

QuickZyme Mouse MMP-9 activity assay

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



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 α_2 macroglobulin.

The MMPs can be grouped according to their domain structure into collagenases, gelatinases, stromelysins, membrane type MMPs and matrilysins. MMP-9 (also known as type neutrophil gelatinase, IV collagenase, Gelatinase B;

EC 3.4.24.35) has a broad range of substrate specificity for denatured collagens (gelatins), native collagens (types IV, V and XI), as well as elastin.

Mouse MMP-9 has a Mw of 103 kDa (pro-form) and 86 kDa (active form). The activity is dependent on Zn^{2+} and Ca^{2+} . MMP-9 is secreted as proMMP-9, and can be activated *in vitro* by organo mercurial compounds such as p-aminophenyl mercuric acetate (APMA).

MMP-9 is produced by a variety of cell types including monocytes, macrophages, fibroblasts, neutrophils, osteoclasts, chondrocytes, keratinocytes, endothelial and epithelial cells.

The QuickZyme mouse MMP-9 activity assay enables you to specifically measure in biological samples both active mouse MMP-9, as well as (pro)MMP-9 which is activated on the plate by APMA. It can be used for the measurement of MMP-9 activity in various biological samples, such as conditioned culture media, tissue homogenates, serum, plasma and urine.

Assay principle

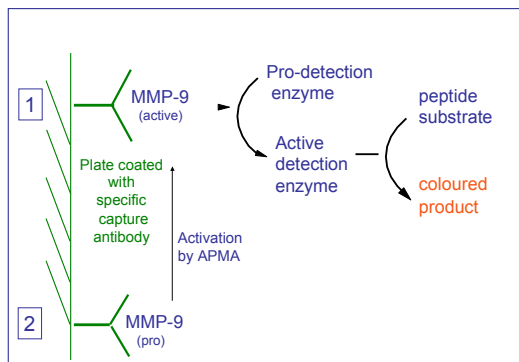


Fig. 1 Assay principle for the measurement of active MMP-9 [1] or total MMP-9 [2]

The QuickZyme mouse MMP-9 activity assay provides a simple, specific and precise quantitative determination of mouse MMP-9 in the active or pro-form in biological samples.

- Specific for mouse MMP-9
- Quantifies active and total (active form + pro-form) of MMP-9 in separate wells.
- Can measure both high and low levels in one plate
- Very high sensitivity (up to 1 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). This multiplication step provides an unique assay sensitivity.

Assay description

Measurement of active mouse MMP-9

Standards, controls and biological samples are pipetted into the pre-coated plate. Mouse MMP-9 present in the biological sample is captured by the antibody. After washing the pro-detection enzyme is added. This is activated by the active MMP-9 into 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 mouse MMP-9

Measurement of total MMP-9 is done similarly to the measurement of active MMP-9. After binding of MMP-9 to the antibody-coated plate, bound MMP-9 is first activated by adding APMA for 30 minutes, resulting in the activation of pro-MMP-9. The activity of total MMP-9 (the newly activated MMP-9 and the already active MMP-9 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 anti-MMP-9
- *Assay buffer* – 125 ml bottle contains 100 ml ready-to-use Tris-HCl buffer
- *Standard* – tube contains 20 µl of approximately 500 ng/ml pro-MMP-9 (mouse), exact concentration can be found on Standard Lot Specification Sheet.
- *p-Aminophenylmercuric acetate (APMA)* – tube contains 17.5 mg APMA, see safety data sheet supplied
- *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 20 ml 25x concentrated phosphate buffer

Safety Warnings and Precautions

Warning: Contains p-Aminophenylmercuric acetate (APMA). See safety data sheet supplied.

Note that the protocol requires the use of Dimethyl Sulphoxide (DMSO).

Warning: Dimethyl Sulphoxide (DMSO) is harmful and an irritant. Please follow the manufacturer's safety data sheet relating to the safe handling and use of this material.

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).
- (Microtitre plate) 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 mouse MMP-9 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 (30 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. Dilution of the plasma with Assay buffer (30 fold or more) might be required for 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-9 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.
4. Rapidly freeze and store at -20 °C or lower.
5. Dilution of the homogenate might be required depending on MMP-9 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.

Reagent preparation

Day 1

Assay buffer

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

Standard

- 1) Add a volume of assay buffer to the standard vial as specified on the Standard Lot Specification Sheet.
- 2) Gently mix, this is the **20 ng/ml stock**
- 3) 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)

-see safety data sheet supplied

- 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 5 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 (0.5 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 17)

Standard preparation

It is important to perform this procedure on ice.

The wide range standard curve is built of 0-0.004-0.008-0.016-0.031-0.063-0.125-0.25-0.5-1-2-4 ng/ml MMP-9.

