



人的金属蛋白酶9定量分析酶联免疫检测试剂盒

本试剂盒仅供科研使用。用于体外定量检测人血清、血浆或细胞培养上清液中的MMP-9浓度。**使用前请仔细阅读说明书并检查试剂组分是否完整。**如有产品包装破损或质量投诉，请在收到货一个月之内联系我们。

MMP-9简介：

MMPs蛋白是一组具有锌指结构的钙依赖的能够降解胞间基质成分的一组蛋白的统称。MMP-9是该蛋白家族成员中的研究较多的一种，是一种明胶酶（明胶酶B）。MMP-9有许多作用底物，如IV、V、VII、X、XI型胶原、蛋白聚糖的核心蛋白、明胶、纤维粘连蛋白、层粘连蛋白、弹性蛋白等。

结构上，MMP-9的催化区包括3个重复的型纤维连接蛋白结构域，与明胶或弹性蛋白有高度的亲和力。MMP-9还有脯氨酸丰富的结构域和含锌指结构的结构域，MMP-9包含一个V型的胶原蛋白结构域，这个结构域有高度的糖基化作用，它影响底物的特异性以及有抗衰变的作用。

MMP-9是以酶原的形式从胞内分泌到胞外，可被MMP-3、MMP-2或者次氯酸裂解，MMP-3可能是MMP-9最有效的激活剂。金属蛋白酶组织抑制因子1（TIMP-1）作为MMP-9的抑制剂，与MMP-9的酶原或活化后酶的催化区的羧末端特异性结合，形成复合物。

MMP-9可由角化细胞、单核细胞、巨噬细胞和多形核白细胞等产生，在一些炎性反应的过程中常伴随着MMP-9的产生。

检测原理：

本试剂盒采用双抗体夹心ELISA法检测样本中MMP-9的浓度。MMP9捕获抗体已预包被于酶标板上，当加入标本或参考品时，其中的MMP-9会与捕获抗体结合，其它游离的成分通过洗涤的过程被除去。当加入生物素化的抗人MMP-9抗体后，抗人MMP-9抗体与MMP-9接合，形成夹心的免疫复合物，其它游离的成分通过洗涤的过程被除去。随后加入辣根过氧化物酶标记的亲合素。生物素与亲合素特异性结合，亲合素连接的酶就会与夹心的免疫复合物连接起来；其它游离的成分通过洗涤的过程被除去。最后加入显色剂，若样本中存在MMP-9将会形成免疫复合物，辣根过氧化物酶会催化无色的显色剂氧化成蓝色物质，在加入终止液后呈黄色。通过酶标仪检测，读其450nm处的OD值，MMP-9浓度与OD₄₅₀值之间呈正比，通过参考品绘制标准曲线，对照未知样本中OD值，即可算出标本中MMP-9浓度。

人MMP-9定量分析酶联免疫检测试剂盒组成：

组分	规格（96T/48T）
人MMP-9预包被板	12条/6条
5X标准品稀释液	10ml/5ml
人MMP-9标准品	2/1支(冻干)
人MMP-9生物素化抗体	10ml/5ml
亲和素连接的HRP酶	10ml/5ml
浓缩洗涤液 20×	30ml/15ml
TMB底物	10ml/5 ml
中止液	5ml/3 ml
封板胶纸	3/2张
说明书	1份

标本收集：

- 标本的收集请按下列流程进行操作：
 - 细胞上清标本离心去除悬浮物后即可；
 - 血清标本应是自然凝固后，取上清，避免在冰箱中凝固血液；
 - 血浆标本，推荐用EDTA的方法收集；
 - 标本收集后请分装，若待测样本不能及时检测，冻存于-20℃，避免反复冻融。
- 血清标本不应添加任何防腐剂或抗凝剂；
- 标本应清澈透明，检测前样本中如有悬浮物应通过离心去除。
- 请勿使用溶血高血脂或污染的标本检测，否则结果将不准确。



