QuickZyme
Human MMP-8 activity assay

QuickZyme Biosciences

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES

Introduction

Matrix metalloproteinases (MMPs) are a family of enzymes that function in the remodeling of extracellular matrix proteins. They are essential for various normal physiological processes such as embryonic development, morphogenesis, reproduction, tissue resorption and tissue remodeling. They also play a role in a number of pathological processes such as inflammation, arthritis, cardiovascular diseases, fibrosis and cancer.

Regulation of MMPs is carried out at various levels. Expression of latent MMPs is regulated at the level of transcription, whereas the proteolytic activity is controlled by specific activation of proMMPs, and by MMP-specific inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) or general circulatory inhibitors, such as $\alpha_2$macroglobulin.

The MMPs can be grouped according to their domain structure into collagenases, gelatinases, stromelysins, membrane type MMPs and matrilysins.

MMP-8 (also known as type neutrophil collagenase or collagenase 2, EC 3.4.24.34) has substrate specificity for native collagens (types I, II and III), MMP-8 was originally found in polymorphonuclear leukocytes (PMN, neutrophils), present in storage granules and is only secreted upon activation of these cells. More recently it was discovered that MMP-8 is also produced by other cells, such as endothelial cells, chondrocytes and synovial fibroblasts.

Human MMP-8 is produced and secreted as an inactive precursor form. Neutrophil pro-MMP-8 has a Mw of 75 kDa, whereas other cells may produce smaller 50-55 kD forms of pro-MMP-8, the differences originate by variations in glycosylation. The activity of MMP-8 is dependent on Zn^{2+} and Ca^{2+}. ProMMP-8, can be activated in vitro by organo mercurial compounds such as p-aminophenyl mercuric acetate (APMA), oxidizing agents like hydrogen peroxide or hypochlorite and proteases such as trypsin, chymotrypsin, cathepsin G, tissue kallikrein and MMP-3.

TIMP-1 or TIMP-2 inhibit MMP-8 activity by binding in a 1 to 1 molar ratio.
The QuickZyme human MMP-8 activity assay enables you to specifically measure in biological samples both active human MMP-8, as well as (pro)MMP-8 which is activated on the plate by APMA.
It can be used for the measurement of MMP-8 activity in various biological samples, such as conditioned culture media, tissue homogenates, serum, plasma and urine.

Assay principle

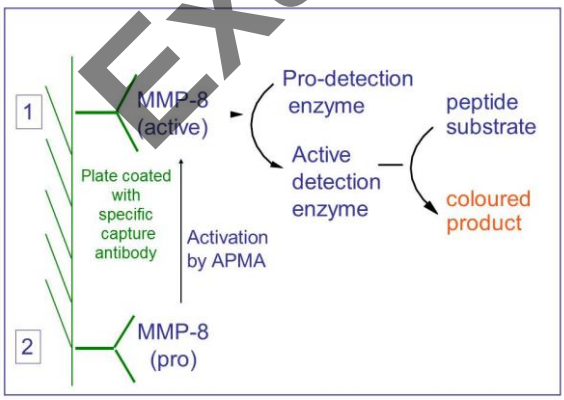


Fig. 1 Assay principle for the measurement of active MMP-8 [1] or total MMP-8 [2]

The QuickZyme human MMP-8 activity assay provides a simple, specific and precise quantitative determination of human MMP-8 in the active or pro-form in biological samples.

- Specific for human MMP-8

- Quantifies active and total (active form + pro-form) of MMP-8 in separate wells.
- Can measure both high and low levels in one plate
- Very high sensitivity (up to 4 pg/ml)
- Can be used for complex biological samples

The assay is based on the QuickZyme technology, using a modified pro-enzyme as a substrate, which upon activation is able to release color from a chromogenic peptide substrate (see Figure 1). This multiplication step provides a unique assay sensitivity.