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

Standard dilution (µl)	assaybuffer (µl)	MMP-9 (ng/ml)
100 (20 ng/ml stock)	400	4.000
250 (4 ng/ml)	250	2.000
250 (2 ng/ml)	250	1.000
250 (1 ng/ml)	250	0.500
250 (0.5 ng/ml)	250	0.250
250 (0.25 ng/ml)	250	0.125
250 (0.125 ng/ml)	250	0.063
250 (0.063 ng/ml)	250	0.031
250 (0.031 ng/ml)	250	0.016
250 (0.016 ng/ml)	250	0.008
250 (0.008 ng/ml)	250	0.004
0	500	0.000

Assay procedure

- 1) Prepare the reagents as described in 'reagent preparation'.
- 2) Prepare the samples as described in 'sample preparation'.
- 3) Prepare the MMP-9 standard as described in 'standard preparation'.
- 4) Set up the microtitre plate with sufficient strips 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.
- 5) Pipette 100 µl assay buffer into appropriate wells for use as blank.
- 6) Pipette 100 µl of unknown samples (or sample dilutions) into the appropriate wells.
- 7) Cover the plate with the lid provided and incubate at 2-8°C overnight.
- 8) Aspirate and wash all wells 4 times with wash buffer, ensuring that the wells are completely filled and emptied at each wash.
- 9) Pipette 50 µl of the ready to use APMA solution (0.5 mM) into wells containing standards and into those wells containing samples where total MMP-9 activity is to be measured. Do NOT add APMA to the wells containing samples where endogenous levels of active MMP-9 is to be measured.
- 10) Pipette 50 µl of assay buffer into wells containing samples in which endogenous levels of active MMP-9 are to be measured.
- 11) Prepare the detection reagent as described in 'reagent preparation'.
- 12) Pipette 50 µl of the detection reagent into all wells.
- 13) Shake the plate for 20 seconds
- 14) Read the plate at 405 nm to obtain a t = 0 value
- 15) Cover the plate with the lid provided and incubate at 37°C for 1 hour in a moist environment (to prevent evaporation).
- 16) Shake the plate for 20 seconds
- 17) Read the plate at 405 nm, this is t=1 hour
- 18) Incubate the plate again at 37°C for another 5 hours (total incubation = 6 hours)
- 19) Read the plate at 405 nm, this is t=6 hours
- 20) Calculate the t= 1 hour data from the standard curve using the following range: 0-0.125-0.25-0.5-1-2-4 ng/ml MMP-9 (see data analysis)
- 21) Calculate the t= 6 hours 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 data analysis)

Data analysis

The MMP-9 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:

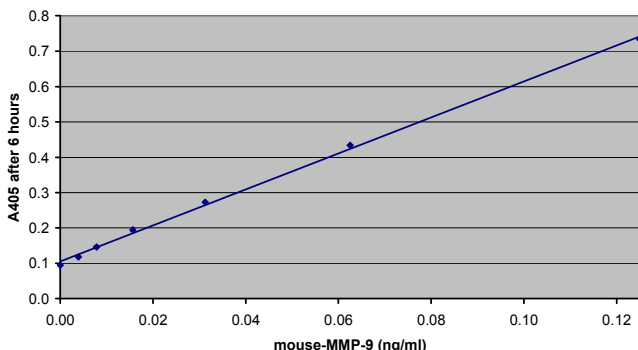
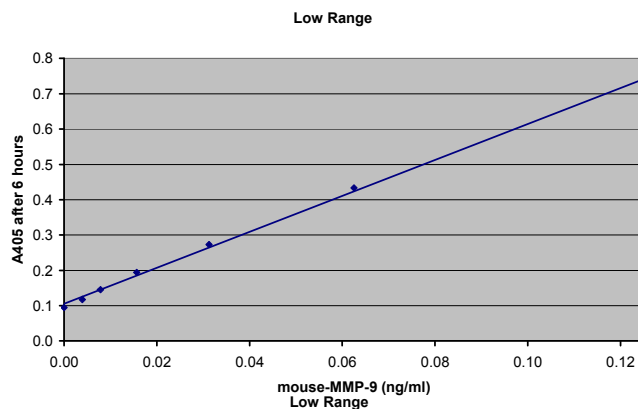
1. Calculate the ΔA for each well (samples and blanks) after 1h and 6h incubation by subtracting the A at t=0 hour from the A at t=1 hour and t=6 hour.
2. Average the ΔA values of multiple blanks to obtain an average blank ΔA value for t=1 hour and t=6 hour incubation.
3. Subtract the average blank ΔA at t=1 hour from the ΔA of the various samples at t=1 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=1 hour 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 t=6 hour 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.

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).

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).

Typical data

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



Storage conditions

Unopened kit: Store at -20°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 -20°C or lower in original foil packaging with desiccant until use.

Undiluted MMP-9 standard should be stored preferably at -70 °C. 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 4 °C for short term storage (less than 1 week), or -20°C for longer storage (several months).

Detection Enzyme should be stored frozen at -20°C or lower.

Substrate solution should be stored frozen at -20°C or lower.

Wash buffer in diluted form should be stored at 4 °C for short term storage (less than 1 week), or -20°C for longer storage (several months), preferably store in concentrated form at -20°C.

Related products

Human MMP-9 activity assay
Human MMP-2 activity assay
Mouse MMP-2 activity assay
Soluble Collagen assay
Total Collagen assay
Hydroxyproline assay
Granzyme B activity Reagent Set

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES

QuickZyme BioSciences

P.O.Box 2215
2301 CE Leiden
The Netherlands

www.QuickZyme.com

E-mail	info@quickzyme.com
Phone	+31888666024 +31888666134 +31888666114
Fax	+31888660609

