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ab100610
Human MMP9 ELISA Kit

For the quantitative measurement of Human MMP9 pro and active forms in serum, plasma, and cell culture supernatants. (Collect plasma using heparin as an anticoagulant. EDTA and Citrate are not recommended).

This product is for research use only and is not intended for diagnostic use.

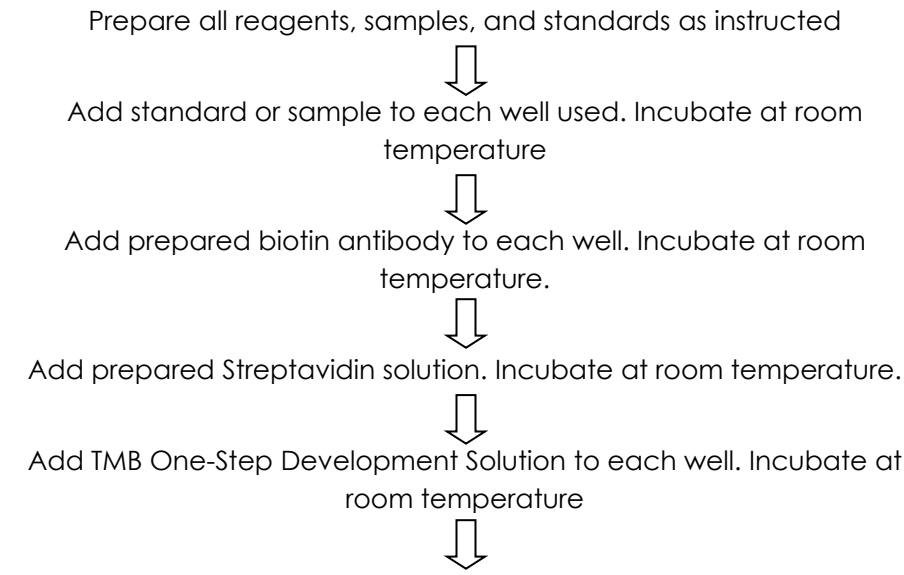
Table of Contents

1. Overview

Abcam's MMP9 Human 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), and cell culture supernatants.

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.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Amount	Storage Condition (Before Preparation)
MMP9 Microplate (12 x 8 wells)	96 wells	-20°C
20X Wash Buffer Concentrate	25 mL	-20°C
Recombinant Human MMP9 Standard	2 vials	-20°C
5X Assay Diluent	15 mL	-20°C
Biotinylated anti-Human MMP9	2 vials	-20°C
400X HRP-Streptavidin Concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 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.

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Assay Diluent

5X Assay Diluent should be diluted 5-fold with deionized or distilled water before use.

9.2 1X Wash Solution

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Solution.

9.3 1X Biotinylated MMP9 Detection Antibody

Briefly spin the Biotinylated anti-Human MMP9 vial 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 either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate must be diluted 80-fold with 1X Assay Diluent prior to use in the Assay Procedure.

9.4 1X HRP-Streptavidin Solution

Briefly spin the 400X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. HRP-Streptavidin concentrate must be diluted 400-fold with 1X Assay Diluent prior to use in the Assay Procedure.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 30 µL of 400X HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent to prepare a 400-fold diluted 1X HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

10. Standard Preparation

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Standard (recombinant protein) should be stored at -20°C or 80°C (recommended at -80°C) after reconstitution.

10.1 Reconstitute Briefly spin the vial of MMP9 Standard. Prepare the 50 ng/mL MMP9 **Stock Standard** by adding 400 µL 1X Assay Diluent into the vial (see table below).

10.2 Dissolve the powder thoroughly by a gentle mix.

10.3 Label tubes #1-7.

10.4 Prepare **Standard #1** by adding 80 µL 50 ng/mL **Stock Standard** to 586.7 µL Assay Diluent into tube #1. Mix thoroughly and gently.

10.5 Pipette 400 µL 1X Assay Diluent into remaining tubes.

10.6 Prepare **Standard #2** by transferring 200 µL from tube #1 to #2, mix thoroughly.

10.7 Prepare **Standard #3** by transferring 200 µL from tube #2 to #3, mix thoroughly.

10.8 Using the table below as a guide, prepare further serial dilutions

10.9 1X Assay Diluent serves as the zero standard (0 pg/mL).

