PRODUCT INFORMATION & MANUAL

Mouse TIM-1/KIM-1/HAVCR Valukine™ ELISA

Catalog Number: VAL646

For the quantitative determination of natural and recombinant mouse T cell-Immunoglobulin-Mucin (TIM-1)/KIM-1/HAVCR 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

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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 (HAVCR), is a member of the TIM family which is involved in the regulation of innate and adaptive immune responses (1, 2). Mouse 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 alternate 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, mouse TIM-1 shares 41% and 82% amino acid (aa) sequence identity with human and rat TIM-1, respectively.

Mouse 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-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 co-stimulates 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 mouse TIM-1 has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse TIM-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse 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 mouse 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 cell culture supernates, mouse plasma, mouse serum and mouse 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 twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Mean (pg/mL)	24.2	61.8	207	23.2	60.0	190
Standard Deviation	0.8	1.6	6.8	2.5	4.2	13.2
CV%	3.3	2.6	3.3	10.8	7.0	6.9

B. RECOVERY

The recovery of mouse TIM-1 spiked into various matrices was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture media (n=4)	100	91-106
Mouse serum (n=4)	95	93-97
Mouse EDTA plasma (n=4)	89	88-91
Mouse heparin plasma (n=4)	87	86-89
Mouse urine* (n=4)	102	94-115

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

C. SENSITIVITY

Fifty-four assays were evaluated and the minimum detectable dose (MDD) of mouse TIM-1 ranged from 0.22-2.18 pg/mL. The mean MDD was 0.71 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 NS0-derived recombinant mouse TIM-1 (Accession # Q3V033 amino acids Y22-T212; isoform 2) 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 TIM-1 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture samples* (n=4)	Mouse serum (n=4)	Mouse EDTA plasma (n=4)	Mouse heparin plasma (n=4)	Mouse urine* (n=4)
1:2	Average % of Expected	98	103	109	103	100
	Range (%)	97-101	101-104	106-115	101-107	96-109
1:4	Average % of Expected	99	102	111	106	100
	Range (%)	98-100	99-104	110-115	101-113	84-110
1:8	Average % of Expected	101	99	108	104	106
	Range (%)	99-104	96-102	101-116	98-113	105-107
1:16	Average % of Expected	103	96	106	107	105
	Range (%)	100-108	91-99	102-108	97-115	104-105

* Samples were diluted prior to assay.

F. SAMPLE VALUES

Mouse serum/ plasma/urine - Samples were evaluated for detectable levels of mouse TIM-1 in this assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Mouse serum (n=20)	17.2	90	ND-42.5
Mouse EDTA plasma (n=20)	25.6	100	7.87-174
Mouse heparin plasma (n=20)	18.0	95	ND-42.8
Mouse urine (n=20)	2248	100	456-8048

ND=Non-detectable

Mouse serum samples from two Balb/c strain mice were evaluated in this assay and measured 27.5 pg/mL and 26.9 pg/mL. Additionally, mouse serum samples from two C57 strain mice were evaluated in this assay and measured 20.4 pg/mL and 14.4 pg/mL.

Mouse urine samples from two Balb/c strain mice and one C57 strain mouse were evaluated in this assay and measured 143,000 pg/mL, 159,000 pg/mL, and 726 pg/mL respectively.

Cell Culture Supernates - Kidneys from mice were removed, rinsed in 1 x PBS, and kept on ice in 1 x PBS. Kidneys were then homogenized using a tissue homogenizer and seeded into media containing RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured for the times indicated. Aliquots of the cell culture supernates were removed and assayed for levels of mouse TIM-1.

Tissue Type	(pg/mL)
NSA mouse kidney (1 day)	1006
NSA mouse kidney (3 days)	756
Balb/c mouse kidney (2 days)	1679
C57 mouse kidney (2 days)	24

G. SPECIFICITY

This assay recognizes natural and recombinant mouse TIM-1.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse TIM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

