# PRODUCT INFORMATION & MANUAL

## Mouse Total MMP-9 Valukine™ ELISA

## VAL621

For the quantitative determination of natural and recombinant Mouse active and Pro-Matrix Metalloproteinase 9 (Total MMP-9) concentration

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

Version202210.1

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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 (ECM). They play an important role in many normal physiological processes such as embryonic development, morphogenesis, reproduction, and tissue remodeling (1, 2). They also participate in many pathological processes such as arthritis, cancer, and cardiovascular disease (3). 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 pro- enzymes or zymogens and the inhibition of active enzymes by endogenous inhibitors, α2-macroglobulin, and tissue inhibitors of metalloproteinases (TIMPs).

MMP-9 (also referred to as gelatinase B, 92 kDa type IV collagenase, 92 kDa gelatinase, and type V collagenase) is secreted as a glycosylated pro-enzyme (4). Activation of the pro-enzyme involves a proteolytic removal of the N-terminal pro region containing the cysteine switch motif conserved in MMPs (5). The resulting 82 kDa active enzyme consists of a catalytic domain with a zinc-binding motif conserved in metzincins (6, 7). The catalytic domain also contains three contiguous fibronectin type II homology units responsible for binding gelatin (8). A proline-rich hinge region links the catalytic domain to the C-terminal hemopexin-like domain. In vitro treatment of the pro-enzyme with 4-aminophenylmercuric acetate (APMA) produces not only the active enzyme but also a C-terminal truncated form with activity comparable to that of the active form (9).

MMP-9 degrades components of the ECM with high specific activity for denatured collagens (gelatin). It can cleave native collagens of type IV, V, and XI, as well as elastin. MMP-9 can also cleave a variety of non-ECM molecules such as IL-1β, IL-8, connective tissue-activating peptide-III, platelet factor-4, GROα, substance P, myelin basic protein, and amyloid β peptide. MMP-9 can increase or decrease the biological activity of these molecules, depending upon the site of cleavage (10, 11).

MMP-9 is produced by a variety of normal and transformed cells including neutrophils, monocytes, macrophages, astrocytes, fibroblasts, osteoclasts, chondrocytes, keratinocytes, endothelial and epithelial cells, and is regulated by various agents. Transgenic mouse models report that MMP-9 regulates growth plate angiogenesis and apoptosis of hypertrophic chondrocytes (12), mediates early bone development through osteoclast recruitment (13), suppresses development of experimental abdominal aortic aneurysms (14), influences sensitization and reward (15), allows cytotrophoblast invasion (16), contributes to skin carcinogenesis (17), triggers the angiogenic switch during carcinogenesis (18), inactivates the serpin α1-proteinase inhibitor (19), and associates with ischemia-induced blood brain barrier permeability (20).

# II. OVERVIEW

## A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse MMP-9 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MMP-9 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for mouse MMP-9 is pipetted into the wells. After washing away any unbound substances, Streptavidin-HRP is pipetted into the wells. Following a wash to remove any unbound reagent, TMB Substrate is added to the wells and color develops in proportion to the amount of mouse MMP-9 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 supernate 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 (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) | 169.9 | 640.4 | 2662.3 | 169.9 | 649.0 | 2679.3 |
| Standard Deviation | 6.8 | 16.5 | 116.6 | 6.6 | 21.2 | 116.5 |
| CV% | 4.0 | 2.6 | 4.4 | 3.9 | 3.3 | 4.3 |

## B. RECOVERY

The recovery of mouse MMP-9 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 84.4-92.6% with an average of 90.1%.

The recovery of mouse MMP-9 spiked to different levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 94.6-101.5% with an average of 98.1%.

