PRODUCT INFORMATION & MANUAL

Human Urinary TIM-1/KIM-1/HAVCR Valukine™ ELISA

Catalog Number: VAL210

For the quantitative determination of natural and recombinant human T cell Immunoglobulin-Mucin (TIM-1) 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

T cell immunoglobulin and mucin domain 1 (TIM-1), also known as Kidney injury molecule 1 (KIM-1) and Hepatitis A virus cellular receptor 1 (HAVcr1), is a member of the TIM family which is involved in the regulation of innate and adaptive immune responses (1, 2). TIM-1 is a type I transmembrane protein that contains an N-terminal immunoglobulin-like domain, a mucin domain with O- and N-linked glycosylations, a transmembrane segment, and a cytoplasmic signaling domain (3, 4). Multiple TIM-1 variants can be produced due to polymorphisms or alternative splicing resulting in deletions in the mucin domain (3). Some of these polymorphisms are associated with susceptibility to atopy, autoimmunity, and severe hepatitis A virus infection in humans (5). Within the extracellular domain, human TIM-1 shares 41% amino acid sequence identity with mouse and rat TIM-1.

In vivo, TIM-1 is expressed on splenic B cells and is a marker for the identification of IL-10+ regulatory B cells (6, 7). TIM-1 is also expressed on $CD4^+$ T cells, mast cells, invariant NKT (iNKT) cells, dendritic cells, kidney epithelium and a broad range of mucosal epithelium (4, 8-15). The expression of TIM-1 is upregulated on activated Th2 cells, after dendritic cell maturation, and on kidney tubular epithelial cells after injury (4, 9, 13, 14, 16, 17). Metalloproteinase-mediated cleavage of TIM-1 at the membrane-proximal region results in the release of a soluble form of TIM-1 which is detectable in the urine and in circulation (18, 19). Urinary TIM-1 is highly elevated in nephropathy and may be a useful biomarker for renal damage (16, 20 - 25).

TIM-1 has been reported to be a receptor for a number of ligands, including phosphatidylserine, leukocyte mono-immunoglobulin-like receptor 5 (LMIR5/CD300b), TIM-1 (homophilic), TIM-4, IgA, and the glycoproteins of a number of enveloped viruses (5, 15, 26-33). Its interaction with phosphatidylserine enables TIM-1 to mediate the phagocytosis of apoptotic cells (26-28). In TIM-1-bearing iNKT cells, interaction with apoptotic cells can also result in iNKT cell activation, proliferation, and cytokine production (11). Interactions between cell-surface or soluble TIM-1 with LMIR5 is proposed to induce LMIR5-mediated activation of myeloid cells including macrophages/monocytes, mast cells, neutrophils, and dendritic cells (29). These interactions contribute to tissue homeostasis and damage during kidney injury (29). Ligand- induced TIM-1 signaling costimulates T cell activation and enhances Th2 cytokine production (9, 31, 34). In humans, TIM-1 serves as a cellular entry receptor for various viruses, including hepatitis A virus, Ebolavirus and Marburgvirus (15, 33).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human TIM-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human TIM-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human TIM-1 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 TIM-1 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 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 (1×) and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

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 forty separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Mean (ng/mL)	0.98	3.04	5.88	1.09	3.19	6.23
Standard Deviation	0.042	0.119	0.259	0.069	0.193	0.484
CV%	4.3	3.9	4.4	6.3	6.1	7.8

B. RECOVERY

The recovery of human TIM-1 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range (%)
Human Urine (n=20)	104	94-112

C. SENSITIVITY

Thirty-nine assays were evaluated and the minimum detectable dose (MDD) of human TIM-1 ranged from 0.003-0.046 ng/mL. The mean MDD was 0.009 ng/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 NS0-expressed recombinant human TIM-1 produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human

TIM-1 were serially diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay. For more information on linearity and handling urine specimens, refer to the Evaluation of the Linearity of Quantitative Analytical Methods: A Statistical Approach (NCCLS) publication EP6-A.

Dilution		Human Urine (n=9)
1:2	Average % of Expected	105
	Range (%)	98-112
1:4	Average % of Expected	109
	Range (%)	100-117
1:8	Average % of Expected	110
	Range (%)	101-118
1:16	Average % of Expected	103
	Range (%)	95-110

F. SAMPLE VALUES

Human Urine - Twenty-six samples from apparently healthy volunteers were evaluated for the presence of human TIM-1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean	Range	Standard Deviation
TIM-1 (ng/mL)	1.35	0.156-5.33	1.09
TIM-1 (µg/g Creatinine)	1.11	0.225-3.20	0.689

G. SPECIFICITY

This assay recognizes natural and recombinant human TIM-1.