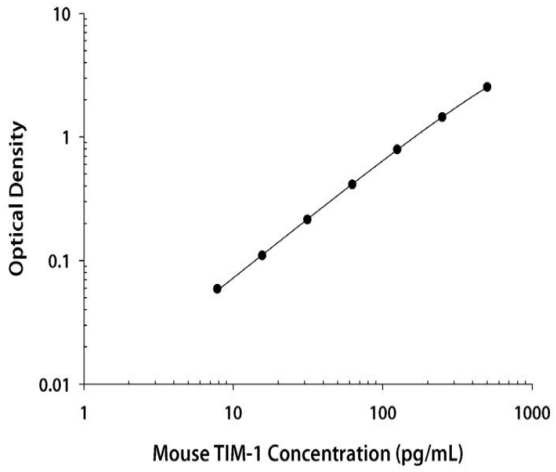
Recombinant mouse:		**Recombinant rat:**
Cystatin C	MMP-12	Lipocalin-2/NGAL
Lipocalin-2/NGAL	TIM-2	TIM-1/KIM-1/HAVCR
MMP-2	TIM-3	**Recombinant human:**
MMP-3	TIM-4	TIM-1/KIM-1/HAVCR
MMP-7	TIM-5	TIM-3
MMP-8	TIM-6	TIM-4
MMP-9	TIM-7	

The antibody components of this kit were raised against the short splice form of mouse TIM-1 (isoform 2) which has a 23 aa deletion following Pro182. This immunoassay detects both the short and long forms of mouse TIM-1.

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	0.016 0.016	0.016	—
7.81	0.070 0.080	0.075	0.059
15.6	0.126 0.126	0.126	0.110
31.3	0.229 0.232	0.231	0.215
62.5	0.425 0.433	0.429	0.413
125	0.802 0.813	0.808	0.792
250	1.462 1.463	1.463	1.447
500	2.533 2.565	2.549	2.533

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse TIM-1 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse TIM-1.	1 plate
Mouse TIM-1 Conjugate	An antibody specific for mouse TIM-1 conjugated to horseradish peroxidase.	1 vial
Mouse TIM-1 Standard	Recombinant mouse TIM-1 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Mouse TIM-1 Control	Recombinant mouse TIM-1 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 RD1-55	A buffered protein base.	1 vial
Calibrator Diluent Concentrate (4×)/ RD5-26	A 4× concentrated 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="9">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>Assay Diluent RD1-55</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Control</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Calibrator Diluent Concentrate (4×)/ RD5-26</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>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.
- 100 mL and 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- 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 (1×).

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x 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 (1×).

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x 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 (1×).

Note: *Citrate plasma has not been validated for use in this assay*.

Hemolyzed samples are not suitable for use in this assay.

Urine - Collect urine using a metabolic cage. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Mouse urine samples recommend a 15-fold dilution. A suggested 15-fold dilution is 10 µL of sample + 140 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

The conjugate must be kept cold during use. Bring all reagents to room temperature before use.

Mouse TIM-1 Control - Reconstitute the control with 1.0 mL of deionized or distilled

water. Mix thoroughly. Assay the control undiluted.

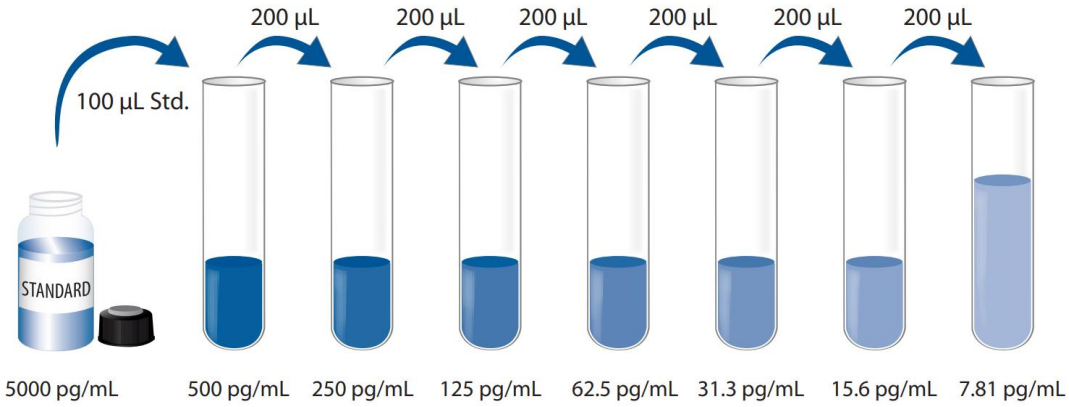
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

Mouse TIM-1 Standard- Refer to the vial label for the reconstitution volume* Reconstitute the Mouse TIM-1 Standard with Calibrator Diluent (1×). This reconstitution produces a stock solution of 5000 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 900 µL of Calibrator Diluent (1×) into the 500 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. The Calibrator Diluent (1×) serves as the zero standard (0 pg/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.

- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep 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

The conjugate must be kept cold during use. 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 RD1-55 to each well.
4. Add 50 µL of standard, control and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.** 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 cold Mouse TIM-1 Conjugate to each well. Cover with a new adhesive strip. **Incubate for 1 hour at 2-8 °C without shaking.**
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 on the benchtop. 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 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 mouse 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

1. Freeman, G.J. *et al.* (2010) Immunol. Rev. 235:172.
2. Waanders, F. *et al.* (2010) J. Pathol. 220:7.
3. McIntire, J.J. *et al.* (2001) Nat. Immunol. 2:1109
4. Kuehn, E.W. *et al.* (2002) Am. J. Physiol. Renal Physiol. 283:F1326.
5. Kim, H.Y. *et al.* (2011) J. Clin. Invest. 121:1111.
6. Ding, Q. *et al.* (2011) J. Clin. Invest. 121:3645.
7. Ma, J. *et al.* (2011) Biochem. Biophys. Res. Commun. 406:223.
8. de Souza, A.J. *et al.* (2005) Proc. Natl. Acad. Sci. 102:17113.
9. Umetsu, S.E. *et al.* (2005) Nat. Immunol. 6:447.
10. Nakae, S. *et al.* (2007) Blood 110:2565.
11. Lee, H.H. *et al.* (2010) J. Immunol. 185:5225.
12. Kim, H.S. *et al.* (2010) J. Immunol. 184:4095.
13. Xiao, S. *et al.* (2011) Eur. J. Immunol. 41:1539.
14. Ichimura, T. *et al.* (1998) J. Biol. Chem. 273:4135.
15. Kondratowicz, A.S. *et al.* (2011) Proc. Natl. Acad. Sci. 108:8426.
16. Ichimura, T. *et al.* (2003) Am. J. Physiol. Renal Physiol. 286:F552.
17. van Timmeren, M.M. *et al.* (2006) Am. J. Physiol. Renal Physiol. 291:F456.
18. Zhang, Z. *et al.* (2007) J. Am. Soc. Nephrol. 18:2704.
19. Bailly, V. *et al.* (2002) J. Biol. Chem. 277:39739.
20. Vaidya, V.S. *et al.* (2006) Am. J. Physiol. Renal Physiol. 290:F517.
21. Kramer, A.B. *et al.* (2009) Am. J. Physiol. Renal Physiol. 296:F1136.
22. Prozialeck, W.C. *et al.* (2009) Toxicol. Appl. Pharmacol. 238:306.
23. Han, W.K. *et al.* (2002) Kidney Int. 62:237.
24. Wasilewska, A. *et al.* (2011) Pediatr. Nephrol. 26:579.
25. Nielsen, S.E. *et al.* (2010) Diabet. Med. 27:1144.
26. Miyanishi, M. *et al.* (2007) Nature 450:435.
27. Kobayashi, N. *et al.* (2007) Immunity 27:927.