## C. SENSITIVITY

The minimum detectable dose (MDD) of mouse MMP-9 is typically less than 2.56 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 MMP-9 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 MMP-9 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

| Dilution | Average % of Expected | Range (%) |
|---|---|---|
| 1:2 | 103.0 | 98.7-113.5 |
| 1:4 | 107.3 | 100.3-120.2 |
| 1:8 | 109.4 | 97.5-127.9 |
| 1:16 | 110.4 | 100.2-122.2 |

## F. SAMPLE VALUES

**Serum -** Three mouse serum samples were evaluated for the presence of mouse MMP-9 in this assay. All samples measured ranged from 52.1 to 69.4 ng/mL with an average of 58.3 ng/mL.

## G. SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

| Recombinant mouse | Other recombinants |
|---|---|
| MMP-2 | human MMP-9 |
| MMP-3 | rat MMP-9 |
| MMP-7 | |
| MMP-8 | |
| MMP-12 | |
| TIMP-1 | |

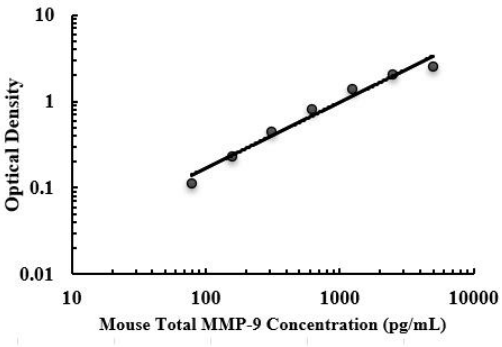
This kit recognizes the Pro form of recombinant mouse (rm) MMP-9 and the mature form of rmMMP-9 by itself or when complexed to rmTIMP-1 or rmTIMP-2.

Recombinant mouse TIMP-2 does not cross-react in this assay but does interfere at concentrations > 6.25 ng/mL.

# 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 | OD | Average | Corrected |
|---|---|---|---|
| 0 | 0.033<br>0.033 | 0.033 | - |
| 78.1 | 0.143<br>0.151 | 0.147 | 0.114 |
| 156.3 | 0.256<br>0.270 | 0.263 | 0.230 |
| 312.5 | 0.461<br>0.487 | 0.474 | 0.441 |
| 625 | 0.822<br>0.890 | 0.856 | 0.823 |
| 1250 | 1.375<br>1.476 | 1.426 | 1.393 |
| 2500 | 2.079<br>2.145 | 2.112 | 2.079 |
| 5000 | 2.485<br>2.593 | 2.539 | 2.506 |

# V. KIT COMPONENTS AND STORAGE

## A. MATERIALS PROVIDED

| Parts | Description | Size |
|---|---|---|
| Mouse Total MMP-9 Microplate | 96 well polystyrene microplate (12 strips of 8 wells) coated with a Rat antibody against Mouse MMP-9. | 1 plate |
| Mouse Total MMP-9 Standard | Recombinant Mouse MMP-9 in a buffered base; lyophilized. Refer to the vial label for reconstitution volume. | 2 vials |
| Mouse Total MMP-9 Detection Antibody | Biotinylated Mouse MMP-9 antibody, lyophilized. Refer to the vial label for reconstitution volume. | 1 vial |
| Calibrator Diluent (1×) | Buffered diluent used to dilute standard and samples. | 2 vials |
| Reagent Diluent (1×) | Buffered protein base used to dilute Detection Antibody and Streptavidin-HRP. | 2 vials |
| Streptavidin-HRP A (200×) | 200× Streptavidin conjugated to horseradish peroxidase. | 1 vial |
| Wash Buffer Concentrate (25×) | A 25× concentrated solution of buffered surfactant with preservatives. | 1 vial |
| TMB Substrate | TMB ELISA Substrate Solution. | 1 vial |
| Stop Solution | 2 N sulfuric acid. | 1 vial |
| Plate Sealers | Adhesive strip. | 3 strips |

## B. STORAGE

| | | |
|---|---|---|
| Unopened Kit | Store at 2-8 °C. Do not use past kit expiration date. | |
| Opened/ Reconstituted Reagents | Streptavidin-HRP A | May be stored for up to 1 month at 2-8 °C.* |
| | Diluted Wash Solution | |
| | TMB Substrate | |
| | Stop Solution | |
| | Standard | Prepare fresh for each assay. |
| | Detection Antibody | Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. * |
| | Reagent Diluent (1×) | May be stored for up to 1 month at 2-8 °C.* |
| | Calibrator Diluent (1×) | May be stored for up to 1 month at 2-8 °C.* |
| | Microplate Wells | 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.* |

* 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.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500±50 rpm.

