PRODUCT INFORMATION & MANUAL

Human Total MMP-7 Valukine™ ELISA

VAL166

For the quantitative determination of natural and recombinant human MMP-7 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

Version202209.1

TABLE OF CONTENTS

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). 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, α2-macroglobulins and tissue inhibitors of metalloproteinases (TIMPs).

MMP-7 (matrilysin) is expressed in the epithelial cells of normal and diseased tissues (3). The protein localizes in normal tissues to secretory and ductal epithelium in the endometrium and in various exocrine glands. It is expressed in a variety of tumors ranging from breast, colon, prostate, stomach, upper aerodigestive tract, lung, and skin. The transcription of the gene is activated by Ets transcription factors of the PEA3 subfamily in intestinal tumors and by Pseudomonas aeruginosa flagellin in airway epithelial cells (4, 5). Knockout mice lacking the gene have suppressed intestinal tumorigenesis (6). Over-expression of the gene results in premature mammary gland differentiation and male infertility (7).

MMP-7 is capable of digesting many proteins of the extracellular matrix such as collagen IV, gelatins, laminin, aggrecan, entactin, elastin, and versican. It activates other proteinases such as urokinase plasminogen activator and pro-MMP-1, -2, and -9, and cleaves additional substrates such as osteopontin (3, 8, 9). In addition to its roles in connective tissue remodeling and cancer, MMP-7 also regulates intestinal α-defensin activation in innate host defense and releases TNF-α in a model of herniated disc resorption (10, 11). MMP-7-mediated cleavage of Fas Ligand protects tumor cells from chemotherapeutic drug cytotoxicity and potentiates epithelial cell apoptosis (12, 13).

Structurally, MMP-7 is one of the smallest MMPs, consisting of two domains, a pro-domain and a catalytic domain (8). Activation of the proenzyme involves a proteolytic removal of the N-terminal pro-region containing the cysteine switch motif conserved in MMPs (14). The resulting mature and active enzyme consists of a catalytic domain with a zinc-binding motif conserved in metzincins (15, 16). Alternatively, activation of the proenzyme can be through oxygenation of the cysteine switch motif by hypochlorous acid (17).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human MMP-7 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human MMP-7 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human MMP-7 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 human MMP-7 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 human 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)	65.4	244.2	1038.3	65.9	244.5	1044.0
Standard Deviation	2.6	9.3	95.6	3.8	13.1	89.3
CV%	3.9	3.8	9.2	5.7	5.4	8.6

B. RECOVERY

The recovery of human MMP-7 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 94.9-122.5% with an average of 109.6%.

The recovery of human MMP-7 spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 78.8-119.0% with an average of 101.1%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human MMP-7 is typically less than 0.74 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 human MMP-7 produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human MMP-7 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	104.6	93.8-118.9
1:4	100.8	86.6-111.5
1:8	106.6	98.2-118.2
1:16	104.9	88.1-118.2

F. SAMPLE VALUES

Serum - Seven human serum samples were evaluated for the presence of human MMP-7 in this assay. All samples measured ranged from 911.8 to 2702.6 pg/mL with an average of 1886 pg/mL.

G. SPECIFICITY

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

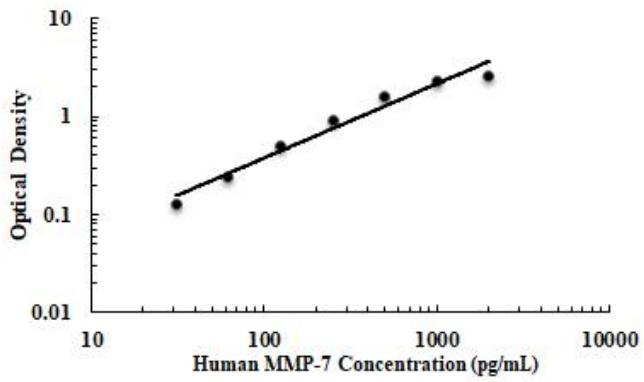
Recombinant human	Recombinant mouse
MMP-1	MMP-7
MMP-2	MMP-9
MMP-3	
MMP-8	
MMP-9	
MMP-10	
MMP-13	
TIMP-1	
TIMP-2	
TIMP-3	
TIMP-4	