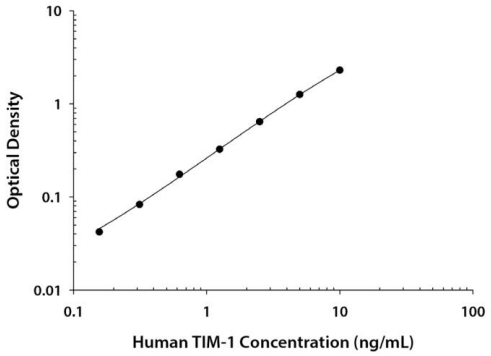
The factors listed below were prepared at 100 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human TIM-1 control were also assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human：	**Recombinant mouse:**	**Recombinant rat:**
TIM-3	TIM-1	TIM-1
TIM-4	TIM-4	

IV. EXPERIMENT

EXAMPLE STANDARD

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



(ng/mL)	O.D.	Average	Corrected
0	0.012 0.012	0.012	—
0.156	0.053 0.055	0.054	0.042
0.313	0.094 0.096	0.095	0.083
0.625	0.187 0.187	0.187	0.175
1.25	0.331 0.343	0.337	0.325
2.5	0.644 0.666	0.655	0.643
5	1.256 1.282	1.269	1.257
10	2.271 2.343	2.307	2.295

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human TIM-1 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human TIM-1	1 plate
Human TIM-1 Conjugate	Solution of antibody against human TIM-1 conjugated to horseradish peroxidase	1 vial
Human TIM-1 Standard	Recombinant human TIM-1 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	1 vial
Assay Diluent RD1-82	A buffered protein base	1 vial
Calibrator Diluent Concentrate (2×) /RD6Q	Animal serum 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	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="7">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="5">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 RD1-82</td></tr>
<tr><td>Standard</td></tr>
<tr><td>TMB Substrate</td><td rowspan="2">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>Calibrator Diluent Concentrate (2×) /RD6Q</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.
- 50 mL and 500 mL graduated cylinder.
- Polypropylene test tubes for dilution of standards.

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.

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 (1×).

B. REAGENT PREPARATION

Bring all reagents to room temperature before use.

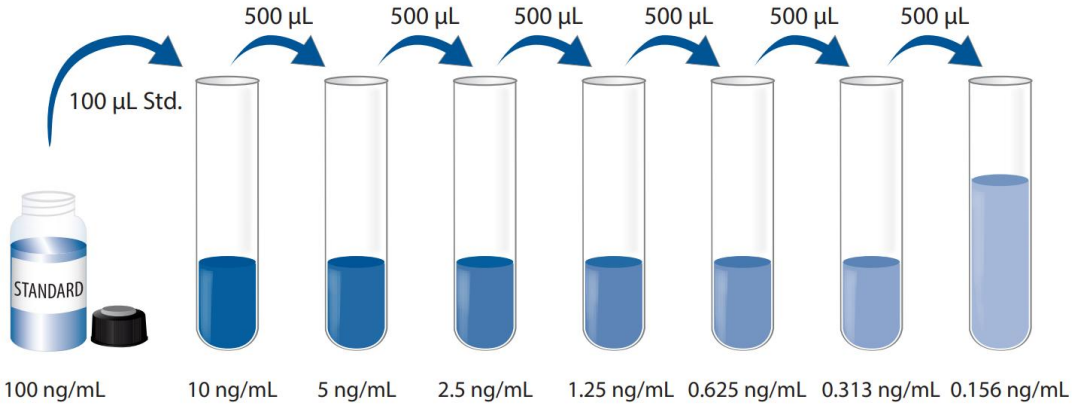
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

Human TIM-1 Standard - Refer to the vial label for the reconstitution volume* Reconstitute the Human TIM-1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/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 900 µL of the Calibrator Diluent (1×) into the 10 ng/mL tube. Pipette 500 µL of the Calibrator Diluent (1×) into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent (1×) serves as the zero standard (0 ng/mL).



