Speedy™ Human MMP-9 One-Step ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: SE50011

Size: 96T

Sensitivity: 8.1 pg/mL

Range: 39-2500 pg/mL

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

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

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

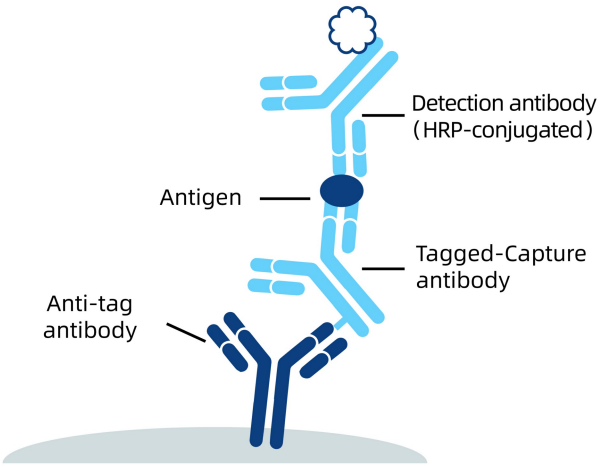
Table of content page

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

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



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the 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. Proteintech data analysis website, https: //www.ptgcn.com/products/elisa-kits/.

3.7 Microplate thermostatic shaker.

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

4. Kit Components and Storage

Microplate - 96 well microplate precoated an anti-tag antibody (8 well × 12 strips)	1 plate	**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 - 5000 pg/bottle; lyophilized	2 bottles	
Capture antibody (100×) - 60 µL/vial*	1 vial	
Detection antibody, HRP-conjugated (100×) - 60 µL/vial*	1 vial	
Sample Diluent PT 4B1 - 30 mL/bottle	1 bottle	
Detection Diluent - 15 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 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.

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

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. Buffer.

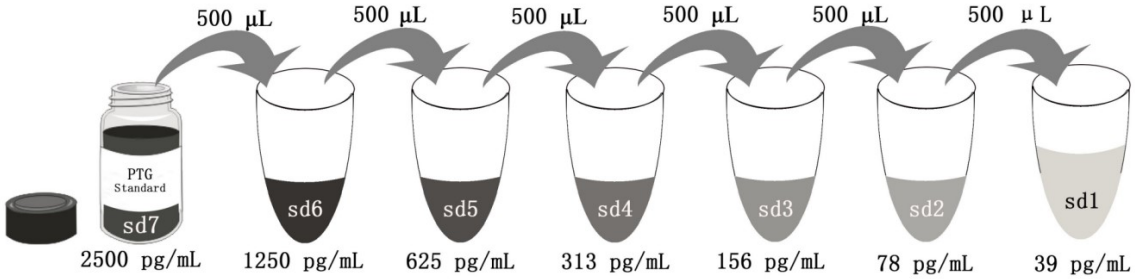
7.2 Antibody Cocktail (1X): Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 µL 100X capture antibody + 50 µL 100X Detection Antibody, HRP-conjugated + 4,900 µLDetection Diluent. Mix gently but thoroughly.

7.3 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: 1:50 or 1:100 is recommended for human serum and plasma; 1:400 or 1:800 is recommended for cell culture supernatant; 1:2 is recommended for urine; 1:50 or 1:100 is recommended for saliva.

7.4 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"

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, 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 Preset the layout of the microplate, including control group, standard group and sample group, 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 Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.3 Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available) .

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 1× 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 TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.6 Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.7 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.8 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, four-parameter logistic curve- fit (4-PL) analysis is recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

Procedure summary

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

Add 50 µL standard or sample to appropriate wells

↓

Add 50 µL antibody cocktail solution (1x) to each well

↓

Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 1 hour

↓

Aspirate and wash each well four times with 400µL 1 x Wash Buffer

↓

Add 100 µL TMB substrate solution to each well

↓

Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 15minutes

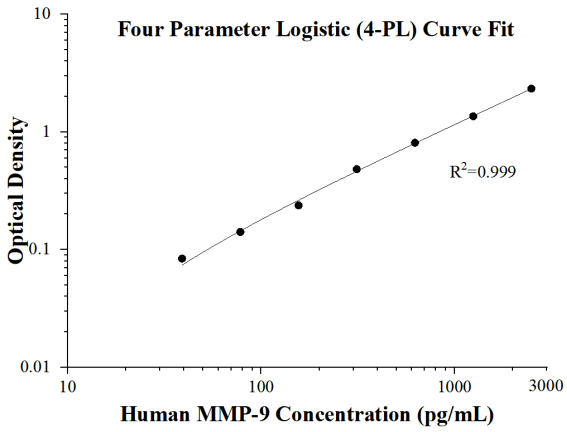
↓

Add 100 µL Stop Solution to each well and Read OD immediately

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	0.0277 0.0276	0.02765	-
39	0.0831 0.0847	0.0839	0.05625
78	0.1461 0.1365	0.1413	0.11365
156	0.2333 0.2413	0.2373	0.20965
313	0.4717 0.4902	0.48095	0.4533
625	0.8387 0.7807	0.8097	0.78205
1250	1.3298 1.3891	1.35945	1.3318
2500	2.3125 2.349	2.33075	2.3031

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	8	1,328.9	34.7	2.6
2	8	339.0	7.9	2.3
3	8	164.4	4.4	2.7

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	16	1,331.5	34.2	2.6
2	16	333.4	8.3	2.5
3	16	160.2	7.5	4.7

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 plasma	1:200	98	86-119
Cell culture supernatant	1:3,200	95	86-101
Urine	1:4	86	80-92
Saliva	1:160	102	90-110

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

9.4 Sample values

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

Sample Type	Mean (**ng/mL**)	Range (**ng/mL**)
Human serum (n=16)	46.3	17.5-104.1
Saliva (n=8)	80.7	7.8-171.3

Sample Type	Mean (pg/mL)	% Detectable	Range (pg/mL)
Urine (n=8)	1,061.9	50	ND-3,483.5

ND*=Non-detectable

Cell culture supernatant - human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented 10% fetal bovine serum, 5 µM 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 1 day. Aliquots of the cell culture supernates were removed and assayed for levels of human MMP-9.

Condition	Day 1 (**ng/mL**)
Unstimulated	194.5
Stimulated	291.2

9.5 Sensitivity

The minimum detectable dose of human MMP-9 is 8.1 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, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.
(The human plasma was initially diluted 1:25. The cell culture supernatant was initially diluted 1:200. The saliva was initially diluted 1:25.)

		Human plasma	Cell culture supernatant	Urine	Saliva
1:2	Average% of Expected	100	100	100	100
	Range (%)	-	-	-	-
1:4	Average% of Expected	113	114	112	104
	Range (%)	100-126	107-120	111-113	98-111
1:8	Average% of Expected	93	117	123	93
	Range (%)	81-106	108-126	122-124	91-95
1:16	Average% of Expected	90	114	123	98
	Range (%)	89-91	109-120	119-127	85-111

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

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 human:
MMP-1	MMP-9
MMP-2	
MMP-3	
MMP-7	
MMP-8	
MMP-12	
MMP-13	

10. References

1. Roy, Roopali et al. (2009) J Clin Oncol. 27(31):5287-5297.
2. Tanindi, Asli et al. (2011) Open Cardiovasc Med J. 5:110-116.
3. Coussens, L M et al. (2000) Cell. 103(3):481-490.
4. Pyo, R et al. (2000) J Clin Invest. 105(11):1641-1649.
5.Bergers, G et al. (2000) Nat Cell Biol. 2(10):737-744.

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com