28. Ichimura, T. *et al.* (2008) J. Clin. Invest. 118:1657.

29. Yamanishi, Y. *et al.* (2010) J. Exp. Med. 207:1501.

30. Santiago, C. *et al.* (2007) Immunity 26:299.

31. Meyers, J.H. *et al.* (2005) Nat. Immunol. 6:455.

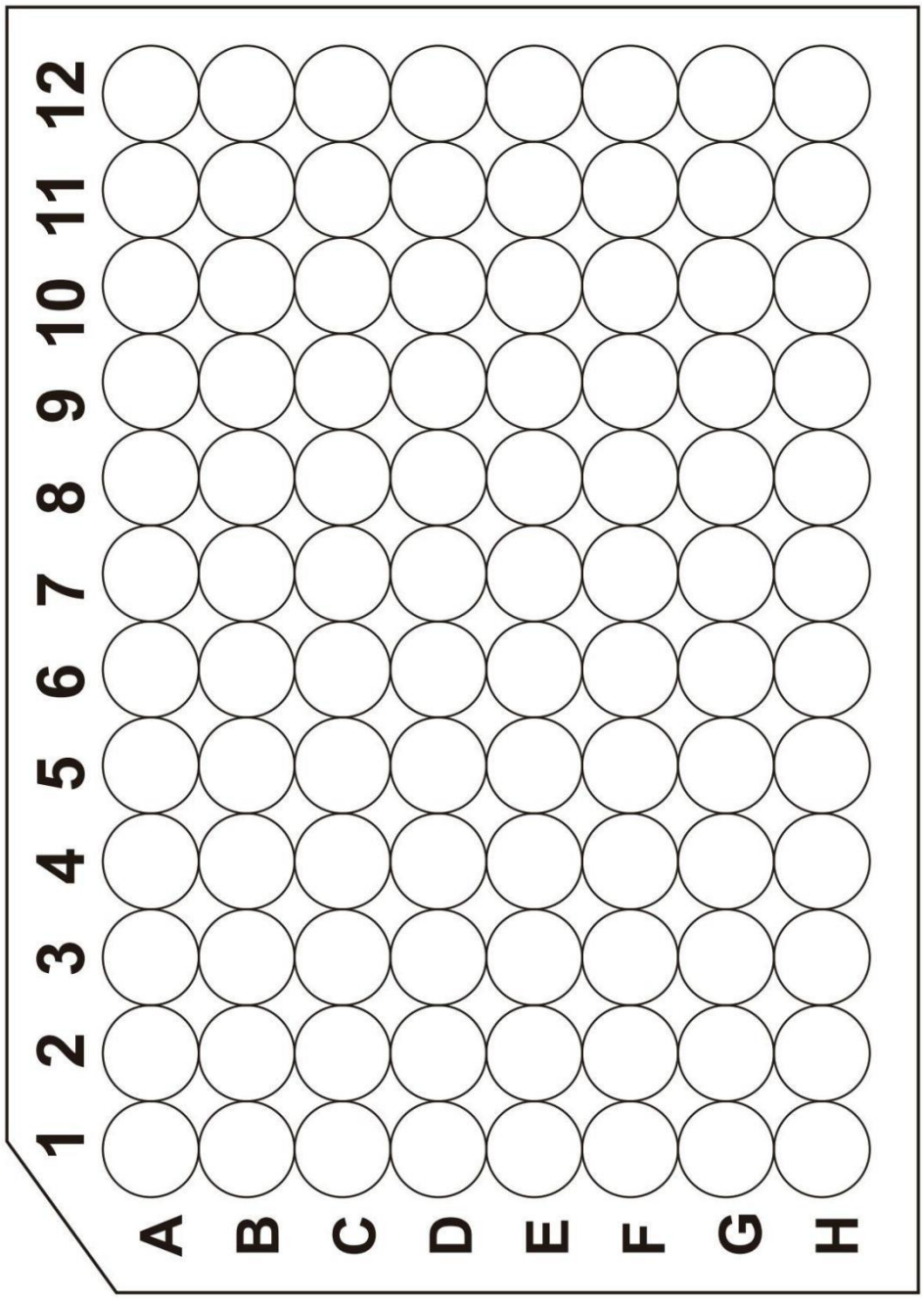
32. Tami, C. *et al.* (2007) J. Virol. 81:3437.

33. Feigelstock, D. *et al.* (1998) J. Virol. 72:6621.

34. Binne, L.L. *et al.* (2007) J. Immunol. 178:4342.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 TIM-1/KIM-1/HAVCR Valukine™ ELISA 试剂盒

目录号：VAL646

适用于定量检测天然和重组小鼠 T 细胞免疫球蛋白(TIM-1)/KIM-1/HAVCR 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

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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（T cell Immunoglobulin and Mucin domain 1，TIM-1）又称肾损伤分子 1（Kidney Injury Molecule 1，KIM-1）和甲型肝炎病毒细胞受体（Hepatitis A Virus Cellular Receptor，HAVCR），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）。小鼠 TIM-1 是 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）。由于多态性或选择性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，小鼠 TIM-1 与人类和大鼠 TIM-1 分别有 41%和 82%的氨基酸序列一致性。

小鼠 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、不变性 NKT（invariant NKT，iNKT）细胞、树突状细胞、肾上皮细胞和多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 的可溶形式释放，这种形式可在尿液和血液循环中检测到（18, 19）。尿液中的 TIM-1 在肾病患者中高度升高，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（leukocyte mono-immunoglobulin-like receptor 5，LMIR5/CD300b）、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（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、嗜中性粒细胞和树突状细胞（29）。这些相互作用有助于组织稳态和肾损伤期间的损害（29）。配体诱导的 TIM-1 信号可共同刺激 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 作为多种病毒的细胞进入受体，包括甲型肝炎病毒、埃博拉病毒和马尔堡病毒（15, 33）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠TIM-1抗体包被于微孔板上，样品，质控品和标准品中的小鼠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小鼠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；**
- 该试剂盒适用于细胞培养上清样本，小鼠血清样本，小鼠血浆样本和小鼠尿液样本；
- 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)	24.2	61.8	207	23.2	60.0	190
标准差	0.8	1.6	6.8	2.5	4.2	13.2
CV%	3.3	2.6	3.3	10.8	7.0	6.9