C. 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 100 µL of Assay Diluent RD1-82 to each well.
4. Add 50 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 three times for a total of four 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 TIM-1 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 30 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 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 human TIM-1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

VIII. REFERENCES

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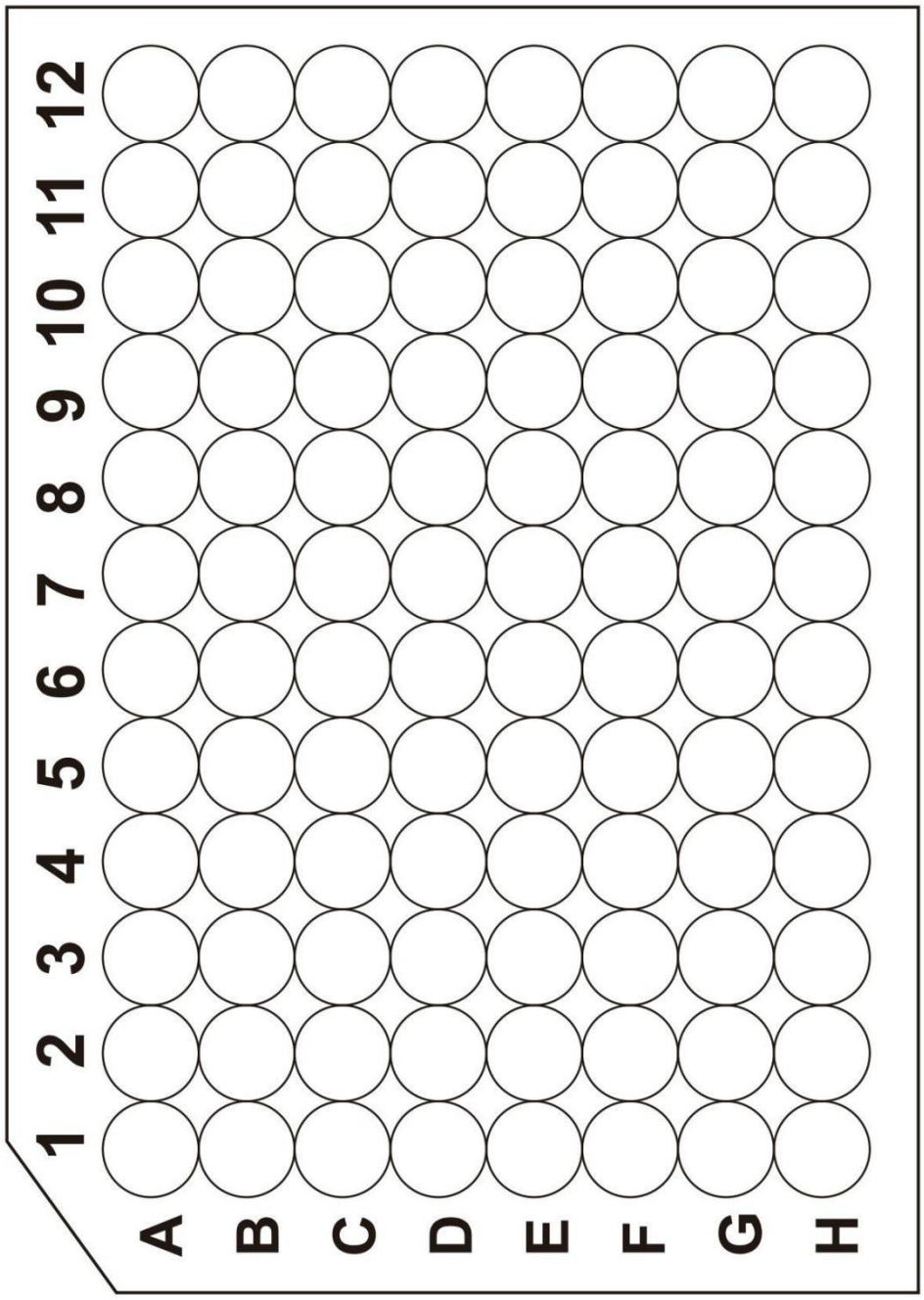
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人尿液 TIM-1/KIM-1/HAVCR Valukine™ ELISA 试剂盒

目录号：VAL210

适用于定量检测天然和重组人 T 细胞免疫球蛋白-粘蛋白（TIM-1）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

T 细胞免疫球蛋白和粘蛋白结构域 1（TIM-1），又称肾损伤分子 1（KIM-1）和甲型肝炎病毒细胞受体 1（HAVcr1）是 TIM 家族的成员，参与先天性和适应性免疫反应的调控（1, 2）。TIM-1 是一种 I 型跨膜蛋白，包含一个 N 端免疫球蛋白样结构域、一个具有 O-和 N-连接糖基化的粘蛋白结构域、一个跨膜区段和一个胞质信号结构域（3, 4）。由于多态性或替代剪接导致粘蛋白结构域缺失，可产生多种 TIM-1 变体（3）。其中一些多态性与人类易患过敏症、自身免疫和严重甲型肝炎病毒感染有关（5）。在细胞外结构域中，人 TIM-1 与小鼠和大鼠 TIM-1 有 41%的氨基酸序列相同。