## 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 protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

# VI. PREPARATION

## A. SAMPLE COLLECTION AND STORAGE

**Cell Culture Supernates -** Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples require dilution with Calibrator Diluent (1×).

**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 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples require dilution with Calibrator Diluent (1×).

## B. SAMPLE PREPARATION

Cell culture supernate samples require a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent (1×).

Serum samples require a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent (1×).

## C. REAGENT PREPARATION

***Note:*** *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×).

**Detection Antibody- Centrifuge briefly before opening. Reconstitution Volume refer to vial label with Reagent Diluent (1×).** Aliquot and store if needed. Allow the detection antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Dilute stock solution in Reagent Diluent (1×) to the working concentration of 100 ng/mL. Prepare at least 15 minutes prior to use.

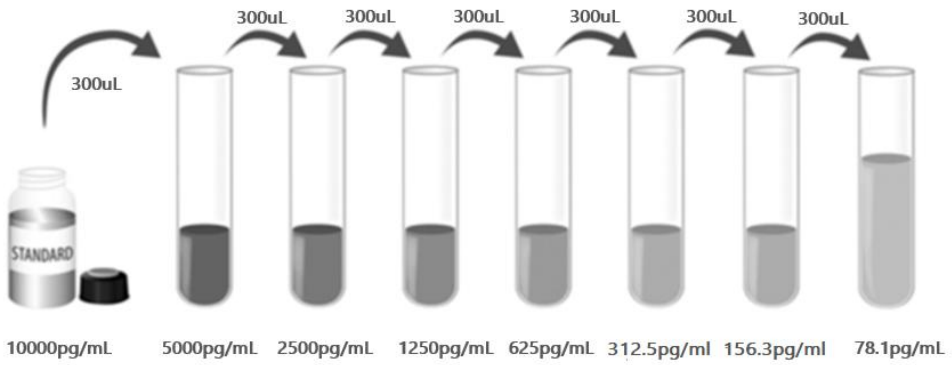
**Streptavidin-HRP A (1×) - Centrifuge briefly before opening.** Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

**Mouse Total MMP-9 Standard - Centrifuge briefly before opening. Refer to the vial label for the reconstitution volume*.** This reconstitution produces a stock

solution of 10,000 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 300 µL of the appropriate Calibrator Diluent (1×) into each tube.** Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 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

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

1. Prepare all reagents and working standards 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 standard, or prepared sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature **on a horizontal orbital microplate shaker set at 500±50rpm.** A plate layout is provided for a record of standards and samples assayed. (Samples may require dilution. See Sample Preparation section.)
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Solution (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.
5. Add 100 µL of the Detection Antibody diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature **on a horizontal orbital microplate shaker set at 500±50rpm.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP A to each well. Cover the plate and incubate for 20 minutes at room temperature. **Avoid placing the plate in direct light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate to each well. Incubate for 20 minutes at room temperature. **Avoid placing the plate in direct light.**
10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. 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.