This Valukine recognizes pro and mature recombinant human MMP-7. It also recognizes recombinant human MMP-7 when complexed to recombinant human TIMP-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	OD	Average	Corrected
0	0.054 0.058	0.056	-
31.3	0.178 0.189	0.184	0.128
62.5	0.297 0.304	0.301	0.245
125	0.543 0.556	0.550	0.494
250	0.966 0.981	0.974	0.918
500	1.636 1.706	1.671	1.615
1000	2.278 2.378	2.328	2.272
2000	2.661 2.674	2.668	2.612

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human MMP-7 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse antibody against human MMP-7.	1 plate
Human MMP-7 Standard	Human MMP-7 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human MMP-7 Detection Antibody	Biotinylated human MMP-7 antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent Concentrate (4×)	Concentrated buffered diluent used to dilute standard and samples.	1 vial
Detection Antibody Diluent Concentrate (4×)	Concentrated buffered diluent used to dilute Detection Antibody.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent Concentrate (10×)	A 10× concentrated buffered protein base used to dilute HRP.	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

<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>Streptavidin-HRP A</td><td rowspan="4">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Reagent Diluent Concentrate (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Calibrator Diluent Concentrate (4×)</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>Detection Antibody Diluent Concentrate (4×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Detection Antibody 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.
- 500 mL graduated cylinder.

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.
- Human MMP-7 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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 may 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.

B. SAMPLE PREPARATION

Human serum samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of **Calibrator Diluent** (1×).

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: Human MMP-7 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

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×).

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

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

Detection Antibody - Centrifuge briefly before opening. Reconstitution Volume refer to vial label with Reagent Diluent (1×). Aliquot and store if needed. Dilute stock solution in Detection Antibody Diluent (1×) to the working concentration of 100 ng/mL. Prepare at least 15 minutes prior to use.

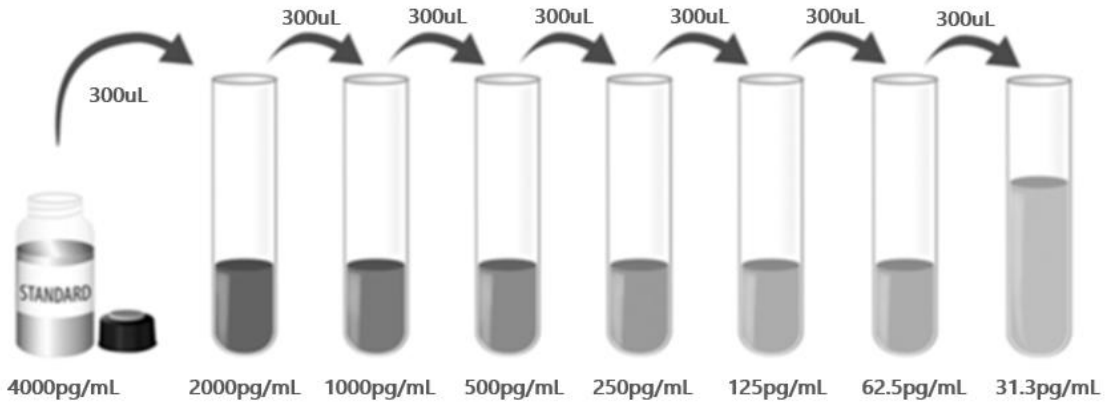
Streptavidin-HRP A (1×) - Centrifuge briefly before opening. Dilute to the working

concentration specified on the vial label using Reagent Diluent (1×).

Human MMP-7 Standard - Centrifuge briefly before opening. Refer to the vial label for the reconstitution volume*. This reconstitution produces a stock solution of 4000 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 2000 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

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

Note: Human MMP-7 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

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. 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 Detection Antibody Diluent, to each well. Cover with a new adhesive strip and incubate for 2 hours at room temperature.
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. **Protect from light**.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate to each well. Incubate for 15 minutes at room temperature. **Protect from 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, 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 human MMP-7 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.

