Human MMP14 Assay Kit

Human MMP14 Assay Kit is a detection kit for the quantification of Human matrix metallopeptidase 14 activity in Human Cell and tissue lysates.

Catalog number: ARG82633

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

MMP14 is one of the MMP14 is a protein of the matrix metalloproteinase (MMP) family, and they are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Most MMP's are secreted as inactive proproteins which are activated when cleaved by extracellular proteinases. However, the protein encoded by this gene is a member of the membrane-type MMP (MT-MMP) subfamily; each member of this subfamily contains a potential transmembrane domain suggesting that these proteins are expressed at the cell surface rather than secreted. This protein activates MMP2 protein, and this activity may be involved in tumor invasion. [provided by RefSeq, Jul 2008]

MMP14 seems to specifically activate progelatinase A. May thus trigger invasion by tumor cells by activating progelatinase A on the tumor cell surface. May be involved in actin cytoskeleton reorganization by cleaving PTK7. Acts as a positive regulator of cell growth and migration via activation of MMP15. Involved in the formation of the fibrovascular tissues in association with pro-MMP2. [UniProt]

PRINCIPLE OF THE ASSAY

This assay kit provides a simple, specific and precise quantitative determination of Human MMP14 activity in biological samples. An antibody specific for MMP14 is coated onto a microtiter plate. The standards (human MMP14) or samples are pipetted into the wells and any MMP14 present is bound by the immobilized antibody. After washing away any unbound substances, the pro-enzyme in detection reagent is then added into the well and then it in turn to active form of enzyme by activated MMP14 protein. The active form of enzyme

catalyzes the reaction of chromogenic substrate to form a colored product and it is measured at a wavelength of 405nm. The MMP14 activity in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped with blue ice. Upon receipt, store the kit at -20°C, and the Standard and antibody concentrate should be stored at-70°C. Use the kit before expiration date.

Component	Quantity	Storage information
Microplate	8 wells X 12 strips	-20°C. Unused strips should be sealed tightly in the air-tight pouch.
Antibody concentrate	20 µl	-70°C
Blocking buffer	15 ml (Ready-to-Use)	-20°C
Extraction buffer	100 ml (Ready-to-Use)	-20°C
Standard (640 ng/ml)	50 µl	-70°C
Detection enzyme	600 µl	-20°C or lower
Assay buffer	20 ml (Ready-to-Use)	-20°C
Substrate	1 ml	-20°C or lower
20 X Wash buffer	25 ml	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Horizontal orbital microplate shaker
- (Microplate) incubator at 37°C or 37°C oven.
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The kit is shipped with blue ice. Upon receipt, it is recommended to aliquot and store to standard stock at -70°C to minimize freeze-thaw cycles. And store antibody concentrate at -70°C and other components at -20°C. Do not use kit, or individual kit components past kit expiration date.
- After opening, microwell plate or individual strips should be stored at -20°C or lower in original foil packaging with desiccant until use.
- Store the kit at -20°C at all times. The kit should not be used beyond the expiration date on the kit label.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Do not let strips dry, as this will inactivate active components in wells.
- It is highly recommended that the standards, samples and controls be assayed in duplicates. A separate standard curve must be run on each plate.
- Change pipette tips between the addition of different reagent or samples.
- Avoid using reagents from different batches.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell lysate - Remove medium from the plate (well), add 250 µl of Extraction Buffer pre cm^2 into the well. Incubate at 4°C for 15 minutes, mild shaking may help to dissolve the MMP14. Transfer extract to a new eppendorf tube and remove particles by centrifugation for at least 5 min at 12,000 x g at 4°C. Collect the supernatants and assay immediately or aliquot and rapidly freeze and store samples at -80°C. Avoid repeated freeze-thaw cycles. It is recommended to dilute the samples at least 10X with Extraction Buffer before assay.

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

Grind the tissue. Freezing the tissue before grinding may help the grinding process. Add 20-50 µl of Extraction Buffer per mg of ground tissue. Mix thoroughly and incubate on ice during 15 min. centrifugation for at least 5 min at 12,000 x g at 4°C. Collect the supernatants and assay immediately or aliquot and rapidly freeze and store samples at -80°C. Avoid repeated freeze-thaw cycles. It is recommended dilute the samples at least 4X with Extraction Buffer before assay.

Note:

a) Rapidly thaw samples in water bath (not higher than 37 °C) and immediately put on ice until use.
b) Thawed sample should be mix thoroughly prior to assay.

REAGENT PREPARATION

- 1X Wash Buffer: It is recommended store concentrated wash buffer at -20°C and diluting only needed volume. Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 25 ml of 20X Wash buffer + 475 ml of distilled water) The diluted Wash buffer is stable for only few days at 2°C to 8°C. For long term storage, the diluted Wash buffer can be stored at-20°C for few months. Keep the 1X wash buffer at RT prior to use.
- 1X Antibody working solution: It is recommended to prepare this reagent immediately prior to use and dilute only needed volume for once used. For total 96 wells are used, dilute 18.6 µl of Antibody concentrate with 11 ml of PBS.
- Extraction Buffer: Thaw the Extraction Buffer and store at 2-8°C prior to use. Extraction Buffer is stable at 2-8°C for few days only, and can be stored at -20°C for few months. It is recommended to aliquot in a small volume and store at -20°C.
- Assay buffer: Thaw the Assay buffer and store at 2-8°C prior to use. Assay buffer is stable at 2-8°C for few days only, and can be stored at -20°C for few months. It is recommended to aliquot in a small volume and store at -20°C.
- Detection enzyme: Thaw Detection enzyme on ice before use, keep the thawed Detection enzyme on ice prior to use.
- Substrate: Thaw Substrate before use, keep the thawed Substrate on ice prior to use.