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	80	586.7	666.7	50,000	6,000
2	200	400	600	6,000	2,000
3	200	400	600	2,000	666.7
4	200	400	600	666.7	222.2
5	200	400	600	222.2	74.07
6	200	400	600	74.07	24.69
7	200	400	600	24.69	8.23
8	0	400	400	0	0

11. Sample Preparation

General Sample Information:

- We recommend preparing serum-free or low-serum medium samples, as serum tends to contain cytokines which may produce significant background signals. If it is necessary to test serum containing medium, we recommend also running an uncultured media blank to assess baseline signals. This baseline can then be subtracted from the cultured media sample data.
- If your samples need to be diluted, 1X Assay Diluent should be used for dilution of serum, plasma and culture supernatants.
- Suggested dilution for normal serum/plasma: 300-3,000 fold.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

Cell culture supernatant preparation:

11.1 On day 0, seed ~1 million cells in 100 mm tissue culture plate with complete medium.*

11.2 On day 3, remove medium and replace medium with 6-8 mL of serum-free or low serum containing medium (e.g. medium containing 0.2% calf serum).

11.3 On day 5, collect medium into 15 mL tube. Centrifuge at 2,000 rpm in centrifuge at 4ºC for 10 minutes. Save the supernatant. Transfer the supernatant into 1.5 mL Eppendorf tubes. Store supernatant at -80ºC until experiment. Most samples can be stored this way for at least a year.

*The optimal number of seeded cells varies from one cell type to another and may need to be empirically determined.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Add 100 µL of each standard (see Standard Preparation section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

13.2 Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with 1X Wash Solution (300 µL) using a multi-channel Pipette or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining liquid by aspirating or decanting. Invert the plate and blot it against clean paper towels.

13.3 Add 100 µL of 1X Biotinylated MMP9 Detection Antibody (Standard Preparation section 10) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4 Discard the solution. Repeat the wash as in step 13.2.

13.5 Add 100 µL of 1X HRP-Streptavidin solution (see Standard Preparation section 10) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

13.6 Discard the solution. Repeat the wash as in step 13.2.

13.7 Add 100 µL of TMB One-Step Substrate Reagent to each well.

13.8 Incubate for 30 minutes at room temperature in the dark with gentle shaking.

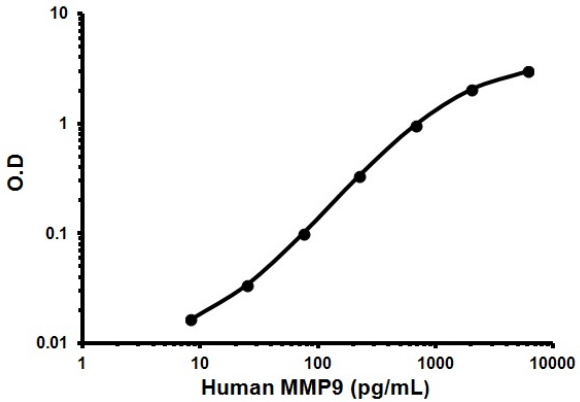
13.9 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

14. Calculations

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, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D. Assay Diluent
8.23	0.017
24.69	0.034
74.07	0.100
222.2	0.336
666.7	0.975
2,000	2.054
6,000	3.000

Figure 1. Example of human MMP standard curve.

16. Typical Sample Values

SENSITIVITY –

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

PRECISION –

	Intra-Assay	Inter-Assay
CV (%)	<10%	<12%

RECOVERY –

Recovery was determined by spiking Human MMP9 into normal Human serum, plasma and cell culture media. Mean recoveries are as follows:

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

Linearity of Dilution

Serum Dilution	Average % Expected Value	Range (%)
1:2	95	84-103
1:4	96	85-104

Plasma Dilution	Average % Expected Value	Range (%)
1:2	93	83-102
1:4	97	86-105

Cell Culture Media Dilution	Average % Expected Value	Range (%)
1:2	94	85-104
1:4	96	83-103

17. Assay Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: Human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN-γ, Leptin (OB), MCP-1, MCP-3, MDC, MIP-1α, MIP-1 β, MIP-1δ, MMP-1, -2, -3, -10, PARC, RANTES, SCF, TARC, TGF-β, TIMP-1, TIMP-2, TNF-α, TNF-β, TPO, VEGF).

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

19. Notes

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