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TIM-1，测定其回收率。

样本类型	平均回收率%	范围 (%)
细胞培养基 (n=4)	100	91-106
小鼠血清 (n=4)	95	93-97
小鼠EDTA血浆 (n=4)	89	88-91
小鼠肝素血浆 (n=4)	87	86-89
小鼠尿液* (n=4)	102	94-115

*样品在检测前按照样品制备部分的指示进行样本稀释

C. 灵敏度

54次检测结果表明，小鼠TIM-1的最低可测剂量（MDD）范围为0.22- 2.18 pg/mL。平均MDD为0.71 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NS0衍生的重组小鼠TIM-1(Accession # Q3V033 amino acids Y22-T212; isoform 2)校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠TIM-1，然后用标准品稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养样本* (n=4)	小鼠血清 (n=4)	小鼠EDTA血浆(n=4)	小鼠肝素血浆 (n=4)	小鼠尿液* (n=4)
1:2	平均值/期待值（%）	98	103	109	103	100
	范围（%）	97-101	101-104	106-115	101-107	96-109
1:4	平均值/期待值（%）	99	102	111	106	100
	范围（%）	98-100	99-104	110-115	101-113	84-110
1:8	平均值/期待值（%）	101	99	108	104	106
	范围（%）	99-104	96-102	101-116	98-113	105-107
1:16	平均值/期待值（%）	103	96	106	107	105
	范围（%）	100-108	91-99	102-108	97-115	104-105

*样品在检测前按照样品制备部分的指示进行样本稀释

F. 样本预值

小鼠血清/血浆/尿液样本 - 在此测定中对样品进行小鼠TIM-1的可检测水平的评估。

样本类型	平均可检测值 (pg/mL)	检测率 %	范围(pg/mL)
小鼠血清样本 (n=20)	17.2	90	ND-42.5
小鼠EDTA血浆样本 (n=20)	25.6	100	7.87-174
小鼠肝素血浆样本 (n=20)	18.0	95	ND-42.8
小鼠尿液样本 (n=20)	2248	100	456-8048

ND=Non-detectable

在该试验中测定两只Balb/c品系小鼠的血清样本分别为 27.5 pg/mL和26.9 pg/mL。另外，本试验还对两只C57品系小鼠的血清样本进行测定，结果分别为 20.4 pg/mL 和 14.4 pg/mL。

该试验对2只 Balb/c品系小鼠和1只C57品系小鼠的尿液样本进行测定，结果分别为 143000 pg/mL、159000 pg/mL 和 726 pg/mL。

细胞上清样本 - 取出小鼠肾脏，置于冰上，用1 × PBS冲洗，并保存在1 × PBS中。然后用组织匀浆器将肾脏匀浆，并将其培养在含有10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和 100 µg/mL 硫酸链霉素的RPMI 1640培养基中。细胞在指定时间内培养。取出等分的细胞培养上清，检测小鼠TIM-1的水平。

组织种类	(pg/mL)
NSA小鼠肾脏（1天）	1006
NSA小鼠肾脏（3 天）	756
Balb/c小鼠肾脏（2天）	1679
C57小鼠肾脏（2天）	24

G. 特异性

此ELISA法可检测天然及重组小鼠TIM-1。

将以下因子用标准品稀释液（1×）配制成50 ng/mL的浓度来检测与小鼠TIM-1的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组小鼠TIM-1对照品中，来检测对小鼠TIM-1的干扰。没有观察到明显的交叉反应或干扰。