在体内，TIM-1 在脾脏 B 细胞上表达，是识别 IL-10^{+}调节性 B 细胞的标志物（6, 7）。TIM-1 还在 CD4^{+} T 细胞、肥大细胞、半不变 NKT 细胞（iNKT）、树突状细胞、肾上皮细胞和各种粘膜上皮细胞上表达（4, 8-15）。活化的 Th2 细胞、树突状细胞成熟后以及肾小管上皮细胞损伤后，TIM-1 的表达都会上调（4, 9, 13, 14, 16, 17）。金属蛋白酶介导的 TIM-1 在膜近端区域的裂解会导致 TIM-1 可溶形式的释放，这种形式的 TIM-1 可在尿液和血液循环中检测到（18, 19）。肾病患者的尿 TIM-1 高度升高，可能是肾损伤的有用生物标志物（16, 20 - 25）。

据报道，TIM-1 是多种配体的受体，包括磷脂酰丝氨酸、白细胞单免疫球蛋白样受体 5（LMIR5/CD300b），TIM-1（嗜同性），TIM-4，IgA 和多种包膜病毒的糖蛋白（5, 15, 26-33）。它与磷脂酰丝氨酸的相互作用使 TIM-1 能够介导对凋亡细胞的吞噬作用（26-28）。在含有 TIM-1 的 iNKT 细胞中，与凋亡细胞的相互作用也会导致 iNKT 细胞的活化、增殖和细胞因子的产生（11）。细胞表面或可溶性 TIM-1 与 LMIR5 之间的相互作用被认为可诱导 LMIR5 介导的髓系细胞活化，包括巨噬细胞/单核细胞、肥大细胞、中性粒细胞和树突状细胞（29）。这些相互作用有助于肾脏损伤过程中的组织稳态和损伤（29）。配体诱导的 TIM-1 信号刺激 T 细胞活化并增强 Th2 细胞因子的产生（9, 31, 34）。在人体中，TIM-1 是甲型肝炎病毒、埃博拉病毒和马尔堡病毒等多种病毒的细胞进入受体（15, 33）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人TIM-1抗体包被于微孔板上，样品和标准品中的人TIM-1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TIM-1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（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40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（ng/mL）	0.98	3.04	5.88	1.09	3.19	6.23
标准差	0.042	0.119	0.259	0.069	0.193	0.484
CV%	4.3	3.9	4.4	6.3	6.1	7.8

B. 回收率

不同类型样本中掺入检测范围内不同水平的人TIM-1，测定其回收率。

样本类型	平均回收率（%）	范围（%）
人尿液样本（n=20）	104	94-112

C. 灵敏度

进行39次测试，人TIM-1的最小可检测剂量（MDD）范围为0.003-0.046 ng/mL。平均MDD为0.009 ng/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NS0表达的重组人TIM-1蛋白校正。

E. 线性

为了评估该检测方法的线性，将含有高浓度人TIM-1样本用标准品稀释液（1×）进行梯度稀释，得到符合检测范围的样本。有关线性及尿液样本处理的信息，请参阅《定量分析方法线性评价：统计方法》（NCCLS）出版物 EP6-A。

稀释倍数		人尿液（n=9）
1:2	平均值/期待值 （%）	105
	范围 （%）	98-112
1:4	平均值/期待值 （%）	109
	范围 （%）	100-117
1:8	平均值/期待值 （%）	110
	范围 （%）	101-118
1:16	平均值/期待值 （%）	103
	范围 （%）	95-110

F. 样本预值

人尿液 - 使用此试剂盒评估 26 份表面健康志愿者尿液样本中 TIM-1 的存在。本研究中使用的供体没有病史。

样品	平均值	范围	标准差
TIM-1 (ng/mL)	1.35	0.156-5.33	1.09
TIM-1 (µg/g Creatinine)	1.11	0.225-3.20	0.689

G. 特异性

此ELISA法可检测天然及重组人TIM-1蛋白。

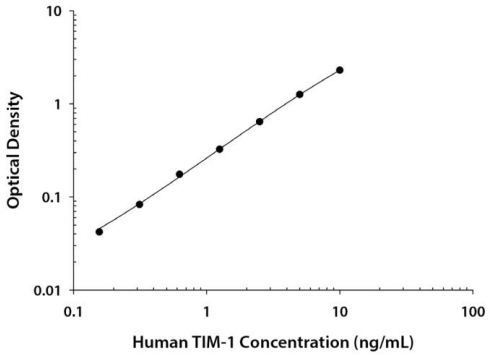
将以下蛋白用标准品稀释液（1×）配制成100 ng/mL的浓度来检测交叉反应。将100 ng/mL的干扰蛋白掺入中间范围的重组人TIM-1对照品中，来检测干扰。没有观察到明显的交叉反应或干扰。

