QuickZyme
Human MMP- 14 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 takes place at various levels. Expression of proMMPs 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-14 (EC 3.4.24.80), also known as MT- MMP or MT1-MMP, is a membrane type MMP and contains a transmembrane domain. MMP-14 has a role in the activation of the proforms of MMP-2 and MMP-13. MMP-14 is believed to be involved in bone and tooth development, tumor cell invasion and cell migration and also in shedding of a variety of cell surface proteins. Furthermore, MMP-14 is able to directly degrade certain matrix proteins such as laminin, fibronectin, vitronectin and interstitial collagens. Recent work points to an involvement of MMP-14 in plaque vulnerability.
Human MMP-14 consists of 562 amino acid residues including a propeptide of 91 residues. and is most likely intracellularly activated by furin-like enzymes and transported to the membrane as an active enzyme. MMP-14 is thought to be released from the cell membrane by proteolytic cleavage. The activity is dependent on Zn^{2+} and Ca^{2+}.
The QuickZyme human MMP-14 activity assay enables you to specifically measure active human MMP-14. It can be used for the measurement of MMP-14 activity in various biological samples, such as cultured cells and tissue homogenates.
(For review see Itoh et al IUBMB Life 58 (2006) 589-596 and Nagase et al. Cardiovascular Research 9 (2006) 562-573).

Assay principle

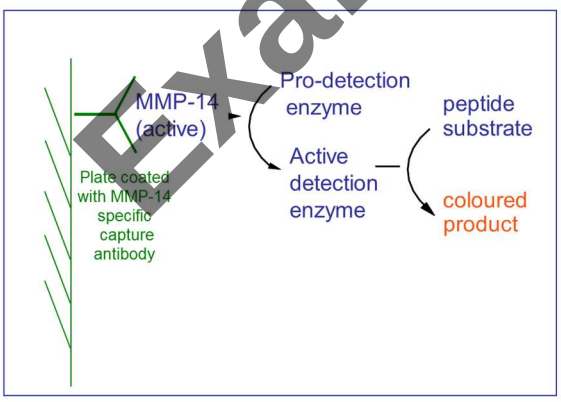


Fig. 1 Assay principle for the measurement of active MMP-14

The QuickZyme human MMP-14 activity assay provides a simple, specific and precise quantitative determination of human MMP-14 in the active form in biological samples.

- Specific for human MMP-14
- Quantifies active MMP-14
- Can measure both high and low levels in one plate

- High sensitivity (up to 0.1 ng/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 amplification step provides a unique assay sensitivity.

Assay description

Measurement of active human MMP-14

Standards, controls and biological samples are pipetted into a plate coated with anti-MMP-14 antibody. Human MMP-14 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-14 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.

What's in the box?

- *96 well microwell plate* - 12x8 well non-coated
- *Blocking buffer* - 15 ml ready to use
- *Antibody stock solution- 20 µl 5.9 mg/ml*
- *Assay buffer* - 30 ml bottle contains 20 ml ready-to-use Tris-HCl buffer
- *Extraction buffer* - 125 ml bottle contains 100 ml ready-to-use Tris-HCl buffer with Triton-X-100
- *Standard* - tube contains 50 µl of 640 ng/ml MMP-14 (human); store at -70°C
- *Detection enzyme* - tube contains 600 µl detection enzyme in Tris-HCl buffer; store at -20°C
- *Substrate* - tube contains 1000 µl peptide substrate in demineralized water
- *Wash buffer* - 30 ml bottle contains 20 ml 25x concentrated phosphate buffer

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.
- Freezer -20 °C and -70°C
- (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-14 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.

Cultured cells

1. Culture cells in e.g. 24-well plates
2. Remove the media and replace with 250 µl of extraction buffer per cm^2
3. Incubate at 4ºC for 15 minutes, mild shaking may help to dissolve the MMP-14.
4. Transfer extract to eppendorf tube
5. Centrifuge the extract at 12000xg for 5 min at 4ºC.
6. Remove the supernatant and use this directly for MMP-14 assay (a dilution of at least 10-fold is recommended), or rapidly freeze and store at -20ºC (short storage), or -70ºC (longer storage).

Tissue samples

Methods to prepare tissue extracts are very dependent on tissue type. The following method is used for extracting RA synovial tissue and is for guidance only.

1. Grind the tissue. Freezing the tissue before grinding may help the grinding process.
2. Add 20-50 µl of extraction buffer per mg of ground tissue.
3. Mix thoroughly and incubate on ice during 15 min.
4. Centrifuge at 12,000xg for 10 min. at 4ºC.
5. Remove the supernatant
6. Use supernatant directly for MMP-14 assay (a dilution of at least 4 fold is recommended), or rapidly freeze and store at -20ºC (short storage), or -70ºC (longer storage).

Reagent preparation

Day 1
Extraction buffer
Thaw the extraction buffer and store at 2-8°C.

Standard
1) Add 950 µl extraction 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.

Sample
Thaw or prepare the sample extracts (see page 6), store on ice until required.

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

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

Substrate
1) Allow the substrate to thaw before use.
2) Store the vial 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 9).

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 ng/ml MMP-14. Prepare a 10 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	0	500 µl	0.000

If high levels of MMP-14 are expected you may limit your range by skipping the 0.062 and 0.125 ng/ml points, if low levels are expected you may skip the 16 and 8 ng/ml points.