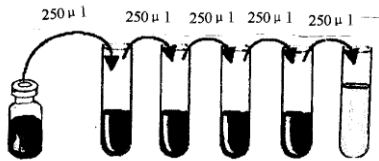
注：人血清或血浆样本请用标准品稀释液稀释后再检测。

注意事项：

1. 试剂盒请保存在2~8℃。
2. 浓缩洗涤液因在低温下可能有结晶，请水浴加热使结晶完全溶解后再配制工作液。
3. 标准品复溶加样后，剩余部份请丢弃。
4. 底物请勿接触氧化剂和金属。
5. 加样时，请及时更换枪头，避免交叉污染。
6. 严禁混用不同批号的试剂盒组份。
7. 充分混匀对保证反应结果的准确性很重要，在加液后请轻轻叩击边缘以保证混匀。
8. 室温反应，请严格控制在25~28℃。
9. 洗涤过程是至关重要的，洗涤不充分会使精确度下降并导致结果误差较大。
10. 试验中标准品和样本检测时建议作双复孔。
11. 加样过程中避免气泡的产生。
12. 血清和血浆标本的检测时，检测抗体的孵育时间应当适当延长。

检测前准备工作：

1. 试剂盒自冰箱中取出后应置室温（25~28℃）平衡20分钟；每次检测后剩余试剂请及时于2~8℃保存。
2. 将浓缩洗涤液用双蒸水或去离子水稀释（1份加19份水）。
3. 将5×标准品稀释液用双蒸水或去离子水稀释（1份加4份水）。
4. 标准品：按标签复溶体积加入1X标准品稀释液复溶使MMP-9终浓度达到2000pg/ml，室温反应，请严格控制在25~28℃，静置15~20分钟后轻轻混悬（建议抽吸几次）待彻底溶解，用标准品稀释液倍比梯度稀释后依次加入检测孔中。（标准曲线取七个点，最高浓度为2000 pg/ml，标准品稀释液直接加入作为0浓度。）



洗涤方法：

自动洗板机或人工洗板：每孔洗涤液为300u1，注入与吸出间隔15-30秒。洗板5次。最后一次洗板完成后将板倒扣着在厚吸水纸上用力拍干。

实验过程需自备的材料：

1. 不同规格的加样枪及相应的枪头；
2. 酶标仪；
3. 自动洗板机；
4. 去离子水或双蒸水；

操作步骤：

1. 通过计算并确定一次性实验所需的板条数，取出所需板条放置在框架内，暂时用不到板条请放回铝箔袋密封，保存于4℃。
2. 建议设置本底校正孔，即空白孔，设置方法为该孔只加TMB显色液和终止液。每次实验均需做标准品对照并画出标准曲线。
3. 分别将标本或不同浓度标准品（100u1/孔）加入相应孔中，用封板胶纸封住反应孔，室温（25~28℃）孵育120分钟。如果是血清血浆样本，不同样本稀释比例不一样，一般范围在400~1500倍，如无明确范围，建议从400倍稀释，如果样本浓度过高，超过检测范围，请加大大稀释倍数后重新稀释检测。
4. 洗板5次，且最后一次置厚吸水纸上拍干。
5. 加入生物素化抗体工作液（100u1/孔）。用封板胶纸封住反应孔，室温（25~28℃）孵育60分钟。
6. 洗板5次，且最后一次置厚吸水纸上拍干。
7. 加入亲和素连接的HRP酶工作液（100u1/孔）。用封板胶纸封住反应孔，避光室温（25~28℃）孵育20分钟。



8. 洗板5次，且最后一次置厚吸水纸上拍干。
9. 加入显色剂TMB100 μ l/孔，避光室温（25~28 $^{\circ}$ C）孵育20分钟。
10. 加入终止液50 μ l/孔，混匀后即刻测量OD₄₅₀值。

