PRODUCT INFORMATION & MANUAL

Mouse TIMP-1 Valukine™ ELISA

Catalog Number: VAL652

For the quantitative determination of natural and recombinant mouse Tissue Inhibitor of Metalloproteinases 1 (TIMP-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

Matrix metalloproteinases (MMPs), also called matrixins, constitute a family of zinc and calcium dependent endopeptidases that function in the breakdown of extracellular matrix and in the processing of a variety of biological molecules. They play an important role in many normal physiological processes such as embryonic development, morphogenesis, reproduction and tissue remodeling (1). They also participate in many pathological processes such as arthritis, cancer and cardiovascular disease (2). While the amounts of newly synthesized MMPs are regulated mainly at the levels of transcription, the proteolytic activities of existing MMPs are controlled through both the activation of proenzymes or zymogens and the inhibition of active enzymes by endogenous inhibitors such as α_2-macroglobulins and tissue inhibitors of metalloproteinases (TIMPs).

The mammalian TIMP family includes four members that share structural similarity (3). All TIMP proteins have 12 conserved cysteine residues that form six intrachain disulfide bonds, resulting in an extremely stable protein with six loops. The TIMP protein has two structurally and functionally distinct domains: the N-terminal domain consisting of loops 1-3 that are responsible for tight but non-covalent binding to the active MMPs in a 1:1 stoichiometry; and the C-terminal domain consisting of loops 4-6 that enhances the enzyme-inhibitor interactions. In the case of TIMP-1, the C-terminal domain has also been shown to bind the hemopexin-like domain of pro MMP-9. TIMP-1 stimulates erythropoiesis, inhibits angiogenesis and is an anti- apoptotic agent for B cells. These TIMP-1 functions may be independent of MMP inhibition (4-6).

Mouse TIMP-1 is a 28-35 kDa secreted glycoprotein (6, 7). The protein is synthesized as a 205 amino acid (aa) precursor that contains a 24 aa signal peptide and a 181 aa mature form (8, 9). Mouse TIMP-1 shares 85%, 77%, 68%, and 67% aa sequence identity with rat, human, porcine, and canine TIMP-1, respectively (10-13). Among the three known mouse TIMPs, TIMP-1 shares 39% and 38% aa sequence identity with TIMP-2 and TIMP-3, respectively (14, 15). TIMP-1 is widely expressed in many cells including fibroblasts, osteoblasts, endothelial cells, granulosa cells, dendritic cells, vascular smooth muscle cells, adipocytes, and monocytes (6, 16-19).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse TIMP-1 has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse TIMP-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse TIMP-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 TIMP-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 and mouse 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 RD5-17 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)	91.8	282	721	89.7	293	721
Standard Deviation	9.4	12.6	23.0	6.5	22.1	41.4
CV%	10.2	4.5	3.2	7.2	7.5	5.7

B. RECOVERY

The recovery of mouse TIMP-1 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture media (n=6)	102	92-112
Mouse serum* (n=6)	101	84-117
Mouse EDTA plasma* (n=4)	107	92-119

*Samples were diluted prior to assay.

C. SENSITIVITY

Seven assays were evaluated and the minimum detectable dose (MDD) of mouse TIMP-1 ranged from 1.4-3.5 pg/mL. The mean MDD was 2.1 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 NS0-expressed recombinant

mouse TIMP-1 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 TIMP-1 and diluted with Calibrator Diluent RD5-17 to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture samples (n=5)	Mouse serum* (n=6)	Mouse EDTA Plasma* (n=4)
1:2	Average % of Expected	96	97	99
	Range (%)	91-102	93-107	97-103
1:4	Average % of Expected	98	98	104
	Range (%)	90-103	93-109	95-107
1:8	Average % of Expected	97	100	107
	Range (%)	85-103	92-113	103-117
1:16	Average % of Expected	98	102	109
	Range (%)	89-108	94-113	101-116

* Samples were diluted prior to assay.

