Human MMP-9 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00298

Size: 5*96T

Sensitivity: 16.6 pg/mL

Range: 125-8000 pg/mL

Usage: For the quantitative detection of human MMP-9 concentrations in serum, plasma, cell culture supernatant, saliva and urine.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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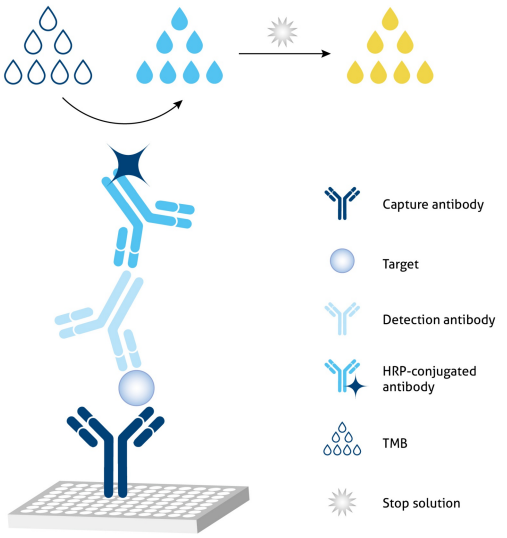
1. Background

MMP9 (matrix metallopeptidase 9), also named as Gelatinase B, is a member of matrix metalloproteinase (MMP) family. The MMP family of enzymes is comprised of critically important extracellular matrix remodeling proteases whose activity has been implicated in normal embryogenesis, tissue remodelling and many diseases such as arthritis, cancer, periodontitis, glomerulonephritis, encephalomyelitis, atherosclerosis and tissue ulceration. These proteases have come to represent important therapeutic and diagnostic targets for the treatment and detection of human cancers. MMP9 is produced by a variety of normal and transformed cells including neutrophils, monocytes, macrophages, astrocytes, fbroblasts, osteoclasts and so on. In tumors, MMP-9 destroys collagen (type IV) in the vascular basal membrane in the vicinity of tumor cells which invade the surrounding tissues and contributes to metastasis. Circulating levels of MMP-9 are increased in many inflammatory disorders such as atherosclerosis, hepatitis C virus infection and colorectal cancer.

2. Principle



Sandwich ELISA structure (HRP conjugated secondary antibody)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody also binds to the analyte. Horseradish peroxidase (HRP)-conjugated secondary antibody binds to the detection antibody. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

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4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	5 plates	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 16000 pg/bottle; lyophilized	10 bottles	
Detection antibody (100×) - 600 µL/vial*	1 vial	
HRP-conjugated antibody (100×) - 600 µL/vial*	1 vial	
Sample Diluent PT 4B1 - 150 mL/bottle	2 bottles	
Detection Diluent - 150 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 150 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 60 mL/bottle	1 bottle	
Stop Solution - 60 mL/bottle	1 bottle	
Plate Cover Seals	15 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.4 Urine: Collect urine samples and centrifuge for 20 minutes at 1000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.5 Saliva: Collect saliva samples and centrifuge for 5 minutes at 10,000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

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7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Detection Antibody (1X): Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody + 990 µL Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).

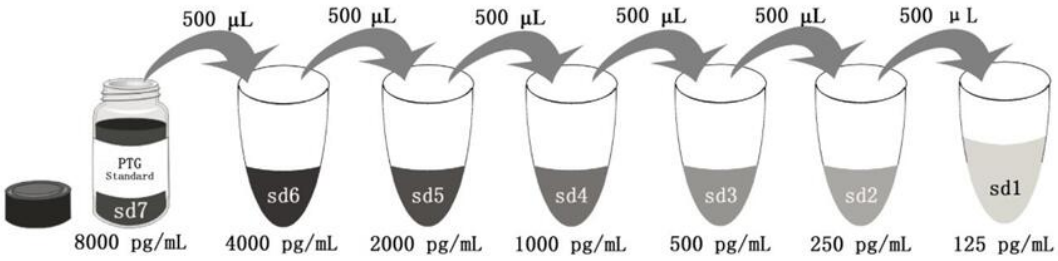
7.3 HRP-conjugated antibody (1X): Dilute 100X HRP-conjugated antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X HRP-conjugated antibody + 990 µL Detection Diluent (Centrifuge the 100X HRP-conjugated antibodyy solution for a few seconds prior to use).

