Human MMP8 Assay Kit

Human MMP8 Assay Kit is a detection kit for the quantification of Human matrix metallopeptidase 8 activity in Human Serum, plasma, cell culture supernatants, urine, saliva and tissue homogenates.

Catalog number: ARG82630

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INTRODUCTION

MMP8 encodes a member of the matrix metalloproteinase (MMP) family of proteins. These proteins are involved in the breakdown of extracellular matrix in embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Proteolysis at different sites on this protein results in multiple active forms of the enzyme with distinct N-termini. This protein functions in the degradation of type I, II and III collagens. The gene is part of a cluster of MMP genes which localize to chromosome 11q22.3. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jan 2015]

MMP8 can degrade fibrillar type I, II, and III collagens. [UniProt]

PRINCIPLE OF THE ASSAY

This assay kit provides a simple, specific and precise quantitative determination of Human MMP8 in the active or pro-form in biological samples. A secondary antibody is pre-coated onto a microtiter plate. An antibody specific for MMP8 is then added in the plate. After incubation and washing, the standards (human pro-MMP8) or samples are pipetted into the wells and any MMP8 present is bound by the immobilized antibody. After washing away any unbound substances, APMA is added in the standard wells and the wells used to determine total MMP8 activity, but not the wells used to detect endogenous MMP8 activity. The pro-MMP8 in samples or standards is activated by APMA. The pro-enzyme in detection reagent is then added into the well and then in turn to active form of enzyme by activated MMP8 protein. The active form of enzyme catalyzes the reaction of chromogenic substrate to form a colored product and it is measured at a wavelength of 405nm. The MMP8 activity in

the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped with blue ice. Upon receipt, store the kit at -20°C, and the Standard and Antibody concentrate should be stored at-70°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 wells X 12 strips	-20°C. Unused strips should be sealed tightly in the air-tight pouch.
200X Antibody concentrate	60 µl	-70°C
Standard (480 ng/ml)	50 µl	-70°C
Detection enzyme	600 µl	-20°C or lower
Assay buffer	100 ml (Ready-to-Use)	-20°C
Substrate	1 ml	-20°C or lower
APMA (17.5 mg)	1 vial	-20°C
20 X Wash buffer	25 ml	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Horizontal orbital microplate shaker
- (Microplate) incubator at 37°C or 37°C oven.
- Dimethyl Sulphoxide (DMSO)
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The kit is shipped with blue ice. Upon receipt, it is recommended to aliquot and store to standard stock and Antibody concentrate at-70°C to minimize freeze-thaw cycles. And store other components at-20°C. Do not use kit, or individual kit components past kit expiration date.
- After opening, microwell plate or individual strips should be stored at -20°C or lower in original foil packaging with desiccant until use.
- Store the kit at -20°C at all times. The kit should not be used beyond the expiration date on the kit label.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Do not let strips dry, as this will inactivate active components in wells.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Avoid using reagents from different batches.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.

- A separate standard curve must be run on each plate.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and rapidly freeze the samples by dry ice, liquid nitrogen or a cold bath is recommended. Store the aliquoted samples at -20°C or lower. Avoid repeated freeze-thaw cycles. Dilution of the serum with Assay buffer (20 fold or more) might be required for a good recovery.

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes 1,000 x g at 4°C. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and rapidly freeze the samples by dry ice, liquid nitrogen or a cold bath is recommended. Store the aliquoted samples at -20°C or lower. Avoid repeated freeze-thaw cycles. Dilution of the plasma with Assay buffer (20 fold or more) might be required for a good recovery.

Cell Culture Supernatants - Remove particulates by centrifugation for at least 10 min at 10,000 x g at 4°C. Collect the supernatants and assay immediately or aliquot and rapidly freeze and store samples at-20°C or lower. Avoid repeated freeze-thaw cycles.

Urine- Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation 10,000 x g at 4°C for at least 10 min. Collect the supernatants and assay immediately or aliquot and rapidly freeze and store samples at -20°C or lower. (Note: Creatinine assay may be used for results

normalization.)

Tissue lysate- *Methods to prepare tissue homogenates are very dependent on tissue type. The following method is for guidance only.*

Cut the tissues in small pieces, homogenize with Tris-HCl buffer (50 mM, pH 7-8) containing a non-ionic detergent e.g. 0.1% (v/v) by Potter homogenizer or other mechanical device (depending on the tissue) on ice. Centrifuge samples 10000 X g at 4 °C for at least 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay. Assay immediately or aliquot and rapidly freeze and store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Note:

a) Rapidly thaw samples in water bath (not higher than 37 °C) and immediately put on ice until use.
b) Thawed sample should be mix thoroughly prior to assay.
c) Do not use haemolytic, icteric or lipaemic specimens.

