Human MMP-9 ELISA KIT

Catalog Number	**Size**
EA100105	48 Tests

Human MMP-9 ELISA KIT

For the quantitative determination of human active (82 kDa) and Pro- (92 kDa) Matrix Metalloproteinase 9 (MMP-9) concentrations in cell culture supernates, serum, and plasma.This package insert must be read in its entirety before using this product. If you have questions or experience problems with this product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that degrade extracellular matrix proteins (1 - 3). They are secreted as zymogens (Pro-MMPs) that are activated by a variety of proteinases or by reaction with organic mercurials. They are inhibited by specific tissue inhibitors of metalloproteinases (TIMPs) and by 2-macroglobulin (3 - 6). The regulation of MMP activity is important in tissue remodeling, inflammation, tumor growth and metastasis (3, 7 - 9).

Human MMP-9 (also known as gelatinase B) is secreted as a 92 kDa zymogen (2, 3). Cleavage of Pro-MMP-9 at or near residue 87 results in the active enzyme with a mass of approximately 82 kDa (1). MMP-9 has three fibronectin type II domains, a hemopexin-like domain and a proline-rich type V collagen-homologous domain (1 - 3). Pro-MMP-9 can be activated by MMP-3 (5) or by certain bacterial proteinases (10). MMP-9 is inhibited by α2-macroglobulin or by TIMP-1 (3 - 6), which binds to Pro-MMP-9 as well as to active MMP-9 (3). In vitro treatment of Pro-MMP-9 with 4-aminophenylmercuric acid (APMA) produces not only the 82 kDa active enzyme but also a C-terminal truncated form of approximately 65 kDa with the activity comparable to that of the 82 kDa form (11).

Pro-MMP-9 is secreted by monocytes, macrophages, neutrophils, keratinocytes, fibroblasts, osteoclasts, chondrocytes, skeletal muscle satellite cells, endothelial cells, and various tumor cells(1, 7- 21). Pro-MMP-9 expression is upregulated by TGF-β1, IL-1β, TGF-α, PDGF-AB, TNF-α, and IL-1α (7, 15, 17). Substrates for MMP-9 include denatured collagen type I (gelatin), native collagens type IV, V, VII, X and XI, fibrinogen, vitronectin, IL-1β, and entactin, a molecule that bridges laminin and type IV collagen (3, 4, 6, 13, 21 - 23).

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for 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. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for MMP-9 is added to the wells and binds to the combination of capture antibody-MMP-9 in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of MMP-9 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven MMP-9 standard dilutions and MMP-9 sample concentration determined.

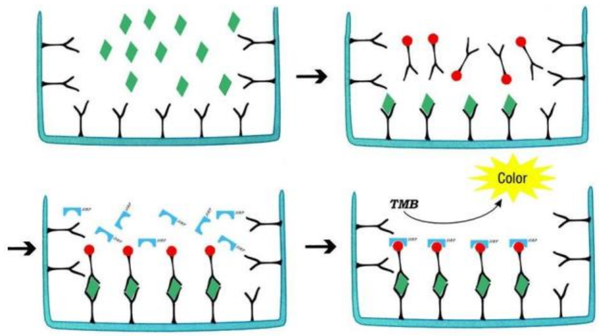

Figure 1:Schematic diagram of the assay

REAGENTS

1. Aluminium pouches with a Microwell Plate coated with antibody to human MMP-9 (8X12)
2. 2 vials human MMP-9 Standard lyophilized, 4000 pg/vial upon reconstitution
3. 2 vials concentrated Biotin-Conjugate anti-human MMP-9 antibody
4. 2 vials Streptavidin-HRP solution
5. 4 bottle Standard /sample Diluent
6. 1 bottle Biotin-Conjugate antibody Diluent
7. 1 bottle Streptavidin-HRP Diluent
8. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)
9. 1 vial Substrate Solution
10. 1 vial Stop Solution
11. 4 pieces Adhesive Films
12. Package insert

NOTE: [96 Tests]

STORAGE

Table 1: Storage of the kit

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 – 8℃. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="10">Opened/ Reconstituted Reagents</td><td>Standard /sample Diluent</td><td rowspan="8">May be stored for up to 1 month at 2 – 8℃.**</td></tr>
<tr><td>Concentrated Biotin-Conjugate</td></tr>
<tr><td>Streptavidin-HRP solution</td></tr>
<tr><td>Biotin-Conjugate antibody Diluent</td></tr>
<tr><td>Streptavidin-HRP Diluent</td></tr>
<tr><td>Wash Buffer Concentrate 20x</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at ≤20℃.
Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.</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℃.**</td></tr>
</table>

**Provided this is within the expiration date of the kit.

THE REQUIRED ITEMS (not provided, but can help to buy):

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000μl.
3. 37 ℃ incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

PRECAUTIONS FOR USE

1. Store kit regents between 2℃ and 8℃. After use all reagents should be immediately returned to cold storage (2℃ to 8℃).

2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.

4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).
   Note: The normal human serum or plasma samples are suggested to make a 1:400 dilution.

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard** - Reconstitute the Standard with 1.0mL of Standard /sample Diluent. 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.

   Pipette 500μl of Standard/sample Diluent into the 2000 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 4000 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).

   If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.
4. Working solution of Biotin-Conjugate anti-human MMP-9 antibody: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.
5. Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.