Assay description

Measurement of active human MMP-8
To a plate precoated with F(ab')2 goat anti-mouse, anti-MMP-8 is pipetted.
After washing, standards and diluted samples are pipetted into the plate. Human MMP-8 present in the standard and in the biological sample is captured by the antibody. After washing, the pro-detection enzyme is added. This is converted by the active MMP-8 to an active detection enzyme.
The active detection enzyme is able to cleave the chromogenic substrate, resulting in generation of a yellow color that can be measured at 405 nm using an ELISA plate reader.

Measurement of total human MMP-8
Measurement of total MMP-8 is done similarly to the measurement of active MMP-8. After binding of MMP-8 to the antibody-coated plate, bound MMP-8 is first activated by adding APMA for 1 hour, resulting in the activation of pro-MMP-8. The activity of total MMP-8 (the newly activated MMP-8 and the already active MMP-8 present in the sample) is measured by adding the detection enzyme, followed by the addition of chromogenic substrate. The released color can be measured at 405 nm using an ELISA plate reader.

What's in the box?

- *96 well microwell plate* - 12x8 well ready-to-use strips coated with F(ab')2 goat anti-mouse
- *Mouse-anti-MMP-8* – 60 µl of 20 µg/ml in assay buffer
- *Assay buffer* – 125 ml bottle contains 100 ml ready-to-use Tris-HCl buffer
- *Standard* – tube contains 50 µl of 480 ng/ml pro-MMP-8 (human)
- *p-Aminophenylmercuric acetate (APMA)* – tube contains 17.5 mg APMA
- *Detection enzyme* – tube contains 600 µl detection enzyme in Tris-HCl buffer
- *Substrate* - tube contains 1000 µl peptide substrate in demineralized water
- *Wash buffer* – 30 ml bottle contains 25 ml 20x concentrated phosphate buffer

Safety Warnings and Precautions

With the kit p-Aminophenylmercuric acetate (APMA) is provided.
Warning: Aminophenylmercuric acetate (APMA) is toxic. See for relevant material safety data sheet: www.quickzyme.com/products/MMP-8-human-activity-assay.

Note that the protocol requires the use of Dimethyl Sulphoxide (DMSO) not supplied
Warning: Dimethyl Sulphoxide (DMSO) is harmful and an irritant. See for relevant material safety data sheet: www.quickzyme.com/products/MMP-8-human-activity-assay

Please follow the manufacturer's safety data sheets relating to the safe handling and use of these materials.
Wear eye, hand, face, and clothing protection when using these materials.

Other materials required

The following materials and equipment are required but not supplied:

- Single and/or multichannel pipettes with disposable polypropylene tips.
- Polypropylene tubes (Eppendorf tubes).
- Glass measuring cylinder 500 ml.
- Distilled or demineralized water.
- Microplate shaker.
- Refrigerator at 2-8°C.
- Dimethyl Sulphoxide (DMSO).
- (Microplate) incubator at 37°C.
- Automatic plate washer or wash bottle (optional).
- Microplate reader capable of measuring at 405 nm.

Sample collection and preparation

The QuickZyme human MMP-8 assay has been tested with various types of samples. Guidelines for the collection and preparation of several types of sample are given below. These procedures are guidelines only and not validated procedures.

Serum

1. Prepare serum by coagulation of blood using established procedures.
2. Rapidly freeze the serum in aliquots (use dry ice, liquid nitrogen or a cold bath, do not put in storage freezer unfrozen).
3. Store frozen at -20°C or lower.
4. Avoid freeze-thaw cycles.
5. Rapidly thaw samples in water bath (not higher than 37°C) and immediately put on ice until use.
6. Dilution of the serum with Assay buffer (20-fold or more) might be required for a good recovery.

