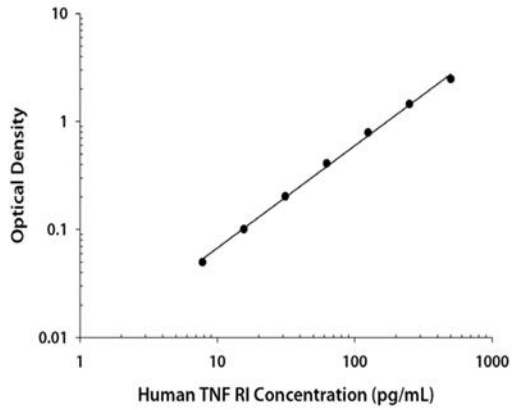
虽然重组人TNF-α、重组小鼠TNF-α、重组大鼠TNF-α和重组猪TNF-α与人TNF- RI无明显交叉反应性，但有低水平的干扰。当这些因子以5.0 ng/mL的浓度掺入中间范围的重组人TNF RI对照品中，免疫测定中得到的观察值降低了10%。

IV. 实验

标准曲线实例

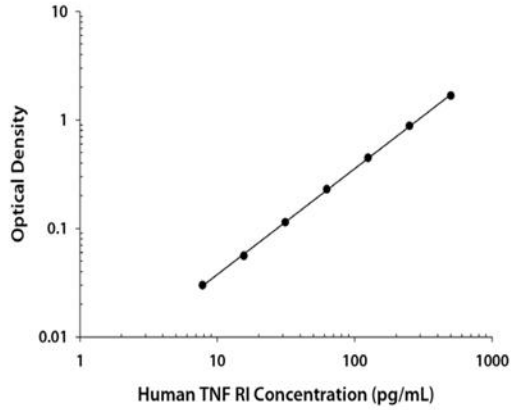
该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.033 0.034	0.033	—
7.8	0.082 0.084	0.083	0.050
15.6	0.132 0.137	0.134	0.101
31.3	0.234 0.238	0.236	0.203
62.5	0.433 0.453	0.443	0.410
125	0.804 0.847	0.826	0.793
250	1.340 1.630	1.485	1.452
500	2.471 2.545	2.508	2.475

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.026 0.026	0.026	—
7.8	0.054 0.057	0.056	0.030
15.6	0.080 0.085	0.082	0.056
31.3	0.138 0.143	0.140	0.114
62.5	0.245 0.266	0.256	0.230
125	0.462 0.486	0.474	0.448
250	0.880 0.935	0.908	0.882
500	1.698 1.701	1.700	1.674

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human TNF RI Microplate	包被抗人TNF RI抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human TNF RI Conjugate	酶标检测抗人TNF RI抗体	1瓶
Human TNF RI Standard	人TNF RI标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent HD1-7	检测液	1瓶
Calibrator Diluent RD5-5	标准品稀释液用于稀释标准品和样品（对于细胞培养上清/尿液样本）	2瓶
Calibrator Diluent RD6O	标准品稀释液用于稀释标准品和样品（对于血清/血浆样本）	2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="7">2-8°C储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>检测液HD1-7</td></tr>
<tr><td>标准品稀释液RD5-5</td></tr>
<tr><td>标准品稀释液RD6O</td></tr>
<tr><td>TMB底物溶液</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 °C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
- 用于稀释标准品和样品的管子

D. 注意事项

- 本试剂盒的某些组件含有叠氮化钠，可能会与铅和铜水管反应生成爆炸性金属叠氮化物。处理时要用大量水冲洗。
- 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. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清液 - 通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤ -20°C的温度下，避免反复冻融。样品可能需要用标准品稀释液RD5-5稀释。

注意：*用于制备细胞培养基的人血清可能含有高水平的TNF RI。由于该试剂盒的物种交叉反应性低，可以在不受干扰的情况下测定含有10%牛或胎牛血清的培养基中的人TNF RI水平。*

血清 - 使用血清分离管（SST），让样本在室温下凝固 30 分钟，然后在 1000 × g 的离心力下离心 15 分钟。分离血清并立即进行检测，或分装并储存在 ≤ -20 °C。避免反复冻融循环。样本可能需要用标准品稀释液 RD6O 进行稀释。

血浆 - 使用EDTA、肝素或枸橼酸钠作为抗凝剂收集血浆。然后1000 × g离心15分钟。需在30分钟内收集血浆样本之后立即检测或分装并储存在 ≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液RD6O 稀释。

尿液 - 无菌收集当天的第一次尿液（中段），直接排入无菌容器中。离心去除颗粒物质，立即检测或分装并储存在 ≤ -20 °C。避免反复冻融循环。样品可能需要用标准品稀释液 RD5-5稀释。