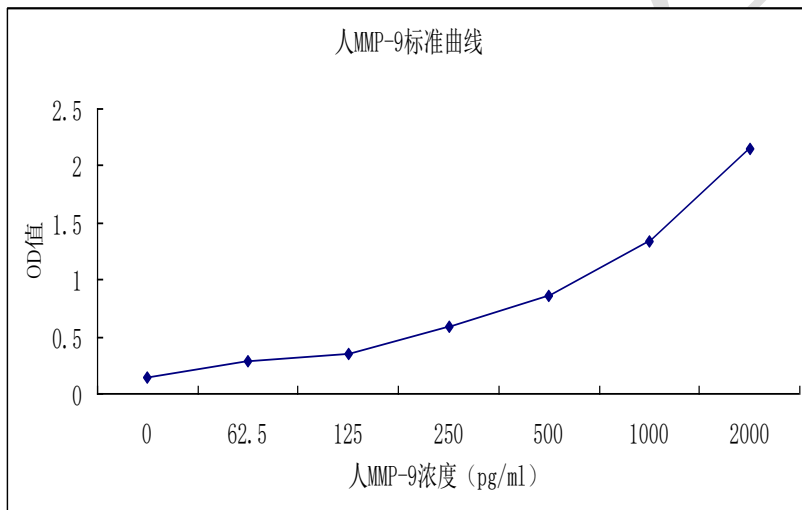
结果判断：

1. 复孔的值在20%的差异范围内结果才有效，复孔的值平均后可作为测量值。
2. 每个标准品或标本的OD值应减去本底校正孔的OD值。
3. 手工绘制标准曲线。以标准品浓度作横坐标，OD值作纵坐标，以平滑线连接各标准品的坐标点。通过标本的OD值可在标准曲线上查出其浓度。
4. 若标本 OD 值高于标准曲线上限，应当适当稀释后重测，计算浓度时应乘以稀释倍数。

典型数值和参考曲线

浓度pg/ml	典型OD值1	典型OD值2	OD平均值
0	0.1273	0.1547	0.141
62.5	0.2852	0.295	0.2901
125	0.3438	0.3632	0.3535
250	0.5779	0.5966	0.58725
500	0.8491	0.8733	0.8612
1000	1.3001	1.3866	1.34335
2000	1.9914	2.304	2.1477

人MMP-9参考标准曲线



注意：本图仅供参考，应以同次试验标准品所绘标准曲线计算标本含量。

灵敏度，特异性和重复性：

1. 灵敏度：多次重复结果表明，最小检出量为7.3pg/ml。
2. 特异性：不与MMP-1、MMP-2、MMP-3、MMP-7、MMP-8、MMP-10、MMP-13、TIMP-2、TIMP-3、TIMP-4等反应。
3. 重复性：板内，板间变异系数均<10%。

参考文献：

1. Tamura, T. et al. (1996) Endocrinology **137**:3729.
2. Okamoto, T. et al. (1997) J. Biol. Chem. **272**:6059.
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4. Maeda, A. and R.A. Sobel (1996) J. Neuropathol. Exp. Neurol. **55**:300.
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ELISA Kit for the Quantitative Analysis of Human MMP-9

The human MMP-9 ELISA (enzyme-linked immunosorbent assay) kit is used for detection of human MMP-9 in cell culture supernatants, human serum and plasma. **THE ELISA KIT IS FOR RESEARCH USE ONLY.** Please read this instruction manual carefully and check out the material provided before use, and you can contact with our company if any questions. You can enter our website or call us for other aim.

Introduction

Matrix metalloproteinases are a family of zinc and calcium dependent endopeptidases with the combined ability to degrade all the components of the extracellular matrix. MMP 9 (gelatinase B) can degrade a broad range of substrates including gelatin, collagen types IV , V , VII , X and XI, [fibronectin](#) , [laminin](#) , elastin and proteoglycan core protein.

Structurally, MMP9 have a gelatinbinding domain consisting of three contiguous fibronectin type II units, which have high appetency with gelatin and elastin . MMP9 have a catalytic domain containing the zinc binding site, a proline rich linker region, and a gelatin V domain with high [glycosylation](#) which can select different substrate and resist degradation .