REAGENT PREPARATION

- 1X Wash Buffer: It is recommended store concentrated wash buffer at -20°C and diluting only needed volume. Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 25 ml of 20X Wash buffer + 475 ml of distilled water) The diluted Wash buffer is stable for only few days at 2°C to 8°C. For long term storage, the diluted Wash buffer can be stored at-20°C for few months. Keep the 1X wash buffer at RT prior to use.
- Assay buffer: Thaw the assay buffer and store at 2-8°C prior to use. Assay buffer is stable at 2-8°C for few days only, and can be stored at -20°C for few months. It is recommended to aliquot in a small volume and store at -20°C.
- 1X Antibody working solution: It is recommended to prepare this reagent immediately prior to use and dilute only needed volume for once used. Dilute 200X Antibody concentrate with Assay buffer. E.g.: Add 50 µl of antibody concentrate into 10 ml of assay buffer, mix well.
- APMA: Reconstitute the APMA with 50 µl of DMSO to yield a stock concentration of 1M. Replace the cap and vortex until the solution is clear. The 1M APMA stock solution should be aliquoted and stored at -20°C. Do not thaw the 1M APMA stock solution for more than once. Prior to use, add 15 µl of 1M APMA stock solution into 10 ml of assay buffer at room temperature and mix well to yield a 1.5 mM APMA working solution.
- Detection enzyme: Thaw Detection enzyme on ice before use, keep the thawed Detection enzyme on ice prior to use.

- **Substrate**: Thaw Substrate before use, keep the thawed Substrate on ice prior to use.
- **Detection reagent**: It is recommended to prepare this reagent immediately prior to addition to the wells.

  For 96 wells: mix 550 µl detection enzyme solution, 880 µl substrate solution, 4070 µl assay buffer together in a vial. Mix gently and add 50 µl to each well of the plate during the assay procedure
- **Standards**: It is important to perform this procedure on ice.

  Add 950 µl of assay buffer to the standard vial, gently mix, to yield a stock concentration of 24,000 pg/ml. Aliquot and store this stock standard at -70˚C. Avoid repeated freeze-thaw cycles. The assay buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with assay buffer as according to the suggested concentration below: 12,000 pg/ml, 6,000 pg/ml, 3,000 pg/ml, 1,500 pg/ml, 750 pg/ml, 375 pg/ml, 187.5 pg/ml, 93.75 pg/ml, 46.875 pg/ml, 23.44 pg/ml and 11.72 pg/ml.

Dilute MMP8 standard as according to the table below:

Standard	MMP8 Conc. (pg/ml)	µl of Assay buffer	µl of standard
S11	12000	300	300 (24,000 pg/ml Stock)
S10	6000	300	300 (S11)
S9	3000	300	300 (S10)
S8	1500	300	300 (S9)
S7	750	300	300 (S8)
S6	375	300	300 (S7)
S5	187.5	300	300 (S6)
S4	93.75	300	300 (S5)

S3	46.875	300	300 (S4)
S2	23.44	300	300 (S3)
S1	11.72	300	300 (S2)
S0	0	300	0

ASSAY PROCEDURE

Standard MMP8 detection curve should be prepared for each experiment. Standards, samples and standard should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Put remaining strips immediately back at-20°C.
2. Add **100 µl** of diluted **1X antibody working solution** into wells**.** Cover the plate with foil and incubate for **2 hours at 37°C** in a moist environment (to prevent evaporation).
3. Prepare the MMP8 standards just before the end of the antibody incubation step.
4. Aspirate each well and wash, repeating the process three times for a total **four washes**. Wash by filling each well with **1X Wash Buffer** (350 µl or completely filled the well with 1X wash buffer) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time.
5. Add **100 µl** of diluted **standards, samples and zero controls** (S0, assay buffer) into wells. Cover the plate with foil and incubate for **overnight at 2-8°C** in a moist environment (to prevent evaporation).

6. Prepare 1.5 mM APMA solution before next wash step.
7. Aspirate each well and **wash** as step 4.
8. Add **50 µl** of **1.5 mM APMA working solution** into standard wells and the wells containing **samples** in which **total MMP8** are to be measured.
9. Add **50 µl of Assay buffer** into the wells containing **samples** in which **endogenous levels of active MMP8** are to be measured.
10. Cover the plate with foil and incubate **at 37°C for 1 hour** in a moist environment (to prevent evaporation).
11. Prepare detection reagent just before the end of APMA incubation.
12. Add **50 µl** of the **detection reagent** into all wells.
13. **Shake** the plate for **20 seconds**. Read the plate at **405 nm** to obtain a **t = 0 value**.
14. Cover the plate with foil and incubate **at 37°C for 2 hours** in a moist environment (to prevent evaporation).
15. **Shake** the plate for **20 seconds**. Read the plate at **405 nm** to obtain a **t = 2 hour value**.
16. Cover the plate with foil and incubate **at 37°C for another 4 hours** in a moist environment (to prevent evaporation).
17. **Shake** the plate for **20 seconds**. Read the plate at **405 nm** to obtain a **t = 6 hour value**.
18. If samples are present with very low activities, it is possible to incubate another 18 hours (total incubation time = 24 hours) **at 37°C** in a moist environment (to prevent evaporation).
19. **Shake** the plate. Read the plate at **405 nm** to obtain a **t = 24 hour value**.