## 12. CALCULATION OF RESULTS

Average the duplicate readings for each standard, 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 MMP-9 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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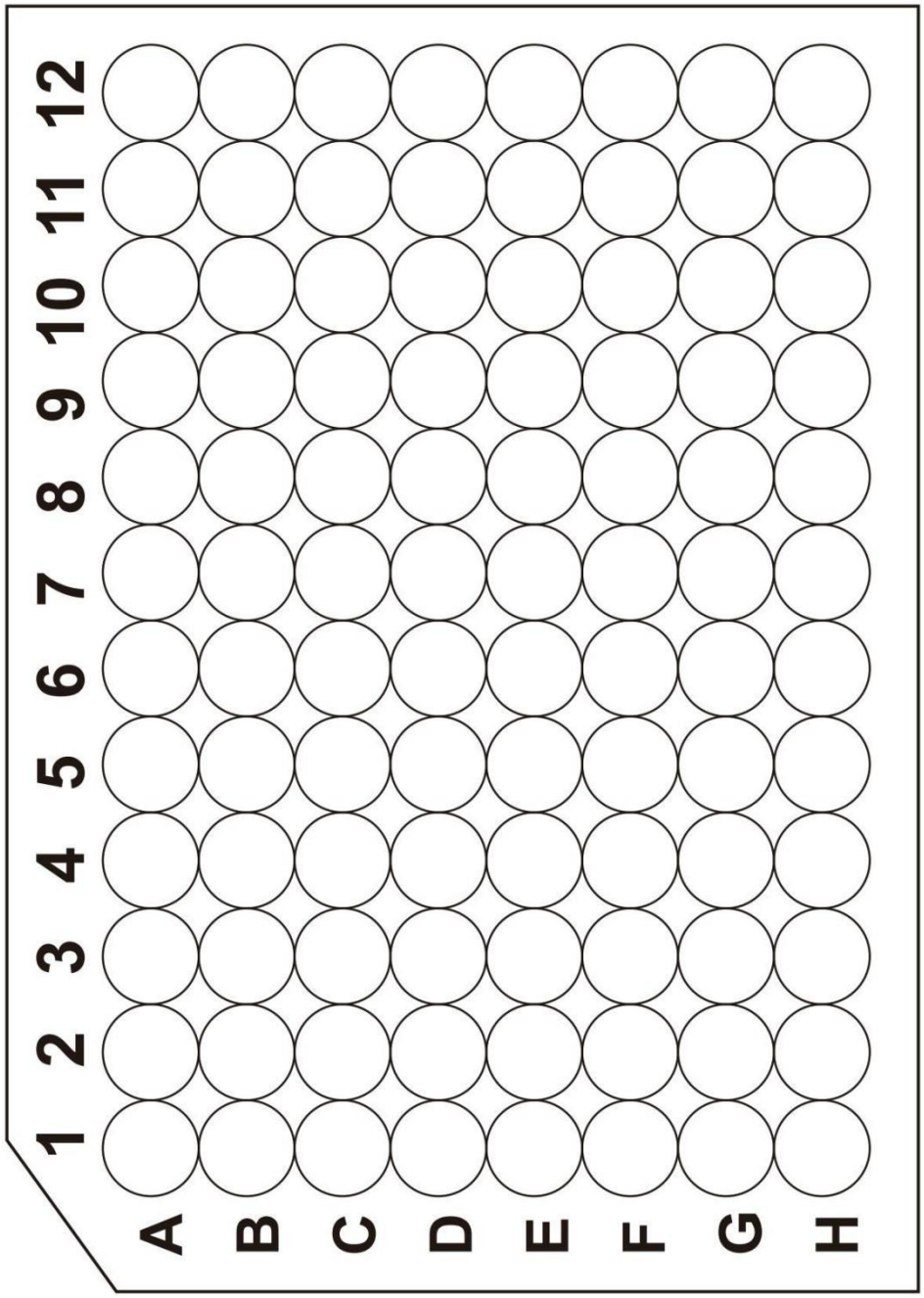
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



# 产品信息及操作手册

## 小鼠总 MMP-9 Valukine™ ELISA 试剂盒

## 目录号：VAL621

适用于定量检测天然和重组小鼠活性和前基质金属蛋白酶 9（总 MMP-9）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