The proenzyme of MMP-9 is secreted into extracellular matrix first, and cleaved into matured MMP-9 by MMP-3, MMP-2 or [hypochlorous acid](#) . It is believed that MMP-3 is the most effective [activator](#) to MMP-9 . The interstitial collagenase (MMP1) as a [inhibitor](#) can combine the carboxyl terminal of MMP-9.

MMP 9 is produced by keratinocytes, monocytes, macrophages and PMN leukocytes. MMP9 is present in most cases of inflammatory responses.

Principles of the Test

The kits is a solid sandwich enzyme-linked immunosorbent assay for detection of human MMP-9. An anti-human MMP-9 monoclonal antibody has been absorbed onto the wells of the microtiter strips provided. Samples including specimens or standards were pipetted into wells. The human MMP-9 in specimens or standards would be captured by the coated antibody and the free others were removed by washing. The human MMP-9 biotin-conjugated antibody were added and binds to human MMP-9 captured by the first antibody, which formed a sandwich. Streptavidin-HRP would be added and binds to the biotin conjugated antibody, then free Streptavidin-HRP would be removed during a wash step. After this, substrate solution would be added and catalyzed by the HRP, and a coloured product is formed. The intensity of the colored product is used to calculate in proportion to the amount of human MMP-9 in the original specimen.

Materials provided with the kits:

reagent	96/48 Test Kit
Human MMP-9 Antibody-Coated Wells	12 strips/6 strips
Standard Diluent	10ml/5ml
Human MMP-9 Standard	2/1 vial(s)
Human Detection Antibody	10ml/5ml
Streptavidin-HRP	10ml/5ml
Wash Buffer Concentrate 20x	30ml/15ml
TMB	10ml/5 ml
Stop Solution	5ml/3 ml
Plate Covers	3/2
Complete Instruction Manual	1

Specimen Collection

- Collecting specimen as following:
 - The particulate of the cell culture supernatants should be removed before use.
 - Serum was obtained from clot at room temperature.
 - Please collect plasma with EDTA.
 - Assay immediately or store samples at -20°C . Avoid free-thaw cycles.
- Antiseptic and anticoagulant should not appear in Serum samples.
- Any particulate should be removed from samples before use.



4. Do not use grossly hemolyzed or lipemic samples.

Note: Strongly recommend that the serum and plasma samples should be diluted as doubling dilution before use.

Precautions for use:

1. Please store the Kit at 2~8°C.
2. Washing buffer concentrate may have crystals in low temperature, and you can melt it in water-bath before use.
3. Please discard the remains after use of the dissolved standard.
4. Avoid contact of substrate solution with oxidizing agents and metal.
5. Usage of disposable pipette tips avoid microbial contamination or cross-contamination of reagents or specimens.
6. Do not mix or substitute reagents with those from other lots or other sources.
7. To ensure the adequate mixture of added reagents, please tap gently the plate after the wells were filled with liquid.
8. Incubation temperature should be 25~28°C.
9. Wash step was crucial for whole assay process.
10. Duplicate wells of the same sample were recommended in assay process.
11. Avoid the foam while pour the liquid into wells.
12. For serum or plasma samples, the biotin-conjugated antibody should be incubated for at least 90 minutes.

Reagent Preparation

1. The reagents should be warmed up to room temperature before use. The remaining reagents must be resealed and put into refrigeration again as soon as possible.
2. Dilute 1ml of wash buffer concentrate into 19ml deionized or distilled water to work.
3. Dilute 1ml of 5× sample diluent into 4ml deionized or distilled water to 1× sample diluent.
4. Add the standard dilution solution to the bottle according to the volume of the label and wait 15 minutes for complete dissolution. Incubation temperature should be 25~28°C. And in turn add the half concentration diluent by standard diluent.

Wash step:

Automated microplate washer or operating by pipette: Each well should be poured into 300ul wash buffer and soaked for 15 or 30 seconds, then be aspirated, five times process were repeated. After the last wash, remove remaining wash buffer by aspirating. Invert the plate and blot it against clean paper towels.

