QuickZyme Human MMP-2 activity assay
Version 2.0

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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-2 (also known as 72 kDa type IV collagenase, Gelatinase A;

EC 3.4.24.24) has a broad range of substrate specificity for denatured collagens (gelatins) and native collagens (types IV, V, VII and X).

Human MMP-2 has a Mw of 72 kDa (pro-form) and 62 kDa (active form). The activity is dependent on Zn^{2+} and Ca^{2+}. MMP-2 is secreted as proMMP-2, and can be activated in vitro by organo mercurial compounds such as p-aminophenyl mercuric acetate (APMA).

MMP-2 is produced by a variety of cell types including fibroblasts, chondrocytes, endothelial and epithelial cells.

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

Assay principle

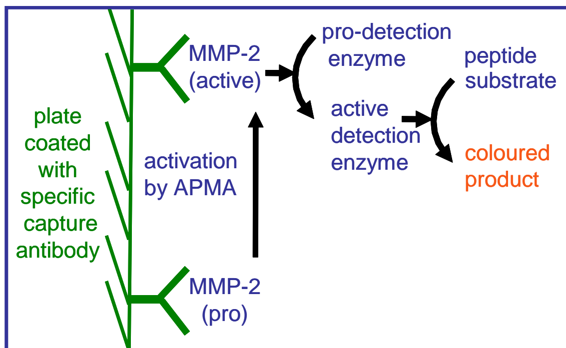


Fig. 1 Assay principle for the measurement of active MMP-2 or total MMP-2

The QuickZyme human MMP-2 activity assay provides a simple, specific and precise quantitative determination of human MMP-2 in the active or pro-form in biological samples.

- Specific for human MMP-2

- Quantifies active and total (active form + pro-form) of MMP-2 in separate wells.
- Can measure both high and low levels in one plate
- Very high sensitivity (down 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-2

Standards, controls and biological samples are pipetted into the pre-coated plate. Human MMP-2 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-2 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 human MMP-2
Measurement of total MMP-2 is done similarly to the measurement of active MMP-2. After binding of MMP-2 to the antibody-coated plate, bound MMP-2 is first activated by adding APMA, resulting in the activation of pro-MMP-2. The activity of total MMP-2 (the newly activated MMP-2 and the already active MMP-2 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-2
- *Assay buffer* – 125 ml bottle contains 100 ml ready-to-use Tris-HCl buffer
- *Standard* – tube contains 50 µl of 640 ng/ml pro-MMP-2 (human)
- *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

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

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-2-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-2-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).
- (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 human MMP-2 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 (100 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 (100 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-2 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-2 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 950 µl assay buffer to the standard vial
2) Gently mix, this is the **32 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 18)

Standard preparation

It is important to perform this procedure on ice.
The wide range standard curve is built of 0-0.008-0.016-0.031-0.063-0.125-0.25-0.5-1-2-4-8-16 ng/ml MMP-2.
Prepare a 13 points standard curve by pipetting the following amounts in Eppendorf tubes:

Standard dilution (µl)	*assaybuffer (µl)*	*MMP-2 (ng/ml)*
250 **(32 ng/ml stock)**	250	16.00
250 (16 ng/ml)	250	8.00
250 (8 ng/ml)	250	4.00
250 (4 ng/ml)	250	2.00
250 (2 ng/ml)	250	1.00
250 (1 ng/ml)	250	0.50
250 (0.5 ng/ml)	250	0.25
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
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-2 standard as described in ‘standard preparation’.
4) Set up the microwell 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 of 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-2

activity is to be measured. Do NOT add APMA to the wells containing samples where endogenous levels of active MMP-2 are to be measured.

