QuickZyme
Human MMP-7 activity assay

QuickZyme Biosciences

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

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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-7 (also known as matrilysin, matrilysin 1 or pump 1;
EC 3.4.24.23) has a broad range of substrate specificity including collagen type IV, elastin, fibronectin, laminin, nidogen, tenascin, osteonectin, MBP, decorin and versican).
Human MMP-7 has a Mw of 29 kDa (pro-form) and 19 kDa (active form). The activity is dependent on Zn^{2+} and Ca^{2+}.
proMMP-7, can be activated in vitro by organo mercurial compounds such as p-aminophenyl mercuric acetate (APMA).
MMP-7 is produced by a variety of cell types including osteoclasts, keratinocytes epithelial cells and macrophages.
The QuickZyme human MMP-7 activity assay enables you to specifically measure in biological samples both active human MMP-7, as well as pro-MMP-7, which is activated on the plate by APMA. It can be used for the quantification of MMP-7 activity in various biological samples, such as conditioned culture media, tissue homogenates, serum, plasma and urine.

Assay principle

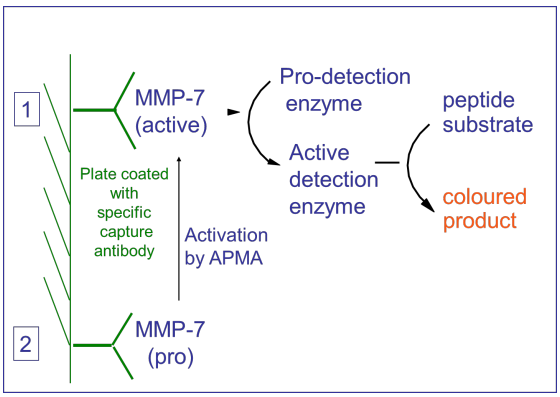


Fig. 1 Assay principle for the measurement of active MMP-7 [1] or total MMP-7 [2]

The QuickZyme human MMP-7 activity assay provides a simple, specific and precise quantitative determination of human MMP-7 in the active or pro-form in biological samples.

- Specific for human MMP-7
- Quantifies active and total (active form + pro-form) of MMP-7 in separate wells.
- Can measure both high and low levels in one plate
- Very high sensitivity (up to 3 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-7

To a plate precoated with rabbit anti-goat antibody, anti-MMP-7 antibody is added. After incubation and washing, standards, controls and biological samples are pipetted into the anti MMP-7 loaded pre-coated plate. Human MMP-7 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-7 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-7

Measurement of total MMP-7 is done similarly to the measurement of active MMP-7. After binding of MMP-7 to the antibody-loaded pre-coated plate, bound MMP-7 is first activated by adding APMA, resulting in the activation of pro-MMP-7. The activity of total MMP-7 (the newly activated MMP-7 and the already active MMP-7 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 Rabbit-anti-Goat IgG (RAG)
- *Antibody stock* – anti-MMP-7 stock solution, dilute 100x before use
- *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-7 (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-7-human-activity-assay.

Note that the protocol requires the use of Dimethyl Sulphoxide (DMSO), which is not supplied with the kit.
Warning: Dimethyl Sulphoxide (DMSO) is harmful and an irritant. See for relevant material safety data sheet: www.quickzyme.com/products/MMP-7-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-7 assay can be used 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-7 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-7 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. creatinine is advisable.

Reagent preparation

Day 1

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

Antibody dilution

1) Thaw antibody stock-solution and prepare a sufficient amount of 100-fold dilution in Assay buffer for the number of wells to be coated (100 µl per well), e.g. : 100 µl stock in 10 ml assay buffer for coating a complete plate.
2) Store dilution on ice until required and store remainder of undiluted antibody stock-solution frozen at -70°C.

Assay buffer

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

Standard stock solution

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 and for future use store remainder aliquoted at -70°C to minimize freeze-thaw cycles.

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 10 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 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 10/11)

Standard preparation

It is important to perform this procedure on ice.

The wide range standard curve is built of 16 - 8 – 4 – 2 – 1 – 0.5 - 0.25 - 0.125 – 0.062 - 0.031 - 0.016 - 0 ng/ml pro-MMP-7.

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 **(32 ng/ml stock)**	250 µl	16.00
S2	250 µl S1	250 µl	8.00
S3	250 µl S2	250 µl	4.00
S4	250 µl S3	250 µl	2.00
S5	250 µl S4	250 µl	1.00
S6	250 µl S5	250 µl	0.50
S7	250 µl S6	250 µl	0.25
S8	250 µl S7	250 µl	0.125
S9	250 µl S8	250 µl	0.062
S10	250 µl S9	250 µl	0.031
S11	250 µl S10	250 µl	0.016
S12	0	500 µl	0.000