- **Detection reagent**: It is recommended to prepare this reagent immediately prior to addition to the wells.

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

- **Standards**: It is important to perform this procedure on ice.

  Add 950 µl of Extraction buffer to the standard vial, gently mix, to yield a stock concentration of 32,000 pg/ml. Aliquot and store this stock standard at-70°C. Avoid repeated freeze-thaw cycles. The Extraction buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with Extraction buffer as according to the suggested concentration below: 16,000 pg/ml, 8,000 pg/ml, 4,000 pg/ml, 2,000 pg/ml, 1,000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml and 62.5 pg/ml.

Dilute MMP14 standard as according to the table below:

Standard	MMP14 Conc. (pg/ml)	µl of Extraction buffer	µl of standard
S9	16000	300	300 (32,000 pg/ml Stock)
S8	8000	300	300 (S9)
S7	4000	300	300 (S8)
S6	2000	300	300 (S7)
S5	1000	300	300 (S6)
S4	500	300	300 (S5)
S3	250	300	300 (S4)
S2	125	300	300 (S3)
S1	62.5	300	300 (S2)
S0	0	300	0

ASSAY PROCEDURE

Standard MMP14 detection curve should be prepared for each experiment. Standards, samples and standard should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Put remaining strips immediately back at-20°C.
2. Add **100 µl** of diluted **1X Antibody working solution** into wells**.** Cover the plate with foil and incubate for **2 hours at 37°C** in a moist environment (to prevent evaporation).
3. Aspirate each well and blot plate dry on paper tissue.
4. Add **100 µl** of **Blocking buffer** into the wells. Cover the plate and incubate **2 hours at 25°C**.
5. Prepare the MMP14 standards on ice with cold Extraction Buffer. And also prepare samples.
6. Aspirate each well and blot plate dry on paper tissue.
7. Aspirate each well and wash, repeating the process three times for a total **four washes**. Wash by filling each well with **1X Wash Buffer** (350 µl or completely filled the well with 1X wash buffer) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time. Put place on ice
8. Add **100 µl** of diluted **standards, samples and zero controls** (S0, Extraction buffer) into wells. (Keep plate on ice during this procedure). If samples have to be diluted, use Extraction buffer.

9. Cover the plate with foil and incubate for **overnight at 2-8°C** in a moist environment (to prevent evaporation).
10. Aspirate each well and **wash** as step 7
11. Add **50 µl of Assay buffer** into all wells.
12. **Shake** the plate for **20 seconds**. Cover the plate with foil and incubate **at 37°C for 2 hour** in a moist environment (to prevent evaporation).
13. Prepare the detection reagent just before the end of Assay Buffer incubation step.
14. Add **50 µl** of the **detection reagent** into all wells.
15. **Shake** the plate for **20 seconds**. Read the plate at **405 nm** to obtain a **t = 0 value**.
16. Cover the plate with foil and incubate **at 37°C for 2 hour** in a moist environment (to prevent evaporation).
17. **Shake** the plate for **20 seconds**. Read the plate at **405 nm** to obtain a **t = 2 hours value**.
18. Cover the plate with foil and incubate **at 37°C for another 3 hours** in a moist environment (to prevent evaporation).
19. **Shake** the plate for **20 seconds**. Read the plate at **405 nm** to obtain a **t = 5 hour value**.

CALCULATION OF RESULTS

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 hour 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 MMP14 standard concentration. Use the OD of zero and standard concentrations of 250-16,000 pg/ml only in the standard curve for this "high level" standard curve.

5. 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 MMP14 standard concentration. Use the OD of zero and standard concentrations of 62.5-4,000 pg/ml only in the standard curve for this "low level" standard curve.

6. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

7. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

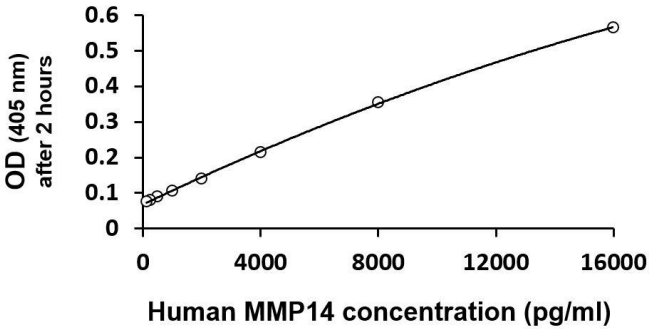
8. If the samples have been diluted, the concentration read from the standard

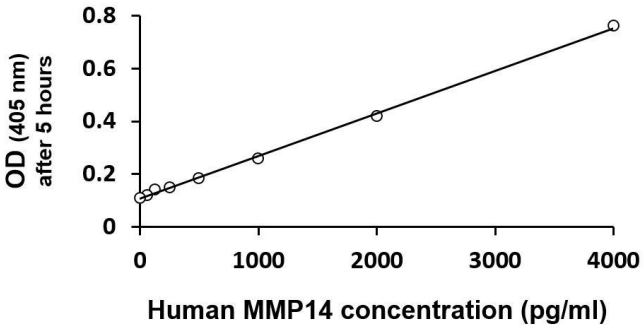
curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

9. If the range of the standard line the samples is known, a shorter standard line with 8 points in the appropriate range can be used, allowing to measure more samples with the kit.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.





QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human MMP14 ranged from 62.5-16000 pg/ml. The mean MDD was

500 pg/ml for 2 hours incubation

100 pg/ml for 5 hours incubation.

Specificity

Measures endogenous active MMP14 (naturally occurring).