F. SAMPLE VALUES

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

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Mouse serum (n=20)	2350	1280-3650	730
Mouse EDTA plasma (n=20)	2270	1250-3390	560

Cell Culture Supernates：

L-929 mouse fibroblast cells (1 × 10^6 cells/mL) were cultured for 3 days in MEM supplemented with 10% equine serum and stimulated with 2.5 ng/mL LPS. An aliquot of the cell culture supernate was removed, assayed for mouse TIMP-1, and measured 26.6 ng/mL.

Mouse lung conditioned media (1-2 mm pieces in 40 mL of medium) were cultured for 7 days in RPMI supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for mouse TIMP-1, and measured 121 ng/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant mouse TIMP-1.

The factors listed below were prepared at 50 ng/mL or 500 ng/mL in Calibrator Diluent RD5-17 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a recombinant mouse TIMP-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:	**Recombinant human:**	
MMP-3	MMP-1	MMP-12
MMP-9 (pro)	MMP-2	MMP-13
MMP-9 (active)	MMP-3	TIMP-1
	MMP-7	TIMP-2
	MMP-8	TIMP-3
	MMP-9	TIMP-4
	MMP-10	

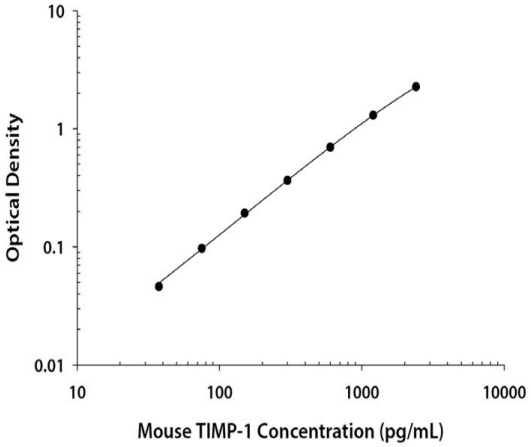
Recombinant rat TIMP-1 does not interfere but does cross-react approximately 0.04% in this assay.

This assay detects approximately 80% of the recombinant mouse TIMP-1 complexed with the pro-form of recombinant mouse MMP-9 and approximately 60% when complexed with active recombinant mouse MMP-9.

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	0.031 0.031	0.031	—
37.5	0.077 0.077	0.077	0.046
75	0.126 0.131	0.128	0.097
150	0.224 0.224	0.224	0.193
300	0.393 0.399	0.396	0.365
600	0.728 0.729	0.728	0.697
1200	1.294 1.369	1.332	1.301
2400	2.245 2.342	2.294	2.263

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse TIMP-1 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse TIMP-1.	1 plate
Mouse TIMP-1 Conjugate	An antibody specific for mouse TIMP-1 conjugated to horseradish peroxidase.	1 vial
Mouse TIMP-1 Standard	Recombinant mouse TIMP-1 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Mouse TIMP-1 Control	Recombinant mouse TIMP-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 RD1-21	A buffered protein base.	1 vial
Calibrator Diluent RD5-17	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="5">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Assay Diluent RD1-21</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Calibrator Diluent RD5-17</td><td rowspan="2">Aliquot and store for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Control</td></tr>
<tr><td>Standard</td><td rowspan="2">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>
<tr><td>Microplate Wells</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.
- Polypropylene 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 RD5-17.

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 RD5-17.

Plasma - Collect plasma using EDTA 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 RD5-17.

Note: *Heparin and citrate plasma have not been validated for use in this assay*.

Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

B. SAMPLE PREPARATION

Use polypropylene tubes.

Cell culture supernate sample may require dilution. Optimal dilutions should be determined by the end user.