Assay procedure

1) Prepare wash buffer, antibody dilution, assay buffer and standard stock solution as described in 'reagent preparation'.
2) Set up the microplate with sufficient wells for running 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 100-fold diluted anti-MMP-7 antibody 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-7 standard curve 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 of the blank and standard dilutions into appropriate wells
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) Prepare the APMA as described in 'reagent preparation'.
11) Prepare the detection reagent as described in 'reagent preparation'.
12) Aspirate and wash all wells 4 times with wash buffer, ensuring that the wells are completely filled and emptied at each wash.
13) Pipet 50 µl of the ready to use APMA solution (1 mM) into wells containing standard (standards contain pro-MMP-7, must be activated)
14) Pipet 50 µl of the ready to use APMA solution (1 mM) into those wells containing samples where total MMP-7 activity is to be measured. Do NOT add APMA to the wells containing samples where endogenous levels of active MMP-7 are to be measured.
15) Pipet 50 µl of assay buffer into wells containing samples in which endogenous levels of active MMP-7 are to be measured.
16) Pipet 50 µl of the detection reagent into all wells.
17) Shake the plate for 20 seconds
18) Read the plate at 405 nm to obtain a t = 0 value
19) Cover the plate with the lid provided and incubate at 37°C for 3 hours in a moist environment (to prevent evaporation).
20) Shake the plate for 20 seconds
21) Read the plate at 405 nm, this is t=3 hours
22) Incubate the plate again at 37°C for another 3 hours (total incubation = 6 hours)
23) Read the plate at 405 nm, this is t=6 hours
24) Incubate the plate again for another 18 hours (only if samples have very low MMP-7 levels)
25) Read the plate at 405 nm, this is t=24 hours
26) Calculate the t= 3 hours data from the standard curve using the following range: 0-0.25-0.5-1-2-4-8-16 ng/ml MMP-7 (see data analysis)
27) Calculate the t= 6 hours data from the standard curve using the following range: of 0-0.0625-0.125-0.25-0.5-1-2-4 ng/ml MMP-7 (see data analysis)
28) Calculate the t= 24 hours data from the standard curve using the following range: of 0-0.016-0.031-0.0625-0.125 ng/ml MMP-7 (see data analysis)

Data analysis

The MMP-7 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 3h and 6h incubation by subtracting the A at t=0 hour from the A at t=3 hour and t=6 hour.

2. Average the ΔA values of multiple blanks to obtain an average blank ΔA value for t=3 hour and t=6 hours
   incubation
3. Subtract the average blank ΔA at t=3 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=3 hour data by plotting the blank subtracted ΔA values at t=3 hour against the MMP-7 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-7 standard concentration. You should only use the 0-0.0625-0.125-0.25-0.5-1-2-4 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 3 h reading and a shorter standard line (0, 0.25, 0.5, 1, 2, 4, 8 and 16 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, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4 ng/ml).

The data analysis described above can also be performed for an incubation time of 24 hours (if samples have very low MMP-7 levels)

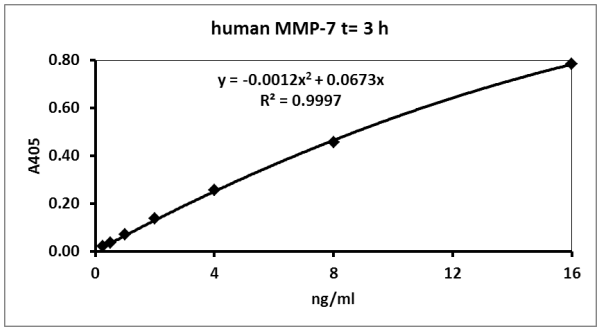
Storage conditions

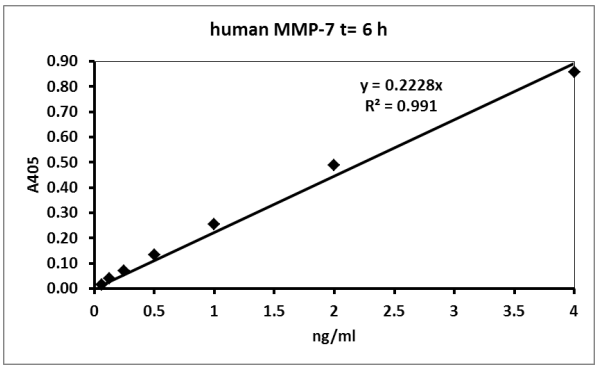
Unopened kit: Store at -20ºC, except for the standard, this vial should be stored at -70°C. Do not use kit, or individual kit components past kit expiration date.

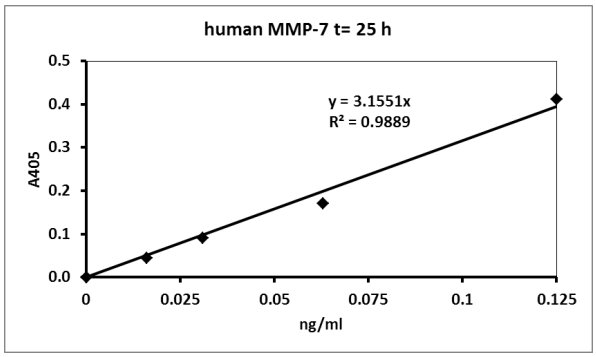
Opened kit / reconstituted reagents:
After opening, microwell plate or individual strips should be stored at
-20ºC or lower in original foil packaging with desiccant until use.
The undiluted MMP-7 stock or 32 ng/ml diluted MMP-7 stock-solution should be stored aliquoted preferably at -70 °C and freeze-thaw cycles should be minimized.
Further diluted standards 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 3, 6 and 25 hours

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-8 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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