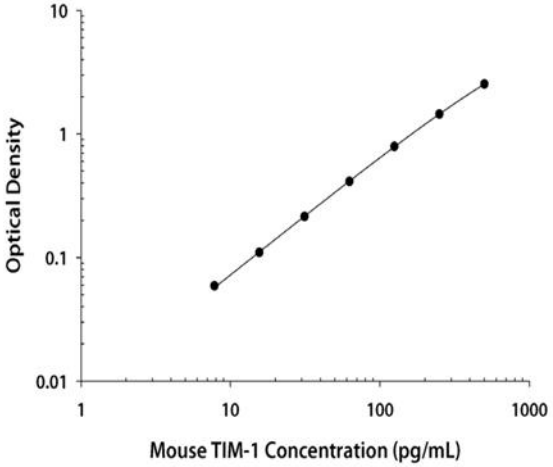
Recombinant mouse:		**Recombinant rat:**
Cystatin C	MMP-12	Lipocalin-2/NGAL
Lipocalin-2/NGAL	TIM-2	TIM-1/KIM-1/HAVCR
MMP-2	TIM-3	**Recombinant human:**
MMP-3	TIM-4	TIM-1/KIM-1/HAVCR
MMP-7	TIM-5	TIM-3
MMP-8	TIM-6	TIM-4
MMP-9	TIM-7	

该试剂盒中的抗体成分是针对小鼠TIM-1的短剪接体（异构体 2），该异构体在 Pro182之后有23个氨基酸缺失。该试剂盒可以检测小鼠TIM-1的短型和长型。

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	0.016 0.016	0.016	—
7.81	0.070 0.080	0.075	0.059
15.6	0.126 0.126	0.126	0.110
31.3	0.229 0.232	0.231	0.215
62.5	0.425 0.433	0.429	0.413
125	0.802 0.813	0.808	0.792
250	1.462 1.463	1.463	1.447
500	2.533 2.565	2.549	2.533

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse TIM-1 Microplate	包被抗小鼠TIM-1抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Mouse TIM-1 Conjugate	酶标检测抗小鼠TIM-1抗体	1瓶
Mouse TIM-1 Standard	小鼠TIM-1标准品（冻干），参考瓶身标签进行重溶	1瓶
Mouse TIM-1 Control	小鼠TIM-1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 RD1-55	检测液	1瓶
Calibrator Diluent Concentrate (4×)/ RD5-26	浓缩标准品稀释液（4×）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RD1-55</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>质控品</td></tr>
<tr><td>标准品</td><td rowspan="2">2-8℃储存，最多 30 天*
请每次使用新鲜配制的1×标准品稀释液，多余的丢弃</td></tr>
<tr><td>浓缩标准品稀释液(4×)/ RD5-26</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
- 100mL和500mL量筒
- 水平振荡器（0.12” 轨道），转速：500±50 rpm
- 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（1×）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（1×） 稀释。

血浆样本：使用 EDTA 或肝素作为抗凝剂收集血浆。然后2000 × g离心20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°C储存备用。避免反复冻融。样本可能需要用稀释液（1×）稀释。

注意： *本试剂盒对枸橼酸钠血浆尚未被验证。*

溶血样本不适合用于本试剂盒。

尿液样本：使用代谢笼收集尿液。立即通过离心和分析去除任何颗粒，或将样本分装并≤ -20°C储存备用。避免反复冻融。在测定前再次离心，以去除储存后可能出现的任何其他沉淀。样本可能需要用稀释液（1×）稀释。

B. 样本准备工作

小鼠尿液样本建议用标准品稀释液（1×）15倍稀释后进行检测，即10 µL血清+140 µL标准品稀释液（1×）。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。酶标检测抗体在使用时保持低温。