Mouse serum and plasma samples recommend a 3-fold dilution prior to assay. A suggested 3-fold dilution is 40 µL of sample + 80 µL of Calibrator Diluent RD5-17. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse TIMP-1 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

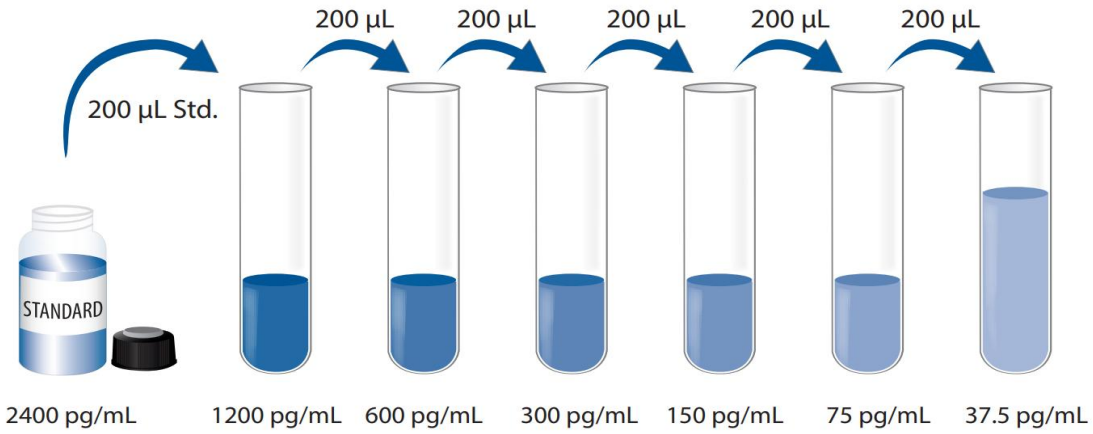
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Mouse TIMP-1 Standard- Refer to the vial label for the reconstitution volume*

Reconstitute the Mouse TIMP-1 Standard with Calibrator Diluent RD5-17. Do not substitute other diluents. This reconstitution produces a stock solution of 2400 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.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-17 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 TIMP-1 Standard (2400 pg/mL) serves as the high standard. The Calibrator Diluent RD5-17 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, standard dilutions, 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 RD1-21 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 TIMP-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 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 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 TIMP-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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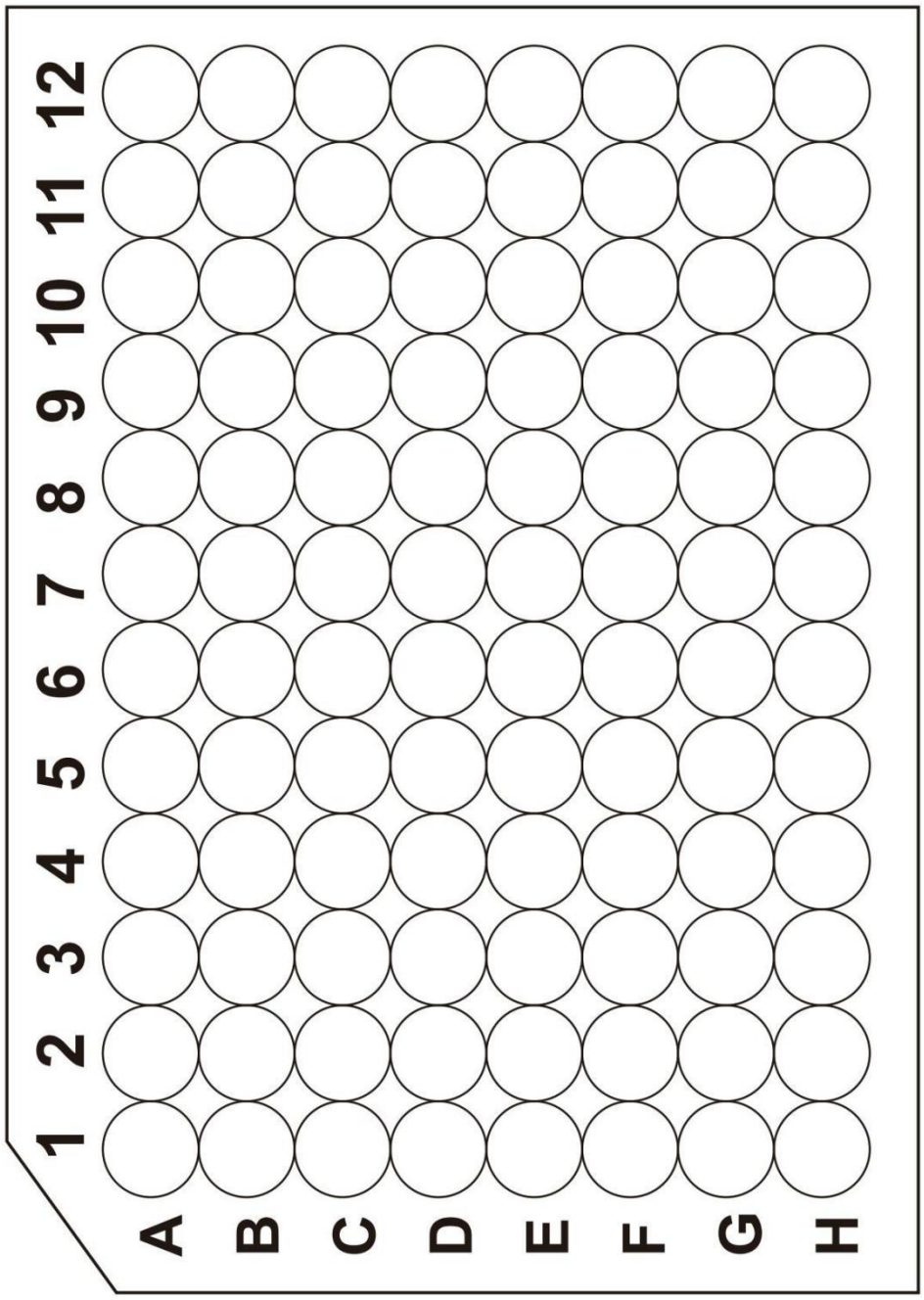
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 TIMP-1 Valukine™ ELISA 试剂盒