10) Pipette 50 µl of assay buffer into wells containing samples in which endogenous levels of active MMP-2 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 4 hours in a moist environment (to prevent evaporation).
16) Shake the plate for 20 seconds
17) Read the plate at 405 nm, this is t=4 hour
18) Incubate the plate again at 37°C for another 2 hours (total incubation = 6 hours)
19) Read the plate at 405 nm, this is t=6 hours
20) If samples are present with very low activities, it is possible to incubate o/n

(total incubation = 22 hours). Read the plate the next morning at 405 nm, this is t=22 hours

21) Calculate the t= 4 hour data from the standard curve using the following range: 0-0.25-0.5-1-2-4-8-16 ng/ml MMP-2 (see data analysis)
22) Calculate the t= 6 hours data from the standard curve using the following range: 0-0.063-0.125-0.25-0.5-1-2-4 ng/ml MMP-2 (see data analysis)
23) If appropiate, calculate the t= 22 hours data from the standard curve using the following range: 0-0.008-0.016-0.031-0.062-0.125-0.25-0.5 ng/ml MMP-2 (see data analysis)

Data analysis

The MMP-2 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 4h and 6h incubation by subtracting the A at t=0 hour from the A at t=4 hour and t=6 hour
2. Average the ΔA values of multiple blanks to obtain an average blank ΔA value for t=4 hour and t=6 hour incubation.
3. Subtract the average blank ΔA at t=4 hour from the ΔA of the various samples at t=4 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=4 hour data by plotting the blank subtracted ΔA values at t=4 hour against the MMP-2 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-2 standard concentration. <u>You should only use the 0-0.063-0.125-0.25-0.5-1-2-4 ng/ml concentrations</u> 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 the activity in the samples is really low, you can do an o/n measurement and create

a standard curve from the t=22 hour data by plotting the blank subtracted ΔA values at t=22 hour against the MMP-2 standard concentration. You should only use the 0-0.008-0.015-0.031-0.062-0.125-0.25-0.5 ng/ml concentrations in the standard curve for this standard curve, since the higher values will be outside the useable range. Draw a best-fit curve through the points in the graph.
Using this standard curve the ΔA values of the t=22h 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 you know in which range of the standard line the samples will be, a shorter standard line with 8 points in the appropriate range can be used, allowing to measure more samples with the kit.

Storage conditions

Unopened kit: Store at -20ºC, <u>except for the standard</u>, this vial should be stored <u>at -70°C.</u> 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-2 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 4 ºC for short term storage (less than 1 week), or -20ºC for longer storage (several months).

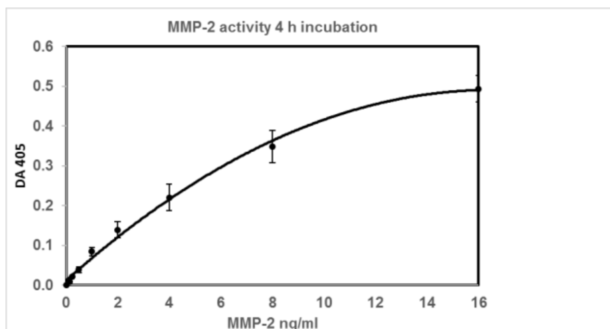
Detection Enzyme should be stored frozen at -20ºC or lower.

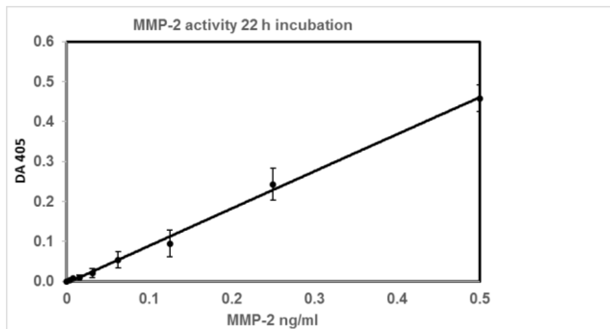
Substrate solution should be stored frozen at -20ºC or lower.

Wash buffer in diluted form should be stored at 4 ºC 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-8 activity assay
- Human and mouse MMP-9 activity assay
- Human MMP-14 activity assay
- Soluble Collagen assay
- Total Collagen assay
- Hydroxy proline assay
- Granzyme B activity Reagent Set
- Total Protein assay

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