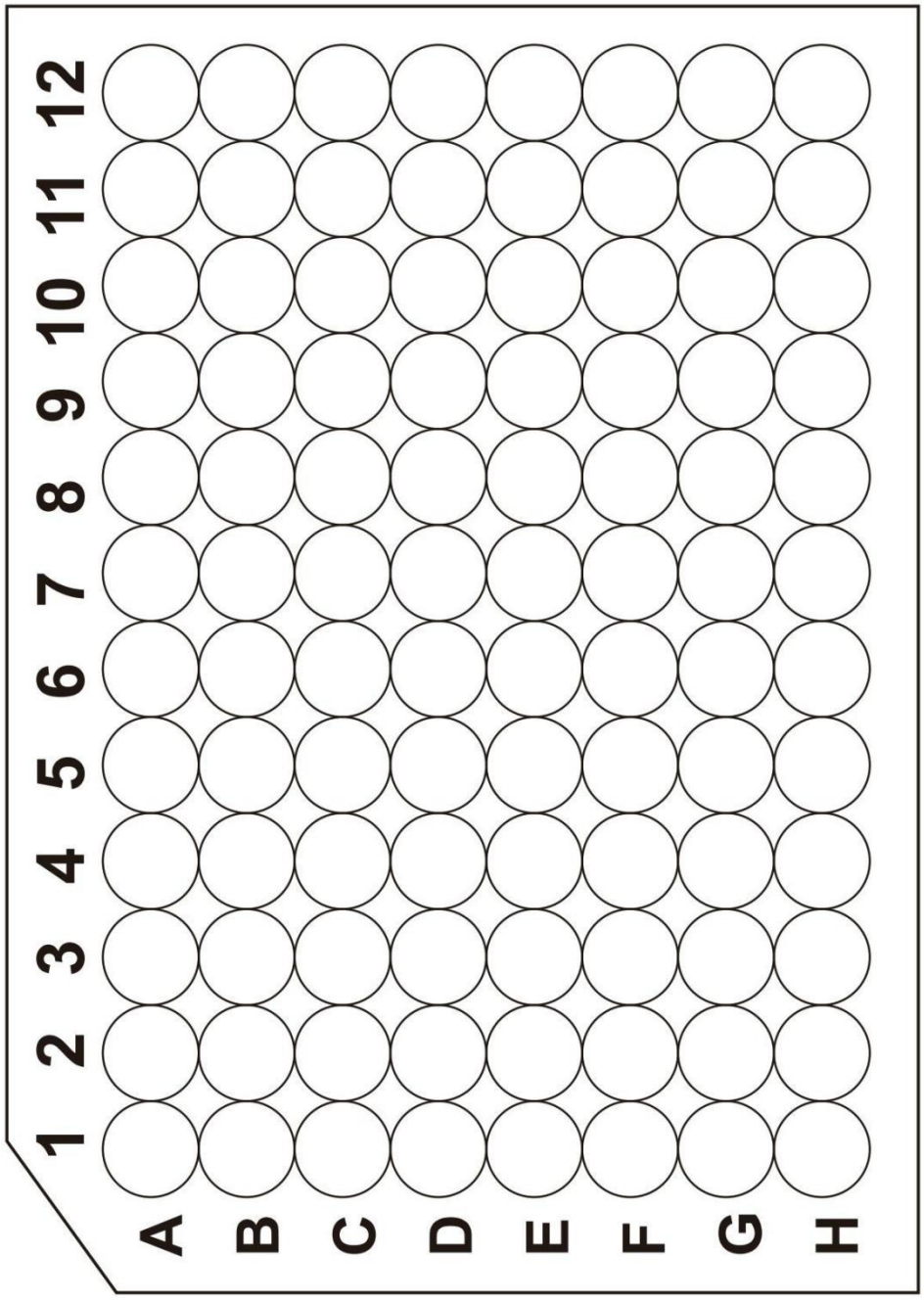
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Nagase, H. and J.F. Woessner Jr. (1999) J. Biol. Chem. 274:2191.
2. Parks, W.C. and R.P. Mecham (1998) in Matrix Metalloproteinases, Academic Press, San Diego.
3. Wilson, C.L. and L.M. Matrisian (1996) Int. J. Biochem. Cell Biol. 28:123.
4. Crawford, H.E. et al. (2001) Mol. Cell. Biol. 21:1370.
5. Lopez-Boado, Y.S. et al. (2001) J. Biol. Chem. 276:41417.
6. Wilson, C.L. et al. (1997) Proc. Natl. Acad. Sci. USA 94:1402.
7. Rudolph-Owen, L.A. et al. (1998) Mol. Biol. Cell 9:421.
8. Woessner, J.F. (1998) in Handbook of Proteolytic Enzymes, Barrett, A.J. et al. Eds, Academic Press, San Diego, pp. 1183-1187.
9. Agnihotri, R.A. et al. (2001) J. Biol. Chem. 276:28261.
10. Wilson, C.L. et al. (1999) Science 286:113.
11. Haro, H. et al. (2000) J. Clin. Invest. 105:143.
12. Mitsiades, N. et al. (2001) Cancer Res. 61:577.
13. Powell, W.C. et al. (1999) Curr. Biol. 9:1441.
14. Van Wart, H.E. and H. Birkedal-Hansen (1990) Proc. Natl. Acad. Sci. USA 87:5578.
15. Jiang, W. and J.S. Bond (1992) FEBS Lett. 312:110.
16. Bode, W. et al. (1993) FEBS Lett. 331:134.
17. Fu, X. et al. (2001) J. Biol. Chem. 276:41279.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人总 MMP-7 Valukine™ ELISA 试剂盒