目录号：VAL652

适用于定量检测天然和重组小鼠基质金属蛋白酶抑制剂 1 (TIMP-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

基质金属蛋白酶（MMPs），也称为基质蛋白酶，是一类依赖锌和钙的内肽酶，它们在细胞外基质的分解和多种生物分子的处理中发挥作用。它们在许多正常的生理过程中扮演重要角色，如胚胎发育、器官发生发育、繁殖和组织重塑（1）。它们也参与许多病理过程，如关节炎、癌症和心血管疾病（2）。新合成的 MMPs 的数量主要在转录水平上受到调节，而现有 MMPs 的蛋白水解活性则通过激活前体酶或酶原和内源性抑制剂如α_2-巨球蛋白和基质金属蛋白酶组织抑制剂（TIMPs）对活性酶的抑制作用来控制。

哺乳动物 TIMP 家族包括四个成员，它们在结构上相似（3）。所有 TIMP 蛋白都有 12 个保守的半胱氨酸残基，形成六个链内二硫键，从而形成具有六个环的极其稳定的蛋白质。TIMP 蛋白在结构和功能上有两个不同的域：N 末端由环 1-3 组成，负责以 1:1 的配比与活性 MMPs 以非共价形式紧密结合；C-末端由环 4-6 组成，可增强酶与抑制剂的相互作用。就 TIMP-1 而言，C-末端已被证实可以结合 pro MMP - 9 的血红蛋白样结构域。TIMP-1 刺激红细胞生成，抑制血管生成，是一种 B 细胞的抗凋亡剂。这些 TIMP-1 的功能可能与 MMP 抑制无关（4-6）。

