Mouse MMP9 Assay Kit

Mouse MMP9 Assay Kit is a detection kit for the quantification of Mouse MMP9 in serum, plasma, cell culture supernatants, urine and tissue homogenates.

Catalog number: ARG82632

Package: 96 wells

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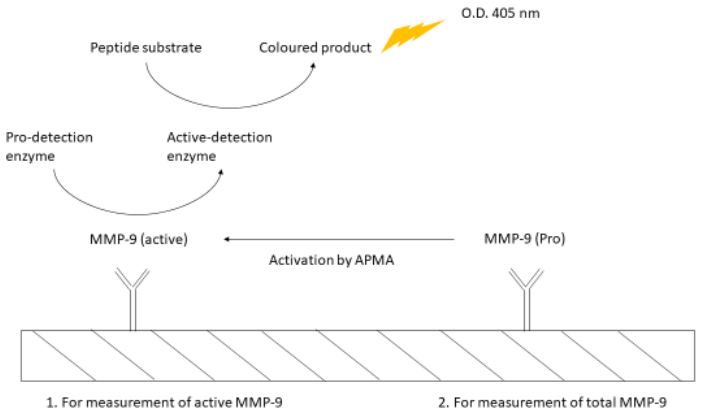
INTRODUCTION

Matrix metallopeptidase 9 (MMP-9), also known as 92 kDa type IV collagenase, 92 kDa gelatinase or gelatinase B (GELB), is a matrixin, a class of enzymes that belong to the zinc-metalloproteinases family involved in the degradation of the extracellular matrix. In humans the MMP9 gene encodes for a signal peptide, a propeptide, a catalytic domain with inserted three repeats of fibronectin type II domain followed by a C-terminal hemopexin-like domain. [Provide by Wikipedia: MMP9]

PRINCIPLE OF THE ASSAY

This Mouse MMP9 Assay Kit is a simple assay that measures the amount of mouse MMP9 in the active or pro-form in biological samples such as serum, plasma, cell culture supernatants, urine and tissue homogenates. This assay is based on using a modified pro-enzyme as a substrate, which upon activation is able to release color from a chromogenic peptide substrate. This multiplication step provides an unique assay sensitivity.



MATERIALS PROVIDED & STORAGE INFORMATION

Store all reagent at -20°C upon receiving except for the Standard, this vial should be stored at -70°C. Do not use kit components past kit expiration data.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	-20°C
Assay Buffer (Tris-HCl Buffer)	100 mL	-20°C
p-Aminophenylmercuric acetate (APMA)	17.5 mg	-20°C
Detection Enzyme (In Tris-HCl Buffer)	600 µL	-20°C
Substrate	1 mL	-20°C
20X Wash Buffer	25 mL	-20°C
Standard (500 ng/mL mouse pro-MMP-9))	20 µL	-70°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 405 nm
- Centrifuge and centrifuge tube
- Incubator at 37°C
- Dimethyl Sulphoxide (DMSO)
- Plate Sealer
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- Aminophenylmercuric acetate (APMA) is toxic. See for relevant material safety data sheet.
- Dimethyl Sulphoxide (DMSO) is harmful and an irritant. See for relevant material safety data sheet.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2000 x g for 10 minutes at 4°C. Store frozen at -20°C or lower. Avoid freeze-thaw cycles. Dilution of the serum with Assay Buffer (30 fold or more) might be required for a good recovery.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Store frozen at -20°C or lower. Avoid freeze-thaw cycles. Dilution of the serum with Assay Buffer (30 fold or more) might be required for a good recovery.

Tissue: Homogenize tissue in Tris-HCl buffer (50 mM, pH 7-8) containing a non-ionic detergent (E.g., 0.1% (v/v) Triton-X-100). Depending on the tissue a Potter homogenizer or other mechanical device might be required. Centrifuge for 10 minutes at 10,000 x g to remove any cell debris. Store frozen at -20°C or lower. Avoid freeze-thaw cycles. Dilution of the homogenate might be required depending on MMP-9 level and other components.

Urine: centrifuge urine sample immediately after collection for 10 minutes at 10,000 x g to remove debris. Store frozen at -20°C or lower. Avoid freeze-thaw cycles.

Cell culture supernatant: centrifuge culture medium immediately after collection for 10 minutes at 10,000 x g to remove debris. Store frozen at -20°C or lower. Avoid freeze-thaw cycles. Dilution of the supernatant might be required depending on MMP-9 level and other components in the medium.

Note:

- Rapidly freeze the samples in aliquots (use dry ice, liquid nitrogen or a cold bath, do not put in storage freezer unfrozen).
- Rapidly thaw samples in water bath (not higher than 37 °C) and immediately put on ice until use.

REAGENT PREPARATION

- **Assay Buffer:** Thaw the Assay Buffer and store at 2-8°C.
- **Standard:** Add a volume of Assay Buffer to the standard vial as specified on the Standard Lot Specification Sheet. Gently mix, this is the 20 ng/mL stock. Store on ice until required.