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Novus 试剂盒确保在你收货日期 3 个月内有效

# 目录

# I. 背景

基质金属蛋白酶（MMPs），也称为基质蛋白，构成一个锌和钙依赖性内肽酶家族，在细胞外基质（ECM）分解中发挥作用。它们在许多正常生理过程中发挥重要作用，如胚胎发育、形态发生、生殖和组织重塑（1，2）。它们还参与许多病理过程，如关节炎、癌症和心血管疾病（3）。虽然新合成的基质金属蛋白酶的数量主要通过转录水平调节，但现有基质金属蛋白酶的蛋白水解活性可以通过激活酶原以及内源性抑制剂、α2-巨球蛋白和金属蛋白酶组织抑制剂（TIMPs）抑制活性酶来控制。

MMP-9（也称为明胶酶B、92 kDa IV型胶原酶、92 kDa明胶酶和V型胶原酶）作为糖基化前酶被分泌（4）。前酶的激活涉及到含有MMPs中保守的半胱氨酸开关基序的N端前区的蛋白水解去除（5）。由此产生的82 kDa活性酶由一个具有保守的锌结合的催化结构域组成（6，7）。催化结构域还包含三个相邻的纤维连接蛋白II型同源结构，负责结合明胶（8）。富含脯氨酸的铰链区将催化结构域连接到C端血红素样结构域。用4-氨基苯汞醋酸盐（APMA）体外处理前酶不仅产生活性酶，而且产生活性与活性形式相当的C端截短形式（9）。

MMP-9能降解ECM成分，对变性胶原蛋白（明胶）具有高比活性。它能切割IV、V、XI型天然胶原，以及弹性蛋白。MMP-9还可切割多种非ECM分子，如IL-1β、IL-8、结缔组织激活肽III、血小板因子-4、GROα、P物质、髓鞘碱性蛋白和淀粉样β肽。MMP-9可以增加或减少这些分子的生物活性，这取决于切割的位置（10，11）。

MMP-9由多种正常细胞和转化细胞产生，包括中性粒细胞、单核细胞、巨噬细胞、星形胶质细胞、成纤维细胞、破骨细胞、软骨细胞、角质形成细胞、内皮细胞和上皮细胞，并受多种因子调节。转基因小鼠模型报告MMP-9调节生长板血管生成和肥大软骨细胞凋亡（12），通过破骨细胞募集介导早期骨发育（13），抑制实验性腹主动脉瘤的发展（14），影响致敏和奖赏效应（15），允许细胞滋养层侵袭（16），促进皮肤癌变（17），在癌变过程中触发血管生成开关（18），使丝氨酸蛋白酶抑制剂失活（19），并与缺血诱导的血脑屏障通透性相关（20）。

# II. 概述

## A. 检测原理

本实验采用双抗体夹心 ELISA 法。抗小鼠MMP-9 抗体包被于微孔板上，样品和标准品中的小鼠MMP-9 会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗小鼠MMP-9 检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶（Streptavidin-HRP）孵育。洗涤去除未结合的试剂后，加入TMB底物溶液。溶液颜色与结合的目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

## B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清样本和小鼠血清样本；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释液（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、试剂盒的储存等。

## 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，在不同板中分别检测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) | 169.9 | 640.4 | 2662.3 | 169.9 | 649.0 | 2679.3 |
| 标准差 | 6.8 | 16.5 | 116.6 | 6.6 | 21.2 | 116.5 |
| CV% | 4.0 | 2.6 | 4.4 | 3.9 | 3.3 | 4.3 |

### B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的小鼠MMP-9，测定其回收率。回收率范围在84.4-92.6%，平均回收率在90.1%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠MMP-9，测定其回收率。回收率范围在94.6-101.5%，平均回收率在98.1%。

### C. 灵敏度

小鼠MMP-9 的最低可测剂量（MDD）一般小于2.56 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

此ELISA试剂盒经由R&D Systems生产的NS0表达的高纯度重组小鼠MMP-9蛋白所校正。

### E. 线性

不同的样本中含有或掺入高浓度的小鼠MMP-9，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 | 平均值(%) | 范围 (%) |
|---|---|---|
| 1:2 | 103.0 | 98.7-113.5 |
| 1:4 | 107.3 | 100.3-120.2 |
| 1:8 | 109.4 | 97.5-127.9 |
| 1:16 | 110.4 | 100.2-122.2 |