小鼠 TIMP-1 是一种 28-35 kDa 的分泌型糖蛋白（6, 7）。该蛋白合成为 205 个氨基酸（aa）的前体，其中包含 24aa 的信号肽和 181aa 的成熟形式（8, 9）。小鼠 TIMP-1 与大鼠、人、猪和犬 TIMP-1 的氨基酸序列同源性分别为 85%、77%、68%和 67%（10-13）。在三种已知的小鼠 TIMPs 中，TIMP-1 与 TIMP-2 和 TIMP-3 的氨基酸序列同源性分别为 39%和 38%（14, 15）。TIMP-1 广泛表达于多种细胞中，包括成纤维细胞、成骨细胞、内皮细胞、颗粒细胞、树突状细胞、血管平滑肌细胞、脂肪细胞和单核细胞（6, 16-19）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠TIMP-1抗体包被于微孔板上。样品，质控品和标准品中的小鼠TIMP-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TIMP-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；
- 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RD5-17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)	91.8	282	721	89.7	293	721
标准差	9.4	12.6	23.0	6.5	22.1	41.4
CV%	10.2	4.5	3.2	7.2	7.5	5.7

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TIMP-1，测定其回收率。

样本类型	平均回收率%	范围 (%)
细胞培养基 (n=6)	102	92-112
小鼠血清* (n=6)	101	84-117
小鼠EDTA血浆* (n=4)	107	92-119

*样品在检测前进行稀释

C. 灵敏度

7次检测结果表明，小鼠TIMP-1的最低可测剂量（MDD）范围为1.4-3.5 pg/mL。平均MDD为2.1 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NS0表达的重组小鼠TIMP-1校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠TIMP-1，然后用标准品稀释液RD5-17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=5)	小鼠血清* (n=6)	小鼠EDTA血浆* (n=4)
1:2	平均值/期待值（%）	96	97	99
	范围（%）	91-102	93-107	97-103
1:4	平均值/期待值（%）	98	98	104
	范围（%）	90-103	93-109	95-107
1:8	平均值/期待值（%）	97	100	107
	范围（%）	85-103	92-113	103-117
1:16	平均值/期待值（%）	98	102	109
	范围（%）	89-108	94-113	101-116

*样品在检测前进行稀释

F. 样本预值

小鼠血清/血浆样本 - 在此测定中对样品进行小鼠TIMP-1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=20)	2350	1280-3650	730
小鼠EDTA血浆样本 (n=20)	2270	1250-3390	560

细胞培养上清样本：

将 L-929 小鼠成纤维细胞（1 x 10^6 cells/mL）培养在含10%马血清的MEM培养基中，培养3 天，然后用 2.5 ng/mL LPS 刺激。取出等分的细胞培养上清，检测小鼠 TIMP-1，结果为 26.6 ng/mL。

小鼠肺组织（1-2 mm块，放于40 mL培养基）培养在含10%胎牛血清的RPMI条件培养基中培养7天。取出等分的细胞培养上清，检测小鼠 TIMP-1，结果为121 ng/mL。

G. 特异性

此ELISA法可检测天然及重组小鼠TIMP-1。

将以下因子用标准品稀释液RD5-17配制成50 ng/mL或500 ng/mL的浓度来检测与小鼠TIMP-1的交叉反应。将50 ng/mL的干扰因子掺入重组小鼠TIMP-1对照品中，来检测对小鼠TIMP-1的干扰。没有观察到明显的交叉反应或干扰。

Recombinant mouse:	**Recombinant human:**	
MMP-3	MMP-1	MMP-12
MMP-9 (pro)	MMP-2	MMP-13
MMP-9 (active)	MMP-3	TIMP-1
	MMP-7	TIMP-2
	MMP-8	TIMP-3
	MMP-9	TIMP-4
	MMP-10	