目录号：VAL166

适用于定量检测天然和重组人 MMP-7 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

info.cn@bio-techne.com

有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

目录

I. 背景

基质金属蛋白酶（MMPs），也称为基质蛋白酶，构成了一个锌和钙依赖性内肽酶家族，在细胞外基质（ECM）分解中起作用。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）。它们也参与许多病理过程，如关节炎、癌症和心血管疾病（2）。虽然新合成的基质金属蛋白酶的数量主要在转录水平上受到调节，但现有基质金属蛋白酶的蛋白水解活性是通过激活酶原以及内源性抑制剂对活性酶的抑制来控制的，如α2-巨球蛋白和金属蛋白酶组织抑制剂（TIMPs）。

MMP-7（matrilysin）在正常和病变组织的上皮细胞中表达（3）。该蛋白在正常组织中定位于子宫内膜和各种外分泌腺中的分泌和导管上皮。它在多种肿瘤中表达，包括乳腺、结肠、前列腺、胃、上呼吸道、肺和皮肤。该基因的转录由肠道肿瘤中PEA3亚家族的Ets转录因子和呼吸道上皮细胞中的铜绿假单胞菌鞭毛蛋白激活（4，5）。缺乏该基因的敲除小鼠抑制了肠道肿瘤的发生（6）。该基因的过度表达导致乳腺过早分化与男性不育（7）。

MMP-7能够消化细胞外基质的许多蛋白质，如IV型胶原，明胶、层粘连蛋白、聚蛋白聚糖、肌动蛋白、弹性蛋白和多功能蛋白聚糖。它激活其他蛋白酶，作为尿激酶纤溶酶原激活剂和前MMP-1、-2和-9，并切割额外的基质，如骨桥蛋白（3、8、9）。除了在结缔组织重塑和癌症中的作用外，MMP-7还调节先天宿主防御中的肠α-防御素激活，并在椎间盘突出吸收模型中释放TNF-α（10，11）。MMP-7介导的脂肪酸合成酶配体保护肿瘤细胞免受化疗药物的细胞毒性，并增强上皮细胞凋亡（12，13）。