## F. 样本预值

**血清样本 -** 使用本试剂盒检测了3份小鼠血清样本中MMP-9的水平。3份样本的检测值范围为52.1-69.4 ng/mL，平均值为58.3 ng/mL。

## G. 特异性

将以下因子配制成50 ng/mL的浓度来检测没有观察到明显的交叉反应或干扰。

| Recombinant mouse | Other recombinants |
|---|---|
| MMP-2 | human MMP-9 |
| MMP-3 | rat MMP-9 |
| MMP-7 | |
| MMP-8 | |
| MMP-12 | |
| TIMP-1 | |

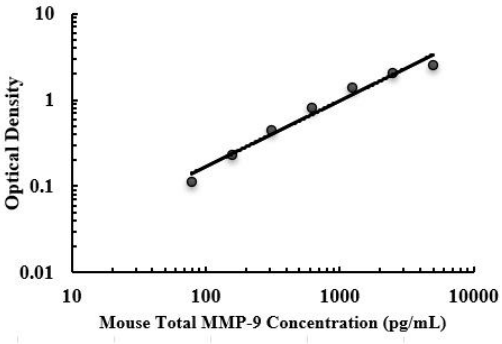
该试剂盒可以识别重组小鼠(rm) MMP-9的前体形式和成熟形式本身，或当其与rmTIMP-1或rmTIMP-2形成复合体时。

重组小鼠TIMP-2在本试验中无交叉反应，但在浓度>6.25 ng/mL时产生干扰。

# 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 | OD | Average | Corrected |
|---|---|---|---|
| 0 | 0.033<br>0.033 | 0.033 | - |
| 78.1 | 0.143<br>0.151 | 0.147 | 0.114 |
| 156.3 | 0.256<br>0.270 | 0.263 | 0.230 |
| 312.5 | 0.461<br>0.487 | 0.474 | 0.441 |
| 625 | 0.822<br>0.890 | 0.856 | 0.823 |
| 1250 | 1.375<br>1.476 | 1.426 | 1.393 |
| 2500 | 2.079<br>2.145 | 2.112 | 2.079 |
| 5000 | 2.485<br>2.593 | 2.539 | 2.506 |

## V. 试剂盒组成及储存

### A. 试剂盒组成

| 组成 | 描述 | 规格 |
|---|---|---|
| Mouse Total MMP-9 Microplate | 包被大鼠抗小鼠 MMP-9 抗体的 96 孔聚苯乙烯板，8 孔×12 条 | 1 块板 |
| Mouse Total MMP-9 Standard | 标准品（冻干粉），参考瓶身标签进行重溶 | 2 瓶 |
| Mouse Total MMP-9 Detection Antibody | 生物素化的 MMP-9 检测抗体，冻干粉，参考瓶身标签进行重溶 | 1 瓶 |
| Calibrator Diluent (1×) | 标准品稀释液（1×），用于稀释标准品和样本。 | 2 瓶 |
| Reagent Diluent (1×) | 试剂稀释液（1×）用于稀释检测抗体和 Streptavidin-HRP A。 | 2 瓶 |
| Streptavidin-HRP A (200×) | 200×浓缩的链霉亲和素标记的 HRP | 1 瓶 |
| Wash Buffer Concentrate (25×) | 浓缩洗涤缓冲液（25×） | 1 瓶 |
| TMB Substrate | TMB ELISA 底物溶液 | 1 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>链霉亲和素-HRP A</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*</td></tr>
<tr><td>检测抗体</td><td>分装，-20℃储存，最多 30 天*</td></tr>
<tr><td>试剂稀释液（1×）</td><td>2-8℃储存，最多 30 天*</td></tr>
<tr><td>标准品稀释液（1×）</td><td>2-8℃储存，最多 30 天*</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
- 振荡器（500±50 rpm）