Summary

	Standard	Total MMP8	Endogenous levels of active MMP8
1X antibody solution	100 µl	100 µl	100 µl
Cover the plate with foil and incubate for 2 hours at 37°C.			
Wash the wells for total 4 washes.			
Standard	100 µl	-	-
Samples	-	100 µl	100 µl
Cover the plate with foil and incubate for overnight at 2-8°C.			
Wash the wells for total 4 washes.			
1.5 mM APMA	50 µl	50 µl	-
Assay buffer	-	-	50 µl
Cover the plate with foil and incubate at 37°C for 1 hour.			
Detection reagent	50 µl	50 µl	50 µl
Shake the plate for 20S. Read the plate at 405 nm to obtain a t = 0 value.			
Cover the plate with foil and incubate at 37°C for 2 hour.			
Shake the plate for 20S. Read the plate at 405 nm to obtain a t = 2 value.			
Cover the plate with foil and incubate at 37°C for another 4 hour.			
Shake the plate for 20S. Read the plate at 405 nm to obtain a t = 6 value.			
Cover the plate with foil and incubate at 37°C for another 18 hour.			
Shake the plate for 20S. Read the plate at 405 nm to obtain a t = 24 value.			

CALCULATION OF RESULTS

1. Calculate the ΔA for each well (samples and blanks) after 2h and 6h incubation by subtracting the A at t=0 hour from the A at t=2 hour and t=6 hour.

2. Average the ΔA values of multiple blanks to obtain an average blank ΔA value for t=2 hour and t=6 hour incubation.

3. Subtract the average blank ΔA at t=2 hour from the ΔA of the various samples at t=2 hour and subtract the average blank ΔA at t=6 hour from the ΔA of the various samples at t=6 hour.

4. Create a "high level" standard curve from the t=2 hour data by plotting the blank subtracted ΔA values at t=2 hour against the MMP8 standard concentration. Use the OD of zero and all concentrations (or zero and standard concentrations of 187.5-12,000 pg/ml only) in the standard curve for this "high level" standard curve.

5. Create a "low level" standard curve from the t=6 hour data by plotting the blank subtracted ΔA values at t=6 hour against the MMP8 standard concentration. Use the OD of zero and standard concentrations of 46.875-3,000 pg/ml only in the standard curve for this "low level" standard curve.

6. If the activity in the samples is really low, and an overnight measurement data is available. Create a "t=24" standard curve from the t=24 hour data by plotting the blank subtracted ΔA values at t=24 hour against the MMP8 standard concentration. Use the OD of zero and standard concentrations of 11.72-375 pg/ml only in the standard curve for this "t=24" standard curve.

7. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration

on the horizontal (X) axis.

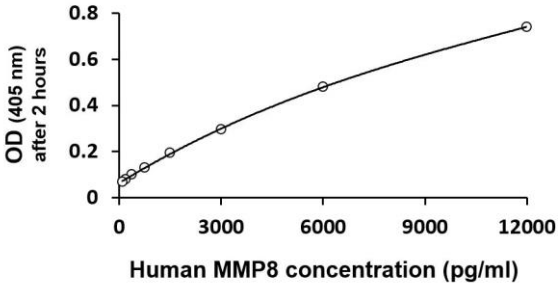
8. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

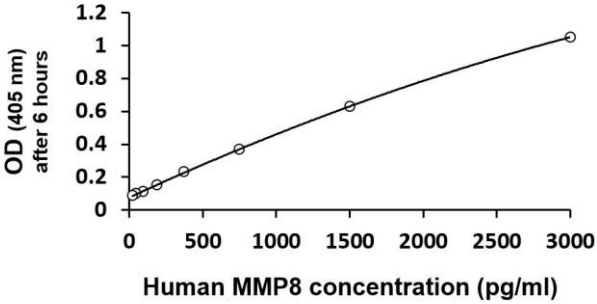
9. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

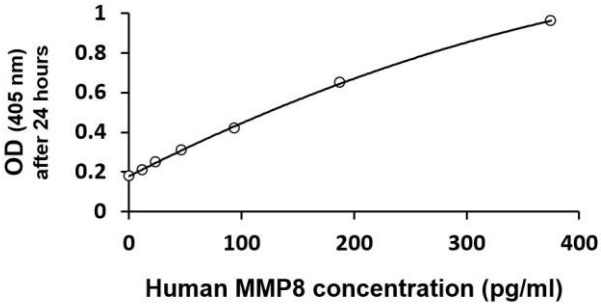
10. If the range of the standard line the samples is known, a shorter standard line with 8 points in the appropriate range can be used, allowing to measure more samples with the kit.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.







QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human MMP8 ranged from 12- 12000 pg/ml. The mean MDD was

100 pg/ml for 2 hours incubation

24 pg/ml for 6 hours incubation

4 pg/ml for 24 hours incubation.

Specificity

Measures endogenous active MMP8 (naturally occurring) or total active MMP8 (following activation with APMA).