B. 样品制备

人血清和血浆样本建议稀释 10 倍。建议的 10 倍稀释为 50 µL 样品 + 450 µL 标准品稀释液 RD6O。最佳稀释倍数应由最终用户决定。

人尿液样本建议稀释 10 倍。建议 10 倍稀释为 50 µL 样品 + 450 µL 标准品稀释液 RD5-5。最佳稀释倍数应由最终用户确定。

C. 检测前准备工作

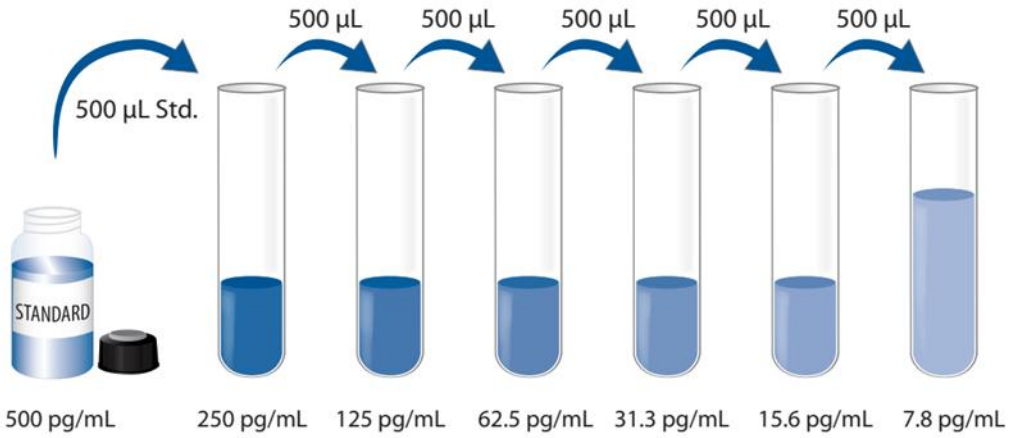
使用前请将所有试剂放置于室温。

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×）。

人TNF RI标准品：重溶体积请参考瓶身标签*，用标准品稀释液 RD5-5（用于细胞培养上清/尿液样品）或标准品稀释液 RD6O（用于血清/血浆样品）重溶人TNF RI标准品，得到浓度为500 pg/mL标准品储备母液。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。

将500 µL标准品稀释液RD5-5（用于细胞培养上清/尿液样本）或标准品稀释液RD6O（用于血清/血浆样本）移入每管中。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。未稀释的人TNF RI（500 pg/m）作标准曲线最高点，标准品稀释液RD5-5（用于细胞培养上清液/尿液样本）或标准品稀释液RD6O（用于血清/血浆样本）移入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，将所有其他试剂和样品带至室温。建议对所有标准品和样品进行复孔检测。

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. 向每个孔中加入50 µL检测液HD1-7。
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔200 µL。用封板膜封住反应孔，**室温下孵育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2次，共洗3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200 µL人TNF RI酶标检测抗体。用封板膜封住反应孔，**室温下孵育2小时**；
7. 重复第5步洗板操作；
8. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育20分钟。注意避光**；
9. 在每个微孔内加入50 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。数据可以通过绘制人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

1. Beutler, B. and A. Cerami (1988) Annu. Rev. Biochem. 57:505.

2. Vilcek, J. and T.H. Lee (1991) J. Biol. Chem. 266:7313.

3. Dembic, Z. et al. (1990) Cytokine 2:231.

4. Schall, T.J. et al. (1990) Cell 61:361.

5. Loetscher, H. et al. (1990) Cell 61:351.

6. Seckinger, P. et al. (1989) J. Biol. Chem. 264:11966.

7. Olsson, I. et al. (1989) Eur. J. Haematol. 42:270.

8. Engelmann, H. et al. (1990) J. Biol. Chem. 265:1531.

9. Aderka, D. et al. (1992) Lymphokine and Cyokine Res. 3:157.

10. Chouaib, S. et al. (1991) Immunology Today 12:141.

11. Austgulen, R. et al. (1992) J. Reproductive Immunol. 22:105.

12. Van Zee, K. et al. (1992) Proc. Natl. Acad. Sci. USA 89:4845.

13. Spinas, G. et al. (1992) J. Clin. Invest. 90:533.

14. Girardin, E. et al. (1992) Immunology 76:20.

15. Kalinkovich, A. et al. (1992) Clin. Exp. Immunol. 89:351.

16. Andus, T. et al. (1992) Hepatology 16:749.

17. Porteu, F. and C. Nathan (1990) J. Exp. Med. 172:599.

18. Aderka, D. et al. (1992) J. Exp. Med. 175:323.

19. Tartaglia, L.A. and D.V. Goeddel (1992) Immunology Today 13:151.

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