Plasma

1. Prepare plasma using established procedures.
2. Rapidly freeze the plasma in aliquots (use dry ice, liquid nitrogen or a cold bath, do not put in storage freezer unfrozen).
3. Store frozen at -20°C or lower.
4. Avoid freeze-thaw cycles.
5. Rapidly thaw samples in water bath (not higher than 37°C) and immediately put on ice until use.
6. Plasma samples prepared with citrate or EDTA as anti-coagulant should be diluted at least 40-fold using Tris-HCl pH 7.6 buffer containing 1.5 mM NaCl and 0.01% Brij 35, in order to prevent clotting in the assay plate. Even in this case recovery may be poor. *We recommend using heparin plasma for this assay, a 20-fold dilution is sufficient to obtain a good recovery.*

Conditioned culture medium

1. It is advisable to centrifuge conditioned culture medium immediately after harvesting at 10,000xg or more for at least 10 min. to remove cell debris.
2. Rapidly freeze and store at -20°C or lower.
3. Dilution of the medium might be required depending on MMP-8 level and other components in the medium.

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

1. 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.
2. Centrifuge at 10,000xg or more for at least 10 min. to remove any cell debris.
3. Rapidly freeze and store at -20°C or lower.
4. Dilution of the homogenate might be required depending on MMP-8 level and other components.

Urine

1. It is advisable to centrifuge urine immediately after collection at 10,000xg or more for at least 10 min. to remove debris.
2. Rapidly freeze and store at -20°C or lower.
3. For expression of results normalization on e.g. creatinin is advisable.

Synovial fluid

1. It is advisable to centrifuge synovial fluid immediately after collection at 10,000xg or more for at least 10 min. to remove debris.
2. Rapidly freeze and store at -20°C or lower.
3. Dilution of synovial fluid samples with assay buffer (40-fold or more depending on the concentration) might be required to obtain a linear response.

Reagent preparation

Day 1

Assay buffer
Thaw the assay buffer and store at 2-8°C.

Antibody dilution
Thaw concentrated mouse anti-human-MMP-8 and dilute 200-fold with assay buffer (50 µl in 10 ml buffer) to 0.1 µg/ml and store at 2-8°C.

Standard

1. Add 250 µl assay buffer to the standard vial
2. Gently mix and transfer to a standard Eppendorf tube
3. Add 700 µl assay buffer and mix gently, this is the **24 ng/ml stock**
4. Store on ice until required

Wash buffer

1. Transfer contents of the bottle to a 500 ml cylinder by repeated washing with distilled water.
2. Adjust the final volume to 500 ml with distilled water and mix thoroughly
3. Store at room temperature in a closed vessel until required

Day 2

p-Aminophenyl mercuric acetate (APMA)

1. Add 50 µl of Dimethyl Sulphoxide

(DMSO) to the vial, replace the cap and vortex until the solution is clear. This is the concentrated APMA solution (1 M).
2. Add 15 µl from the 1 M APMA solution to a vial containing 10 ml of assay buffer at room temperature and mix well. This is the ready to use APMA solution (1.5 mM).

The concentrated APMA (1 M) can be stored at -20°C in aliquots (thaw not more than once, then dispose according to local regulations).

Detection enzyme

1. Allow the vial containing the detection enzyme to thaw before use.
2. Store on ice until required.

Substrate

1) Allow the vial containing the substrate to thaw before use.
2) Store on ice until required.

Detection reagent

This reagent should be prepared immediately prior to addition to the wells.

1. For 96 wells: mix 550 µl detection enzyme solution, 880 µl substrate solution and 4070 µl assay buffer together in a vial.
2. Mix gently and add 50 µl to each well of the plate during the assay procedure (see page 11)

Standard preparation

It is important to perform this procedure on ice.

The wide range standard curve is built of 12 - 6 – 3 – 1.5 – 0.75 – 0.38 - 0.19 - 0.094 - 0.047 - 0.023 - 0.012 – 0 ng/ml pro-MMP-8.