Materials Required But Not Provided

1. pipettes and pipette tips
2. Microwell strip reader capable of reading at 450 nm (540 nm as optional reference wave length)
3. automated microplate washer
4. Glass-distilled or deionized water

Assay procedure

1. The needed strips were put into the frame, the remains were returned into foil pouch and resealed.
2. Blank well were recommended, which only color reagent and stop solution be added. It is suggested that each testing with gradient density of standard for standard curve.
3. Add 100ul of standard or sample. Cover with the Plate Covers provided. Incubate for 2 hours at room temperature. If it is plasma serum samples, different sample dilution ratio is different, generally in the range of 400 ~ 1500 times, if there is no definite scope, advice from 400 times dilution, if the sample concentration is too high, more than test scope, please increase after diluted times dilution test again.
4. Five times wash process were repeated.
5. Add 100ul of detection antibody. Cover with the Plate Covers provided. Incubate for 1 hour at room temperature.
6. Five times wash process were repeated.
7. Add 100ul of Streptavidin-HRP. Cover with the Plate Covers provided. Luciferal incubation for 20 minutes at room temperature.
8. Five times wash process were repeated.
9. Add 100ul of TMB, Luciferal incubation for 20 minutes at room temperature.
10. Add 50ul of stop solution to each well, determine the optical density of each well within 10 minutes.

Calculation of Results

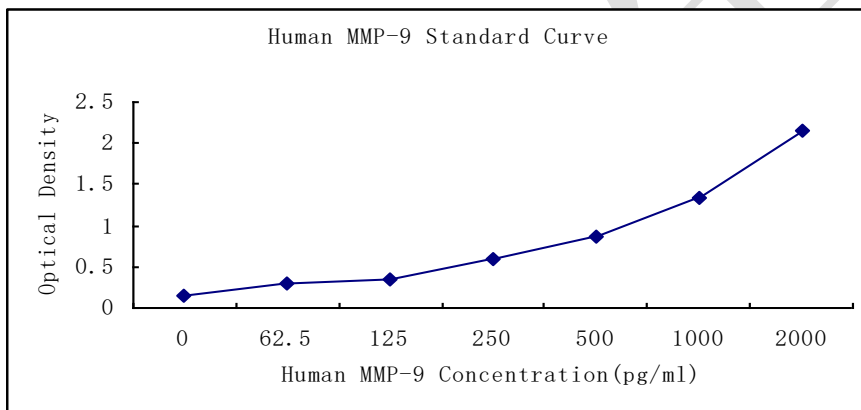


1. Duplicates should be within 20 per cent of the mean. Average absorbance values for each set of duplicate samples were used as detection results.
2. The blank absorbance values of subtract should be deducted.
3. Drawing a best fit curve through the points of graph. Draw the standard curve by plotting assayed OD value (on the Y axis) vs. concentration (on the X axis). The sample concentration was obtained based on its OD value founding in the standard concentration curve.
4. If the values obtained are not within the expected range of the standard, Samples should be dilute and assay again.

Typical Data and Standard Curve

concentration (pg/ml)	Typical data 1	Typical data 2	Average
0	0.1273	0.1547	0.141
62.5	0.2852	0.295	0.2901
125	0.3438	0.3632	0.3535
250	0.5779	0.5966	0.58725
500	0.8491	0.8733	0.8612
1000	1.3001	1.3866	1.34335
2000	1.9914	2.304	2.1477

Human MMP-9 standard curve



Sensitivity, Specificity, Repeatability

Sensitivity: repeated assays were evaluated and the minimum detectable dose was 7.3pg/ml.

Specificity : No significant cross-reactivity or interference with human MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-10, MMP-13, TIMP-2, TIMP-3, TIMP-4

Repeatability: The coefficient of variation between wells or plates is less than 10 percent.

REFERENCES:

1. Tamura, T. et al. (1996) *Endocrinology* **137**:3729.
2. Okamoto, T. et al. (1997) *J. Biol. Chem.* **272**:6059.
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