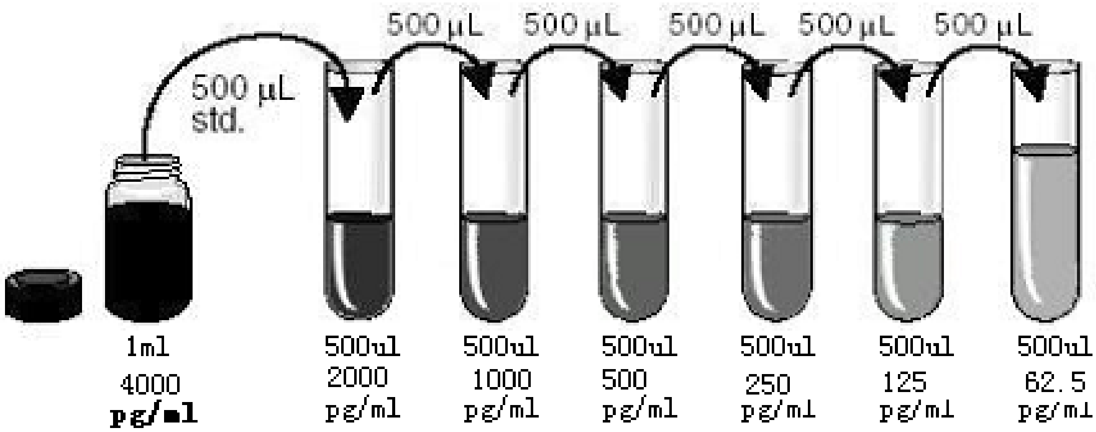

Figure 2: Preparation of MMP-9 standard dilutions

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.
3. Add 100µl of Standard, control, or sample, per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at 37℃.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350µl) using a squirt bottle, manifold dispenser or auto-washer. 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 100µl of the working solution of Biotin-Conjugate to each well. Cover with a new adhesive strip and incubate 1 hour at 37℃.
6. Repeat the aspiration/wash as in step 4.
7. Add 100µl of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at 37℃. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.

9. Add 100µl of Substrate Solution to each well. Incubate for 10-20 minutes at 37℃. Avoid placing the plate in direct light.
10. Add 100µl of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.(optionally 630nm as the reference wave length;610-650nm is acceptable)

ASSAY PROCEDURE SUMMARY

Prepare all reagents and standards as directed

⇩

Add 100µl standard or samples to each well, incubate 90 minutes,37℃

⇩ Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human MMP-9 antibody to each well, incubate 60 minutes,37℃

⇩ Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes,37℃

⇩ Aspirate and wash 4 times

Add 100µl Substrate solution to each well, incubate 10-20 minutes,37℃.Protect from light.

⇩

Add 100µl Stop solution to each well. Read at 450nm within 30 minutes.

Figure 3: Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. 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.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. 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.
3. The data may be linearized by plotting the log of the 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.
4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 2:Typical data using the MMP-9 ELISA (Measuring wavelength: 450nm, Reference wavelength: 630nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.098	0.090	0.094	——
62.5	0.208	0.216	0.212	0.197
125	0.269	0.253	0.261	0.262
250	0.414	0.402	0.408	0.389
500	0.603	0.618	0.611	0.630
1000	1.113	1.127	1.120	1.063
2000	1.701	1.685	1.693	1.733
4000	2.287	2.297	2.292	2.285

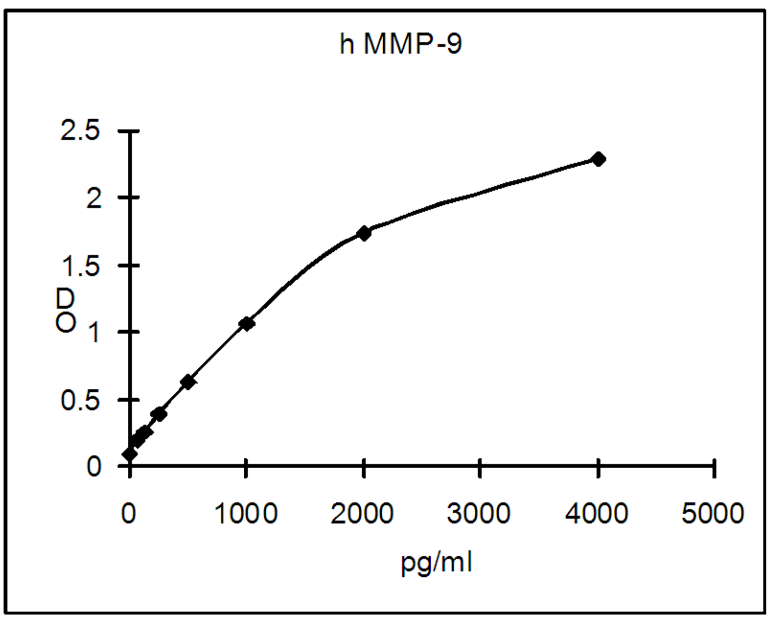

Figure 4:Representative standard curve for MMP-9 ELISA. MMP-9 was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

SENSITIVITY, SPECIFICITY AND REPEATABILITY

1. **REPEATABILITY**: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
2. **SENSITIVITY**: The minimum detectable dose was 31 pg/mL.
3. **SPECIFICITY:** This assay recognizes both natural and recombinant human MMP-9. The factors listed below were prepared at 200 ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 3: Factors assayed for cross-reactivity

Recombinant human	**Recombinant mouse**	
MMP-1	MMP-2	
MMP-2	MMP-3	
MMP-3	MMP-9	
MMP-7	TIMP-1	
MMP-8		
MMP-10		
MMP-12		

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If you have any questions, please tell us!