Recombinant human：	**Recombinant mouse:**	**Recombinant rat:**
TIM-3	TIM-1	TIM-1
TIM-4	TIM-4	

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。



(ng/mL)	O.D.	Average	Corrected
0	0.012 0.012	0.012	—
0.156	0.053 0.055	0.054	0.042
0.313	0.094 0.096	0.095	0.083
0.625	0.187 0.187	0.187	0.175
1.25	0.331 0.343	0.337	0.325
2.5	0.644 0.666	0.655	0.643
5	1.256 1.282	1.269	1.257
10	2.271 2.343	2.307	2.295

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human TIM-1 Microplate	包被抗人TIM-1抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human TIM-1 Conjugate	酶标检测抗人TIM-1抗体	1瓶
Human TIM-1 Standard	重组人TIM-1标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1-82	检测液	1瓶
Calibrator Diluent Concentrate (2×) / RD6Q	标准品稀释液用于稀释标准品和样品	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="7">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="5">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RD1-82</td></tr>
<tr><td>标准品</td></tr>
<tr><td>TMB底物溶液</td><td rowspan="2">2-8°C储存，最多30天*
请每次使用新鲜配制的1×标准品稀释液，多余的丢弃</td></tr>
<tr><td>浓缩标准品稀释液（2×）/ RD6Q</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
- 50 mL和500 mL量筒
- 标准品稀释用聚丙烯管

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. 样品收集及储存

注：*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。*

尿液 - 采用无菌技术收集晨尿（中段），直接排入无菌容器。离心分离去除颗粒物。立即检测，或分装并≤ -20 °C储存。避免反复冻融循环。样品可能需要用标准品稀释液（1×）稀释。

B. 检测前准备工作

使用前请将所有试剂放置于室温。

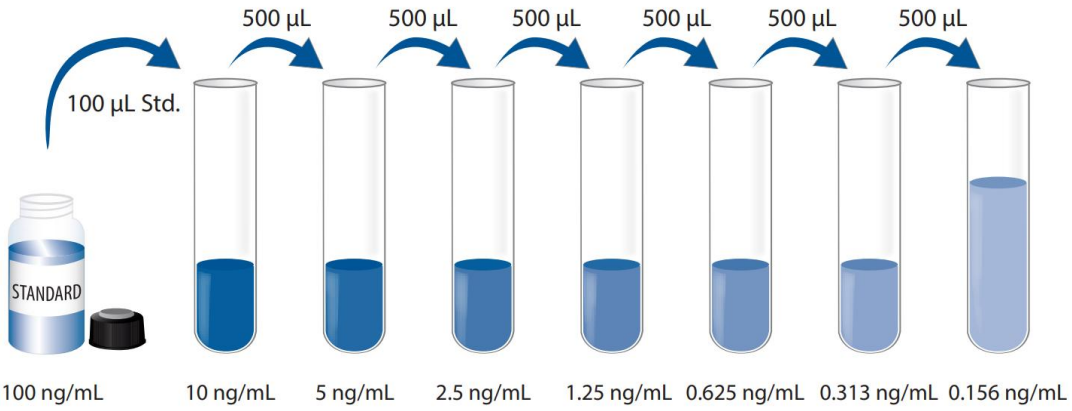
洗涤液（1×）- 从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液（1×）- 使用去离子水或蒸馏水配制标准品稀释液（1×）。

人TIM-1标准品：复溶体积请参考瓶身标签*，用去离子水或蒸馏水复溶人TIM-1标准品，得到浓度为100 ng/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900 µL标准品稀释液（1×）移入10 ng/mL管中。在剩余管中均加入500 µL标准品稀释液（1×）。将标标准品储备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。10 ng/mL作标准曲线最高点，标准品稀释液（1×）可用作标准曲线零点（0 ng/mL）。



C. 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100 µL检测液RD1-82。
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔50 µL。用封板膜封住反应孔，**在室温下孵育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；
6. 在每个微孔内加入200 µL人TIM-1酶标检测抗体。用新的封板膜封住反应孔，**在室温下孵育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30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.），使用计算机软件作四参数逻辑（4-PL）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，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人TIM-1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。该程序将产生足够但不太精确的数据拟合。

    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。

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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