## 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，使用时请做好眼镜、手、面部及衣服的防护。使用后请彻底洗手。

# VI. 实验前准备

## A. 样品收集及储存

**细胞培养上清液：**颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于-20℃ 冰箱内，避免反复冻融。样本需要用标准品稀释液（1×）稀释。

**血清样本:** 用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后1000 x g离心15分钟。吸取血清样本之后即刻用于检测，或者分装，-20℃贮存备用。避免反复冻融。样本需要用标准品稀释液（1×）稀释。

## B. 样本准备工作

细胞上清样本需要用标准品稀释液（1×）4倍稀释后进行检测，例如：50 µL样本+150 µL标准品稀释液（1×）。

血清样本需要用标准品稀释液（1×）50倍稀释后进行检测，例如：10 µL样本+490 µL标准品稀释液（1×）。

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

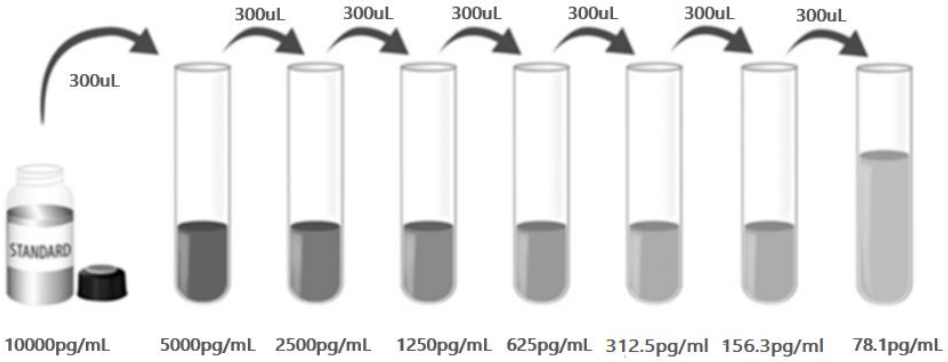
**检测抗体：开盖前请瞬时离心。**参考检测抗体瓶标签指示，用试剂稀释液（1×）将冻干粉进行重溶，轻轻震摇至少15分钟，使其充分溶解。再用试剂稀释液（1×）稀释至工作浓度100 ng/mL，至少在使用前15分钟准备。

**链霉亲和素- HRP A（1×）：开盖前请瞬时离心。**用试剂稀释液（1×）将链霉亲和素- HRP A（200×）稀释至工作浓度链霉亲和素- HRP A（1×）。

**小鼠MMP-9标准品：开盖前请瞬时离心。冻干标准品的重溶体积请参考瓶身标签**，得到浓度为10,000 pg/mL标准品母液。轻轻震摇至少15分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

向各稀释管中加入300 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。5000 pg/mL管作标准曲线最高点，标准品稀释液（1×）可用作标准品零点（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和标准品；
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. 分别将不同浓度标准品、实验样本加入相应孔中，每孔100 µL。用封板膜封住反应孔，**室温500±50rpm水平振荡孵育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；（样本需要稀释，详情参见样本制备部分。）
4. 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；
5. 在每个微孔内加入100 µL配制好的检测抗体工作液。用封板胶纸封住反应孔，**室温500±50rpm水平振荡孵育2小时**；
6. 重复第4步洗板操作；
7. 在每个微孔内加入100 µL稀释好的链霉亲和素- HRP A 工作液。用封板膜封住反应孔，室温孵育20分钟，**注意避光；**
8. 重复第4步洗板操作；
9. 在每个微孔内加入100 µL TMB底物溶液，室温孵育20分钟，**注意避光**；
10. 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。孔内溶液颜色会从蓝色变为黄色；
11. 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。如果没有使用双波长校正，结果准确度可能会受影响；
12. **计算结果：**将每个标准品和样品的校正吸光度值（OD450-OD540/OD570），复孔读数取平均值，然后减去平均零标准品OD值。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。另一种方法是，可以通过绘制标准品浓度做对数与相应OD值对数生成曲线，并通过回归分析确定最佳拟合线。通过样本的OD值，可从标准曲线上得到样本中小鼠MMP-9的浓度。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