Prepare a 12 points standard curve by pipetting the following amounts in Eppendorf tubes:

Standard label	*Sample from*	*Assay buffer (add)*	*Final conc (ng/ml)*
S1	250 µl **(24 ng/ml stock)**	250 µl	12.00
S2	250 µl S1	250 µl	6.00
S3	250 µl S2	250 µl	3.00
S4	250 µl S3	250 µl	1.50
S5	250 µl S4	250 µl	0.75
S6	250 µl S5	250 µl	0.38
S7	250 µl S6	250 µl	0.19
S8	250 µl S7	250 µl	0.094
S9	250 µl S8	250 µl	0.047
S10	250 µl S9	250 µl	0.023
S11	250 µl S10	250 µl	0.012
S12	0	500 µl	0.000

Assay procedure

1. Prepare the reagents as described in ‘reagent preparation’.
2. Set up the microplate with sufficient wells for running of all zero (blanks), standards and samples as required.
   Put remaining strips immediately back at -20°C in original foil packaging with desiccant.
3. Pipet 100 µl 200-fold diluted anti-MMP-8 antibody (0.1 µg/ml) into all wells.
4. Cover the plate with the lid provided and incubate at 37°C for 2 hours.
5. Just before the end of this incubation period, prepare the MMP-8 standard as described in ‘standard preparation’.
6. Aspirate and wash all wells 4 times with wash buffer, ensuring that the wells are completely filled and emptied at each wash.
7. Pipet 100 µl assay buffer into appropriate wells for use as blank.
8. Pipet 100 µl of (diluted) standards and unknown samples/ sample dilutions into the appropriate wells
9. Cover the plate with the lid provided and incubate at 2-8°C overnight.
10. Just before the end of this incubation period, prepare the APMA solution as described in ‘reagent preparation’.
11. Aspirate and wash all wells 4 times with wash buffer, ensuring that the wells are completely filled and emptied at each wash.
12. Pipette 50 µl of the ready to use APMA solution (1.5 mM) into wells containing standard (standard contain pro-MMP-8, must be activated)
13. Pipet 50 µl of the ready to use APMA solution (1.5 mM) into those wells containing samples where total MMP-8 activity is to be measured. Do NOT add APMA to the wells containing samples where endogenous levels of active MMP-8 are to be measured.
14. Pipet 50 µl of assay buffer into wells containing samples in which endogenous levels of active MMP-8 are to be measured.
15. Cover the plate with the lid provided and incubate at 37°C for 1 hour in a moist environment (to prevent evaporation).
16. Just before the end of this incubation period, prepare the detection reagent as described in ‘reagent preparation’.
17. Pipet 50 µl of the detection reagent into all wells.
18. Shake the plate for 20 seconds
19. Read the plate at 405 nm to obtain a t = 0 value
20. Cover the plate with the lid provided and incubate at 37°C for 2 hours in a moist environment (to prevent evaporation).
21. Shake the plate for 20 seconds
22. Read the plate at 405 nm, this is t=2 hours
23. Incubate the plate again at 37°C for another 4 hours (total incubation = 6 hours)
24. Read the plate at 405 nm, this is t=6 hours
25. If you observe that the OD values of the samples are very low, you can extend the incubation time to 24 hours
26. Calculate the t= 2 hour data from the standard curve using the following range: 0-0.19-0.38-0.75-1.5-3-6-12 ng/ml MMP-8 (see data analysis)
27. Calculate the t= 6 hours data from the standard curve using the following range: 0-0.047-0.094-0.19-0.38-0.75-1.5-3-ng/ml MMP-8 (see data analysis)

Data analysis

The MMP-8 concentration in the assay samples can be calculated in various ways. The use of a software package 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 2h and 6h incubation by subtracting the A at t=0 hour from the A at t=2 hours and t=6 hours.
2. Average the ΔA values of multiple blanks to obtain an average blank ΔA value for t=2 hours and t=6 hours incubation.
3. Subtract the average blank ΔA at t=2 hours from the ΔA of the various samples at t=2 hours and subtract the average blank ΔA at t=6 hours from the ΔA of the various samples at t=6 hours.
4. Create a “high level” standard curve from the t=2 hours data by plotting the blank subtracted ΔA values at t=2 hours against the MMP-8 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 t=6 hours data by plotting the blank subtracted ΔA values at t=6 hours against the MMP-8 standard concentration. You should only use the 0-0.047-0.094-0.19-0.38-0.75-1.5 and 3 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.