Assay procedure

Day 1

1) Set up the microplate with sufficient wells for running of all zero (blanks), standards and samples as required.
2) Coating anti-MT1-MMP: Dilute the 5.9 mg/ml antibody stock to 10 µg/ml in PBS: e.g. 18.6 µl stock + 11 ml PBS. Pipet 100 µl into the required wells.
3) Cover the plate and incubate for 2 hours at 37°C in a humidified chamber.
4) Decant the coating solution, blot plate dry on paper tissue.
5) Pipet 100 µl ready-to-use **blocking buffer** into the wells.
6) Cover the plate and incubate 2 hours at 25°C.
7) Prepare a standard curve on ice of MMP-14 diluted in cold **extraction buffer**. 16 – 0 ng/ml or 4 – 0 ng/ml, depending on expected concentration in sample.
8) Prepare your samples.
9) Decant the blocking buffer from plate, blot plate dry on paper tissue.
10) Wash the wells 4 times with **wash buffer** and put place on ice.
11) Apply 100 µl standard or (diluted) sample to the wells (keep plate on ice during this procedure). If samples have to be diluted, use **extraction buffer.**
12) Incubate overnight at 4°C in a humidified chamber.

Day 2

13) Aspirate and wash all wells 4 times with **wash buffer**, ensuring that the wells are completely filled and emptied at each wash.
14) Pipet 50 µl of the **assay buffer** into all wells
15) Shake the plate for 20 seconds
16) Cover the plate with the lid provided and incubate at 37°C for 2 hours in a moist environment (to prevent evaporation).

17) Just before the end of the incubation time, prepare the detection reagent (see page 7) and 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 3 hours (total incubation = 5 hours)
24) Read the plate at 405 nm, this is t=5 hours
25) Make a graph of the A405 versus the MMP-14 concentration for data at 2 hours and 5 hours incubation.
26) Draw best-fit linearized curves through the points on the graphs. Using these standard curves the A_{405} values of the test samples can be calculated to ng/ml MMP-14. From the sample volume used in the assay the MMP-14 concentrations can be calculated.

Data analysis

The MMP-14 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 5h incubation by subtracting the A at t=0 hour from the A at t=2 hour and t=5 hour.
2. Average the ΔA values of multiple blanks to obtain an average blank ΔA value for t=2 hour and t=5 hours 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=5 hour from the ΔA of the various samples at t=5 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 MMP-14 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=5 hour data by plotting the blank subtracted ΔA values at t=5 hour against the MMP-14 standard concentrations. You should only use the 0, 0.0625, 0.125, 0.25, 0.5, 1, 2 and 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 2 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 5 h reading and a shorter standard line (0, 0.0625, 0.125, 0.25, 0.5, 1, 2 and 4 ng/ml).

Storage conditions

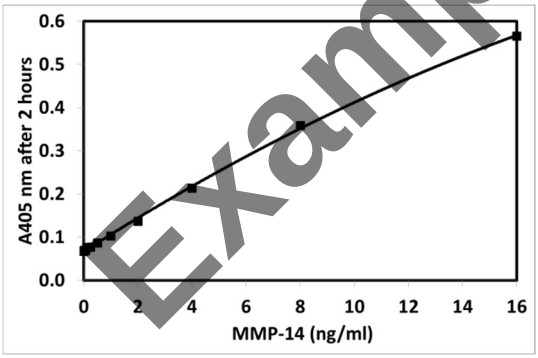
Unopened kit: Store at -20ºC, except for the standard, this vial should be stored at -70°C. Do not use kit, or individual kit components past kit expiration date.

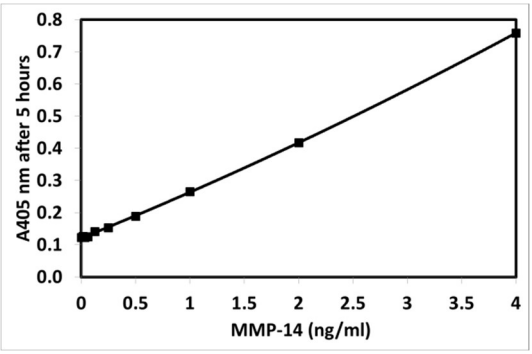
Opened kit / reconstituted reagents:
After opening, microwell plate or individual strips can be stored at room temperature or at -20°C or lower in original foil packaging with desiccant until use

- Undiluted MMP-14 antibody should be stored at -20°C or below and aliquoted to minimize freeze-thaw cycles.
Diluted antibody should be used immediately and thereafter discarded
- Undiluted MMP-14 standard should be stored preferably at -70°C and aliquoted to minimize freeze-thaw cycles.
Diluted standard should be used immediately and thereafter discarded.
- Extraction buffer should be store at 2-8ºC for short term storage (less than 1 week), or -20ºC for longer storage (several months).
- Assay Buffer should be stored at 4ºC for short term storage (less than 1 week), or -20ºC for longer storage (several months).
- Blocking buffer should be stored frozen at -20ºC or lower.
- Detection Enzyme should be stored frozen at -20ºC or lower.
- Substrate solution should be stored frozen at -20ºC or lower.
- Preferably store wash buffer in concentrated form at -20ºC. Wash buffer in diluted form should be stored at 4ºC for short term storage (less than 1 week), or at -20ºC for longer storage (several months).

Typical data

The data curves shown 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 MMP-9 activity assay
- Mouse MMP-9 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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