Standard tube	MMP-9 (ng/mL)	Assay Buffer (µL)	Standard stock, 20 ng/mL (µL)
S1	4.0	400	100
S2	2.0	250	250 of S1
S3	1.0	250	250 of S2
S4	0.5	250	250 of S3
S5	0.25	250	250 of S4
S6	0.125	250	250 of S5
S7	0.63	250	250 of S6
S8	0.031	250	250 of S7
S9	0.016	250	250 of S8
S10	0.008	250	250 of S9
S11	0.004	250	250 of S10
S0	0	500	0

Note: It is important to perform this procedure on ice.

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 25 mL of 20X Wash Buffer into 575 mL of distilled water to a final volume of 500 mL). Store at room temperature in a closed vessel until required.
- Working APMA Solution: Add 50 µL of DMSO to the vial, replace the cap and vortex until the solution is clear. This is the concentrated APMA Solution (1 M). Then add 5 µL from the 1M APMA Solution to a vial containing 10 mL of Assay Buffer at room temperature and mix well. This is the Working APMA Solution (0.5 mM). The concentrated APMA Solution (1 M) can be stored at -20°C. Thaw not more than once, then dispose according to local regulation.
- Detection Reagent: This reagent should be prepared immediately prior to addition to the wells. For 96 wells, mix 550 µL of Detection Enzyme, 880 µL of Substrate and 4070 µL of Assay Buffer.

ASSAY PROCEDURE

It is recommended that all samples and standards be assayed in duplicate.

	Sample well (Pro-MMP-9)	Sample well (active MMP-9)	Standard well	Blank well
Assay Buffer				100 µL
Each sample	100 µL	100 µL		
Each diluted standard			100 µL	
Cover the plate and incubate at **2-8°C overnight.**				
Aspirate and wash all wells **4 times** with **350 µL** of **1X Wash Buffer**. Ensuring that the wells are completely filled and emptied at each wash.				
Working APMA Solution	50 µL		50 µL	
Assay Buffer		50 µL		
Detection Reagent	50 µL	50 µL	50 µL	50 µL
Shake the plate for **20 seconds**. Read the absorbance at **O.D. 405 nm** to obtain **T_0 value.**				
Cover the plate and incubate at **37°C** for **1 hour** in a moist environment (to prevent evaporation).				
Shake the plate for **20 seconds**. Read the absorbance at **O.D. 405 nm** to obtain **T_1 value.**				
Cover the plate and incubate at **37°C** for **another 5 hours (total 6 hours)** in a moist environment (to prevent evaporation).				
Shake the plate for **20 seconds**. Read the absorbance at **O.D. 405 nm** to obtain **T_6 value.**				
Calculate the **T_1 value** data from the standard curve using the following range: 0, 0.125, 0.25, 0.5, 1, 2, 4 ng/ml MMP-9 (see CALCULATION OF RESULTS)				
Calculate the **T_6 value** data from the standard curve using the following range: of 0, 0.004, 0.008, 0.016, 0.031, 0.063, 0.125 ng/ml MMP-9 (see CALCULATION OF RESULTS)				

CALCULATION OF RESULTS

The MMP-9 concentration in the assay samples can be calculated in various ways. The use of a software employing a regression curve fitting algorithm is recommended. Manual calculation can be done as follows:

1. Calculate the ΔA for each well (samples and blanks) after 1h and 6h incubation by subtracting the T_0 value from the T_1 value and T_6 value.
2. Average the ΔA values of multiple blanks to obtain an average blank ΔA value for t=1 hour and t=6 hour incubation.
3. Subtract the average blank ΔA T_1 value from the ΔA of the various samples T_1 value and subtract the average blank ΔA T_6 value from the ΔA of the various samples T_6 value.
4. Create a "high level" standard curve from the ΔT_1 value data by plotting the blank subtracted ΔA values at t=1 hour against the MMP-9 standard concentration. You can use the zero and all concentrations in the standard curve for this "high level" standard curve.
5. Draw a best-fit curve through the points in the graph.
6. Using this standard curve the ΔA values of the "high level" test samples can be calculated in ng/ml either graphically, or by using the curve fitting software. Be aware of including dilution factors of your samples to calculate the final results.
7. Create a "low level" standard curve from the ΔT_6 value data by plotting the blank subtracted ΔA values at t=6 hour against the MMP-9 standard concentration. You should only use the 0, 0.004, 0.008, 0.016, 0.031, 0.063 and 0.125 ng/ml concentrations in the standard curve for this "low level" standard curve, since the higher values will be outside the useable range.

8. Draw a best-fit curve through the points in the graph.
9. Using this standard curve the ΔA values of the "low level" test samples can be calculated in ng/ml either graphically or by using the curve fitting software. Be aware of including dilution factors of your samples to calculate the final results.
10. If all your test samples can be read on the "high level" standard curve you could simplify future assays by using only the 1 h reading and a shorter standard line (0, 0.125, 0.25, 0.5, 1, 2 and 4 ng/ml).
11. If all your test samples can be read on the "low level" standard curve you could simplify future assays by using only the 6 h reading and a shorter standard line (0, 0.004, 0.008, 0.0016, 0.0031, 0.0063 and 0.125 ng/ml).

QUALITY ASSURANCE

20 pg/ml for 1 hour incubation

1 pg/ml for 6 hours incubation

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Mouse MMP9 Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.