DATA SHEET

MMP9
Human Matrix Metallopeptidase 9 ELISA Kit
Catalog No: CKH176 Size: 1 x 96 wells

Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that degrade extracellular matrix proteins. MMPs have been linked with a wide array of biological activities and play important roles during organ development and pathological processes. Collectively MMPs are key enzymes for the metabolism of extracellular matrix proteins, including fibrillar and non-fibrillar collagens, fibronectin, laminin and basement membrane or interstitial stroma glycoproteins. Under physiological conditions MMPs are involved in extracellular degradation and breakdown of matrix proteins during normal tissue remodelling processes such as wound healing, pregnancy, and angiogenesis. Human MMP9 is a 92 kDa glycoprotein that plays a significant role in matrix remodeling, enzyme modulation, and cytokine/growth factor activation. MMP9 is also known as gelatinase B based on its ability to degrade gelatin.

The Cell Sciences Human MMP9 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human MMP9 pro and active forms in serum, plasma (Collect plasma using heparin as an anticoagulant. EDTA and Citrate are not recommended), cell culture supernatants and urine. This assay employs an antibody specific for human MMP9 coated on a 96-well plate. Standards and samples are pipetted into the wells and MMP9 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human MMP9 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of MMP9 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

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Reagents and materials supplied with the kit:

Items	Quantity
A. MMP9 Microplate coated with anti-human MMP9	96 wells (12 strips x 8 wells)
B. Wash Buffer Concentrate (20x)	25 ml
C. Recombinant human MMP9 Standards	2 vials
E. Assay Diluent (5x) for Standard/Sample (serum, plasma, cell culture medium/urine) diluent	15 ml
F. Detection Antibody-Biotinilyated anti-human MMP9	2 vials (each vial is enough to assay half microplate)
G. Streptavidin-HRP Concentrate (20,000x)	8 µl
H. TMB One-Step Substrate Reagent (TMB in buffered solution)	12 ml
I. Stop Solution (2 M sulfuric acid)	8 ml

Storage of Kit Reagents

Stable for up to 6 months from date of shipment at 2-4 ^{o}C. Store reconstituted standard (recombinant protein) at -80 ^{o}C. Opened Microplate Wells and reagents are stable for 1 month at 2-4 ^{o}C. Return unused wells to the pouch containing desiccant pack and reseal along the entire edge. Kit is stable for one year if entire kit stored at -20 ^{o}C.

Materials/reagents required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µl to 1 ml volumes
- Adjustable 1-25 ml pipettes for reagent preparation
- 100 ml and 1 liter graduated cylinders
- Absorbent paper
- Distilled or deionized water
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare standard or sample dilutions

Preparation of Kit Reagents

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Sample dilution: If your samples need to be diluted, Assay Diluent (Item E) is used for dilution of serum/plasma/culture supernatants/urine.
3. Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use.
4. Preparation of standard: **Briefly spin the vial of Item C**. Add 400 µl 1x Assay Diluent (Item E) into Item C vial to prepare a 50 ng/ml standard. **Dissolve the powder thoroughly by a gentle mix**. Add 80 µl MMP-9 standard from the vial of tem C, into a tube with 586.7 µl 1x Assay Diluent Buffer to prepare a 6000 pg/ml stock standard solution. Pipette 400µl 1x Assay Diluent into each tube. Use the stock standard solution to produce a Dilution series (shown below). Mix each tube thoroughly before the next transfer. Gently vortex to mix. 1x Assay Diluent serves as the zero standard (0 pg/ml).

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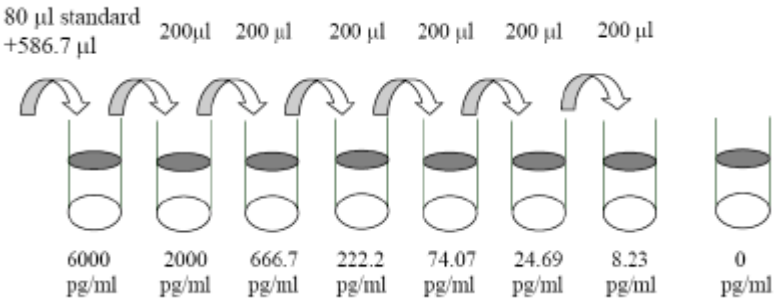

5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 100-fold with 1x Assay Diluent and used in step 4 of Part VI Assay Procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 20,000-fold with 1x Assay Diluent. *For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 2 µl of HRP-Streptavidin concentrate into a tube with 198.0 µl 1x Assay Diluent to prepare a 100-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix through and then pipette 70 µl of prepared 100-fold diluted solution into a tube with 14 ml 1x Assay Diluent to prepare a final 20,000 fold diluted HRP-Streptavidin solution.*

ELISA Method
Be sure to read **'Preparation of Kit Reagents'** before carrying out the assay.

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard (see **Preparation of Kit Reagents: MMP9 Standard**) and sample into appropriate wells. Cover and incubate for 2.5 hours at room temp. or overnight at 2-4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel pipette 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.
4. Add 100 µl of 1x prepared biotinylated antibody (see **Preparation of Kit Reagents: Detection Antibody**) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution and wash as in step 3 above.
6. Add 100 µl of prepared Streptavidin solution (see **Preparation of Kit Reagents: Streptavidin-HRP Concentrate**) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution and wash as in step 3 above.

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8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

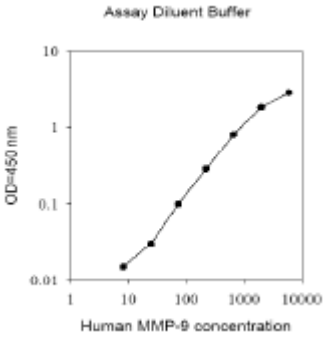
Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature, or over night at 4°C.
3. Add 100 µl prepared Biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µl TMB One-step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Figure 2: Typical Data
These standard curves are for demonstration only. A standard curve must be run with each assay.



Performances and Characteristics

Sensitivity

The minimum detectable dose of MMP9 is typically less than 10 pg/ml.

Recovery

Recovery was determined by spiking various levels of human MMP9 into human serum, plasma and cell culture media. Mean recoveries are as follows:

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Sample Type	Average % Recovery	Range (%)
Serum	96.23	84-103
Plasma	94.64	83-102
Cell culture media	95.38	84-104

Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected Range (%)	95 84-103	93 83-102	94 85-104
1:4	Average % of Expected Range (%)	96 85-104	97 86-105	96 83-103
1:8	Average % of Expected Range (%)	94 84-103	96 85-105	101 87-105

Reproducibility

Intra-assay: CV<10%
Inter-assay: CV<12%

Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (*e.g.*, human Angiogenin, BDNF, BLC, ENA-78, FGF4, IL1α, IL1β, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12 p70, IL12 p40, IL13, IL15, IL309, IP10, G-CSF, GM-CSF, IFN-γ, Leptin (OB), MCP1, MCP3, MDC, MIP1α, MIP1 β, MIP1δ, MMP1, -2, -3, -10, PARC, RANTES, SCF, TARC, TGF-β, TIMP1, TIMP2, TNF-α, TNF-β, TPO, VEGF).

Troubleshooting Guide

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times	1. Ensure sufficient incubation time; ELISA Method Step 2 may change to overnight.
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation.
3. Large CV	1. Inaccurate pipetting	1. Check pipettes.
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer.
5. Low sensitivity	1. Improper storage of the ELISA Kit	1. Store your standard at < -20°C after reconstitution, others at 2-4°C. Keep substrate solution protected from light.
	2. Stop solution	2. Stop solution should be added to each well before measure.

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