在结构上，MMP-7是最小的MMP之一，由两个结构域组成，一个前结构域和一个催化域（8）。酶原的激活涉及蛋白水解去除含有MMP中保守的半胱氨酸开关基序的N末端pro区域（14）。由此产生的成熟和活性酶由一个催化结构域组成，该结构域具有锌结合基序（15，16）。另外，可以通过次氯酸氧化半胱氨酸开关基序来激活酶原（17）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人MMP-7抗体包被于微孔板上，样品和标准品中的人MMP-7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人MMP-7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人血清样本；
- 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)	65.4	244.2	1038.3	65.9	244.5	1044.0
标准差	2.6	9.3	95.6	3.8	13.1	89.3
CV%	3.9	3.8	9.2	5.7	5.4	8.6

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人MMP-7，测定其回收率。回收率范围在94.9-122.5%，平均回收率在109.6%。

在人血清样本中掺入检测范围内不同水平的人MMP-7，测定其回收率。回收率范围在78.8-119.0%，平均回收率在101.1%。

C. 灵敏度

人MMP-7的最低可测剂量（MDD）一般小于0.74 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生产的高纯度NS0表达的重组人MMP-7蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人MMP-7，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	104.6	93.8-118.9
1:4	100.8	86.6-111.5
1:8	106.6	98.2-118.2
1:16	104.9	88.1-118.2

F. 样本预值

血清样本 - 使用本试剂盒检测了7份人血清样本中人MMP-7的水平。7份样本的检测值在911.8-2702.6 pg/mL之间，平均值为1886 pg/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。

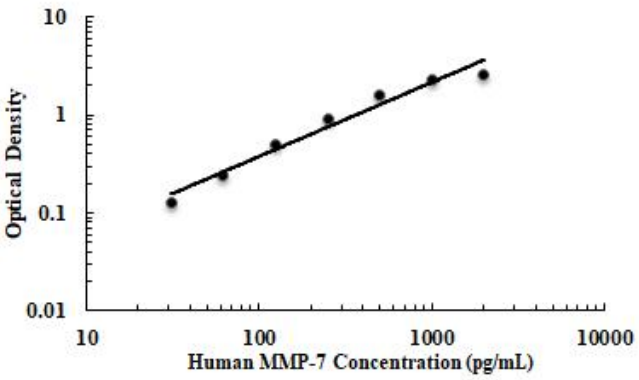
Recombinant human	Recombinant mouse
MMP-1	MMP-7
MMP-2	MMP-9
MMP-3	
MMP-8	
MMP-9	
MMP-10	
MMP-13	
TIMP-1	
TIMP-2	
TIMP-3	
TIMP-4	

该试剂盒识别前体和成熟的重组人MMP-7。当与重组人TIMP-1复合时，它还能识别重组人MMP-7。

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	0.054 0.058	0.056	-
31.3	0.178 0.189	0.184	0.128
62.5	0.297 0.304	0.301	0.245
125	0.543 0.556	0.550	0.494
250	0.966 0.981	0.974	0.918
500	1.636 1.706	1.671	1.615
1000	2.278 2.378	2.328	2.272
2000	2.661 2.674	2.668	2.612

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human MMP-7 Microplate	包被小鼠抗人 MMP-7 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human MMP-7 Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Human MMP-7 Detection antibody	生物素化的人 MMP-7 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Calibrator Diluent Concentrate (4×)	浓缩的标准品稀释液（4×）	1 瓶
Detection Antibody Diluent Concentrate (4×)	浓缩的检测抗体稀释液（4×）	1 瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液（10×）	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
TMB Substrate	TMB 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="9">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（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液</td></tr>
<tr><td>标准品稀释液（4×）</td><td>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</td></tr>
<tr><td>检测抗体稀释液（4×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×检测抗体稀释液</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

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。使用后请彻底洗手。
- 唾液中含有人MMP-7，为防止试剂盒在检测过程中产生污染，请采取防护措施。

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。样本可能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。

B. 样本准备工作

血清样本需要用**标准品稀释液（1×）**2倍稀释后进行检测，例如：100 µL血清样本+100 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。
注：*唾液中含有人MMP-7，为避免污染，实验时请带口罩、手套。*

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20mL浓缩洗涤液用去离子水或蒸馏水稀释配制成500mL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稀释配制成标准品稀释液（1×）。