7.4 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:160 or 1:320 is recommended for human serum, plasma and saliva; 1:80 or 1:160 is recommended for cell culture supernatant; 1:2 is recommended for urine.

7.5 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 4B1 in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and HRP-conjugated antibody can be used immediately). 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.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection Antibody solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 µL of 1X HRP-conjugated antibody solution (refer to Reagent Preparation7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C .

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

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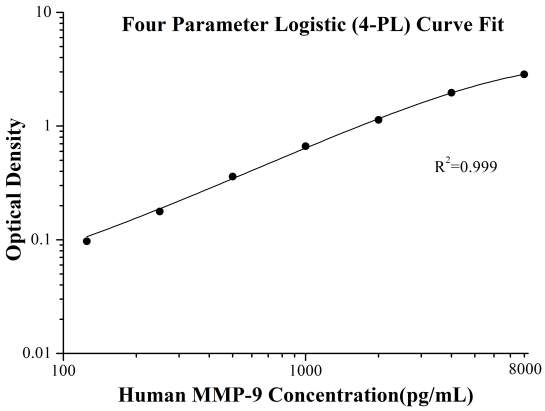
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.055 0.055	0.055	-
125	0.155 0.149	0.152	0.097
250	0.233 0.232	0.232	0.177
500	0.420 0.411	0.416	0.360
1000	0.725 0.716	0.720	0.665
2000	1.168 1.210	1.189	1.134
4000	1.992 2.044	2.018	1.963
8000	2.912 2.900	2.906	2.851

9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	3,875.1	199.6	5.2
2	20	957.3	26.1	2.7
3	20	250.3	5.4	2.1

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	3,831.8	137.2	3.6
2	24	917.2	28.5	3.1
3	24	241.6	8.5	3.5

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9.3 Recovery

The recovery of human MMP-9 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:500	85	82-91
	1:1,000	85	79-90
Cell culture supernatant	1:320	94	86-100
	1:640	90	87-98
Saliva	1:320	90	75-107
	1:640	92	87-100
Urine	1:2	84	81-86
	1:4	86	84-87

9.4 Sample values

Human serum, urine and saliva samples were evaluated for the presence of human MMP-9 in this assay.

Sample Type	%Detectable	Mean (ng/mL)	Range (ng/mL)
Human serum (n=16)	100	421.8	18.7-1,583.6
Saliva (n=8)	100	779.1	73.4-1,579.1
Urine (n=8)	50	0.08	ND*-0.29

ND*=Non-detectable

Cell culture supernatant - Human peripheral blood mononuclear cells (PBMC) (1 x 10^6cells/mL) were cultured in DMEM supplemented with 8% fetal bovine serum, 5μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μg/mL PHA for 3 days. Aliquots of the cell culture supernatant were removed and assayed for levels of human MMP-9.

Condition	3 day (ng/mL)
Unstimulated	10.6
Stimulated	141.3

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9.5 Sensitivity

The minimum detectable dose of human MMP-9 is 16.6 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, human serum, cell culture supernatant and saliva samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Urine samples were spiked with high concentrations of human MMP-9 and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(Human serum and saliva were initially diluted 1:80, cell culture supernatant was initially diluted 1:40.)

		Human serum	Cell culture supernatant	Saliva	Urine
1:2	Average% of Expected	100	100	100	91
	Range (%)	-	-	-	78-99
1:4	Average% of Expected	104	105	102	92
	Range (%)	99-109	102-108	97-105	85-95
1:8	Average% of Expected	105	108	106	99
	Range (%)	100-109	106-109	105-107	92-104
1:16	Average% of Expected	103	108	110	98
	Range (%)	92-114	108-109	105-118	94-102

9.7 Specificity

This assay recognizes natural and recombinant human MMP-9.

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

Recombinant human:		Recombinant mouse:	Recombinant rat:
MMP-1	MMP-13	MMP-9	MMP-9
MMP-2	MMP-14		
MMP-3	TIMP-2		
MMP-7	TIMP-3		
MMP-8	TIMP-4		
MMP-12			

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10. References

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