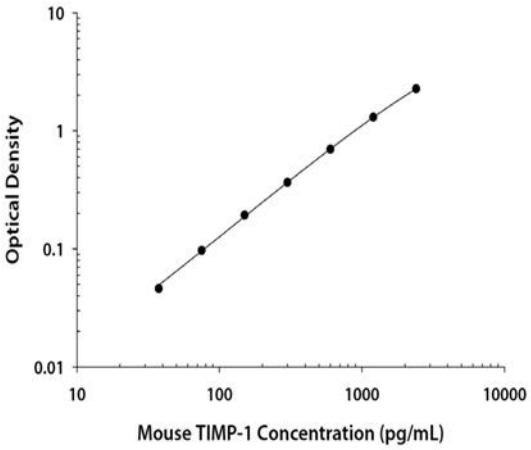
重组大鼠TIMP-1在本试验中没有干扰，但会有约0.04%的交叉反应。

本试剂盒检测约80%的重组小鼠TIMP-1与重组小鼠MMP-9前体复合，约60%与活性的重组小鼠MMP-9复合。

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	0.031 0.031	0.031	—
37.5	0.077 0.077	0.077	0.046
75	0.126 0.131	0.128	0.097
150	0.224 0.224	0.224	0.193
300	0.393 0.399	0.396	0.365
600	0.728 0.729	0.728	0.697
1200	1.294 1.369	1.332	1.301
2400	2.245 2.342	2.294	2.263

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse TIMP-1 Microplate	包被抗小鼠TIMP-1抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Mouse TIMP-1 Conjugate	酶标检测抗小鼠TIMP-1抗体	1瓶
Mouse TIMP-1 Standard	小鼠TIMP-1标准品（冻干），参考瓶身标签进行重溶	1瓶
Mouse TIMP-1 Control	小鼠TIMP-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 RD1-21	检测液	1瓶
Calibrator Diluent RD5-17	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="7">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RD1-21</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RD5-17</td></tr>
<tr><td>质控品</td><td rowspan="2">分装并 2-8℃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RD5-17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RD5-17稀释。

血浆样本：使用 EDTA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RD5-17稀释。

注意： *本试剂盒对枸橼酸钠血浆和肝素血浆尚未被验证。*

溶血或血脂过高样本不适合用于本试剂盒。

B. 样本准备工作

使用聚丙烯管。

细胞培养上清样本可能需要稀释。最佳稀释度应由最终用户确定。

小鼠血清和血浆样本建议用标准品稀释液RD5-17 3倍稀释后进行检测，即40 µL样品+80 µL标准品稀释液RD5-17。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。

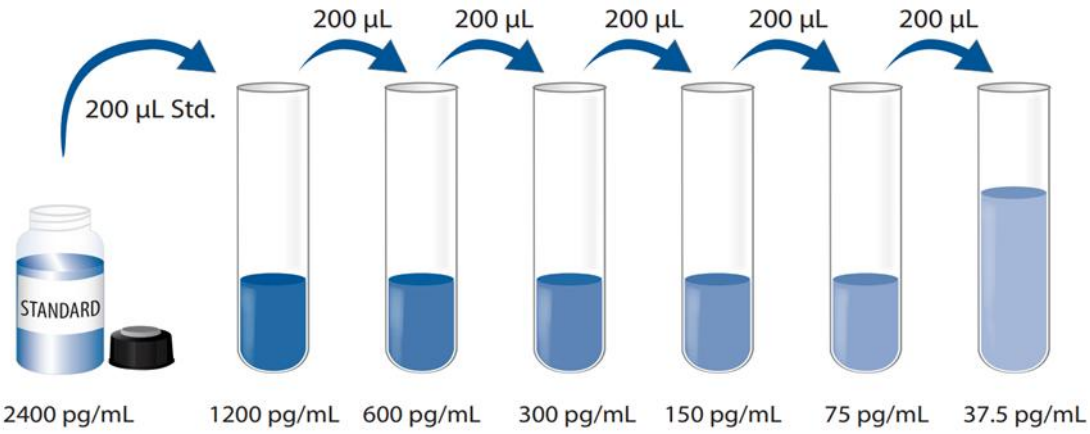
小鼠TIMP-1质控品：使用1.0 mL去离子水或蒸馏水重溶质控品。混合均匀，测定时不稀释质控品。

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×）。

小鼠TIMP-1标准品：重溶体积请参考瓶身标签*，用标准品稀释液RD5-17重溶小鼠TIMP-1标准品，请勿使用其他稀释液。得到浓度为2400 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。

使用聚丙烯管，向每一稀释管中加入200 µL标准品稀释液RD5-17。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 TIMP-1 标准品可用作标准曲线最高点（2400 pg/mL），标准品稀释液RD5-17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50μL检测液RD1-21。
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TIMP-1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TIMP-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

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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