If all your test samples can be read on the “high level” standard curve you could simplify future assays by using only the 2 h reading and a shorter standard line (0-0.19-0.38-0.75-1.5-3-6 and 12 ng/ml).
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 (0-0.047-0.094-0.19-0.38-0.75-1.5-3-ng/ml).
The data analysis described above can also be performed for an incubation time of 24 hours (if samples have very low MMP-8 levels)

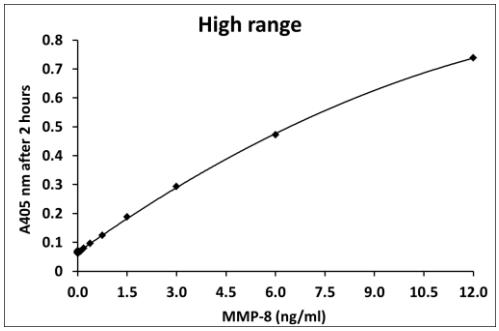
Storage conditions

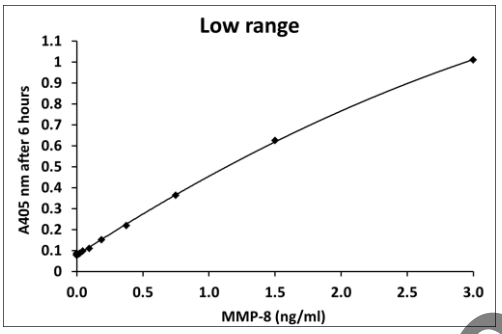
Unopened kit: Store at -20ºC, except for the standard, this vial should be stored at -70°C. Do not use kit, or individual kit components past kit expiration date.

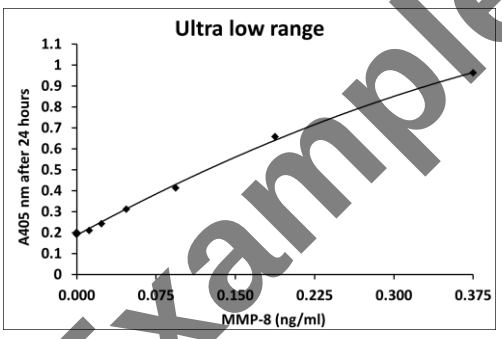
Opened kit / reconstituted reagents:
After opening, microwell plate or individual strips should be stored at -20ºC or lower in original foil packaging with desiccant until use.
Undiluted MMP-8 standard should be stored preferably at -70°C and aliquoted to minimize freeze-thaw cycles.
Diluted standard should be used immediately and thereafter discarded.
Concentrated APMA solution (1M) should be stored aliquoted at -20°C. The diluted working solution should be discarded after use and not refrozen. Discard this organo- mercurial according to local regulations.
Assay Buffer should be stored at 4ºC for short term storage (less than 1 week), or -20ºC for longer storage (several months).
Detection Enzyme should be stored frozen at -20ºC or lower.
Substrate solution should be stored frozen at -20ºC or lower.
Wash buffer in diluted form should be stored at 4ºC for short term storage (less than 1 week), or -20ºC for longer storage (several months), preferably store in concentrated form at -20ºC.

Typical data

The shown data curves are provided for demonstration only. The exact A_{405} values can vary per experiment and kit.







Related products

- Human MMP-2 activity assay
- Human MMP- 7 activity assay
- Human MMP-9 activity assay
- Mouse MMP-9 activity assay
- Human MMP-14 activity assay
- Granzyme B activity Reagent Set
- Total Collagen assay
- Sensitive Tissue Collagen assay
- Hydroxyproline assay
- Sensitive Tissue Hydroxyproline assay
- Soluble Collagen assay
- Total Protein assay
- Human TIMP-1 ELISA
- Human Urokinase (uPA) ELISA

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