检测抗体稀释液（1×）：使用蒸馏水或去离子水稀释配制成检测抗体稀释液（1×）。

试剂稀释液（1×）：使用去离子水或蒸馏水稀释配制成试剂稀释液（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。再用检测抗体稀释液（1×）稀释至工作浓度100 ng/mL，至少在使用前15分钟准备。

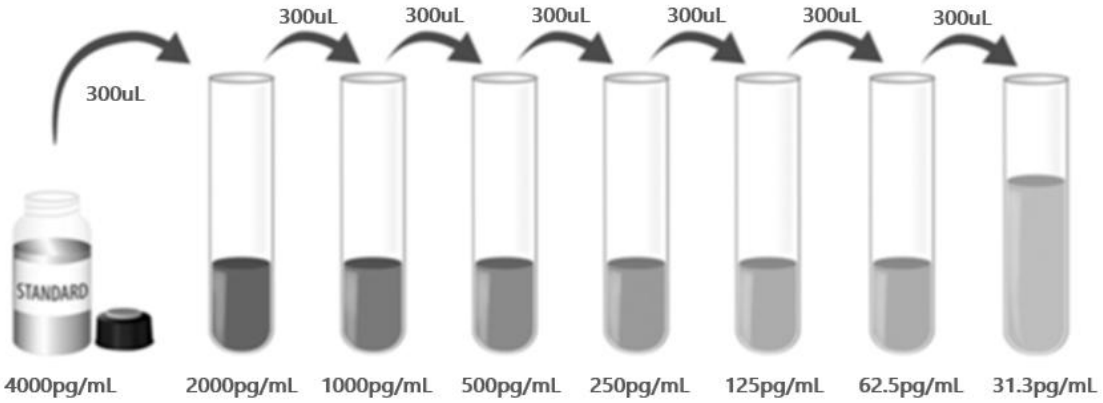
链霉亲和素- HRP A：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP A（200×）稀释至工作浓度链霉亲和素- HRP A（1×）。

人MMP-7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4000 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。2000 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，TMB底物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。

注：唾液中含有人MMP-7，为避免污染，实验时请带口罩、手套。

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，实验样本或者质控品加入相应孔中，每孔100 µL。用封板膜封住反应孔，在室温孵育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；（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。用封板胶纸封住反应孔，在室温孵育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，在室温孵育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，在室温孵育15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人MMP-7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

1. Nagase, H. and J.F. Woessner Jr. (1999) J. Biol. Chem. 274:2191.
2. Parks, W.C. and R.P. Mecham (1998) in Matrix Metalloproteinases, Academic Press, San Diego.
3. Wilson, C.L. and L.M. Matrisian (1996) Int. J. Biochem. Cell Biol. 28:123.
4. Crawford, H.E. et al. (2001) Mol. Cell. Biol. 21:1370.
5. Lopez-Boado, Y.S. et al. (2001) J. Biol. Chem. 276:41417.
6. Wilson, C.L. et al. (1997) Proc. Natl. Acad. Sci. USA 94:1402.
7. Rudolph-Owen, L.A. et al. (1998) Mol. Biol. Cell 9:421.
8. Woessner, J.F. (1998) in Handbook of Proteolytic Enzymes, Barrett, A.J. et al. Eds, Academic Press, San Diego, pp. 1183-1187.
9. Agnihotri, R.A. et al. (2001) J. Biol. Chem. 276:28261.
10. Wilson, C.L. et al. (1999) Science 286:113.
11. Haro, H. et al. (2000) J. Clin. Invest. 105:143.
12. Mitsiades, N. et al. (2001) Cancer Res. 61:577.
13. Powell, W.C. et al. (1999) Curr. Biol. 9:1441.
14. Van Wart, H.E. and H. Birkedal-Hansen (1990) Proc. Natl. Acad. Sci. USA 87:5578.
15. Jiang, W. and J.S. Bond (1992) FEBS Lett. 312:110.
16. Bode, W. et al. (1993) FEBS Lett. 331:134.
17. Fu, X. et al. (2001) J. Biol. Chem. 276:41279.

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