小鼠TIM-1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×）。

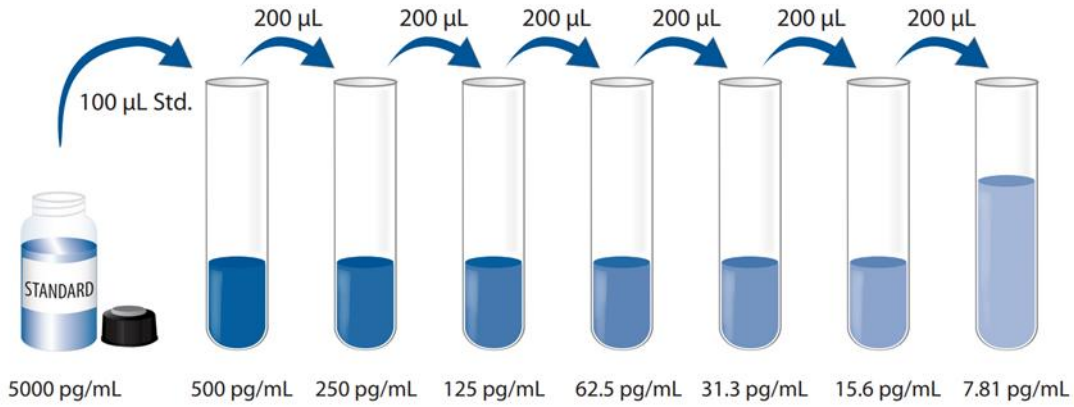
标准品稀释液（1×）：使用蒸馏水或去离子水稀释配制成标准品稀释液（1×）。

小鼠TIM-1标准品：重溶体积请参考瓶身标签*，用标准品稀释液（1×）重溶小鼠TIM-1标准品。得到浓度为5000 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。

取900 µL标准品稀释液（1×）移入500 pg/mL稀释管中。剩余每管中加入200 µL标准

品稀释液（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。500 pg/mL标准品可用作标准曲线最高点，标准品稀释液（1×）可用作标准曲线零点（0 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，酶标检测抗体在使用时保持低温。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50μL检测液RD1-55。
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。用封板膜封住反应孔，**在水平振荡器（0.12”轨道）上，转速：500±50 rpm，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冰的TIM-1小鼠酶标检测抗体。用封板膜封住反应孔，**2-8°C孵育1小时无需振荡**；
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。数据可以通过绘制小鼠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。

VIII. 参考文献

1. Freeman, G.J. et al. (2010) Immunol. Rev. 235:172.
2. Waanders, F. et al. (2010) J. Pathol. 220:7.
3. McIntire, J.J. et al. (2001) Nat. Immunol. 2:1109
4. Kuehn, E.W. et al. (2002) Am. J. Physiol. Renal Physiol. 283:F1326.
5. Kim, H.Y. et al. (2011) J. Clin. Invest. 121:1111.
6. Ding, Q. et al. (2011) J. Clin. Invest. 121:3645.
7. Ma, J. et al. (2011) Biochem. Biophys. Res. Commun. 406:223.
8. de Souza, A.J. et al. (2005) Proc. Natl. Acad. Sci. 102:17113.
9. Umetsu, S.E. et al. (2005) Nat. Immunol. 6:447.
10. Nakae, S. et al. (2007) Blood 110:2565.
11. Lee, H.H. et al. (2010) J. Immunol. 185:5225.
12. Kim, H.S. et al. (2010) J. Immunol. 184:4095.
13. Xiao, S. et al. (2011) Eur. J. Immunol. 41:1539.
14. Ichimura, T. et al. (1998) J. Biol. Chem. 273:4135.
15. Kondratowicz, A.S. et al. (2011) Proc. Natl. Acad. Sci. 108:8426.
16. Ichimura, T. et al. (2003) Am. J. Physiol. Renal Physiol. 286:F552.
17. van Timmeren, M.M. et al. (2006) Am. J. Physiol. Renal Physiol. 291:F456.
18. Zhang, Z. et al. (2007) J. Am. Soc. Nephrol. 18:2704.
19. Bailly, V. et al. (2002) J. Biol. Chem. 277:39739.
20. Vaidya, V.S. et al. (2006) Am. J. Physiol. Renal Physiol. 290:F517.
21. Kramer, A.B. et al. (2009) Am. J. Physiol. Renal Physiol. 296:F1136.
22. Prozialeck, W.C. et al. (2009) Toxicol. Appl. Pharmacol. 238:306.
23. Han, W.K. et al. (2002) Kidney Int. 62:237.
24. Wasilewska, A. et al. (2011) Pediatr. Nephrol. 26:579.
25. Nielsen, S.E. et al. (2010) Diabet. Med. 27:1144.
26. Miyanishi, M. et al. (2007) Nature 450:435.

27. Kobayashi, N. et al. (2007) Immunity 27:927.

28. Ichimura, T. et al. (2008) J. Clin. Invest. 118:1657.

29. Yamanishi, Y. et al. (2010) J. Exp. Med. 207:1501.

30. Santiago, C. et al. (2007) Immunity 26:299.

31. Meyers, J.H. et al. (2005) Nat. Immunol. 6:455.

32. Tami, C. et al. (2007) J. Virol. 81:3437.

33. Feigelstock, D. et al. (1998) J. Virol. 72:6621.

34. Binne, L.L. et al. (2007) J. Immunol. 178:4342.

96 孔模板图

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