



## Human MMP9 Assay Kit

Human MMP9 Assay Kit is a detection kit for the quantification of Human matrix metalloproteinase 9 activity in Human Serum, plasma, cell culture supernatants, urine, synovial fluid and tissue homogenates.

Catalog number: ARG82631

---

For research use only. Not for use in diagnostic procedures.

## **TABLE OF CONTENTS**

<b>SECTION</b>	<b>Page</b>
INTRODUCTION .....	3
PRINCIPLE OF THE ASSAY .....	3
MATERIALS PROVIDED & STORAGE INFORMATION.....	4
MATERIALS REQUIRED BUT NOT PROVIDED .....	4
TECHNICAL HINTS AND PRECAUTIONS .....	5
SAMPLE COLLECTION & STORAGE INFORMATION .....	6
REAGENT PREPARATION.....	8
ASSAY PROCEDURE .....	9
CALCULATION OF RESULTS .....	12
EXAMPLE OF TYPICAL STANDARD CURVE .....	13
QUALITY ASSURANCE.....	14

### **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](mailto:info@arigobio.com)

### INTRODUCTION

MMP9 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. The enzyme encoded by this gene degrades type IV and V collagens. Studies in rhesus monkeys suggest that the enzyme is involved in IL-8-induced mobilization of hematopoietic progenitor cells from bone marrow, and murine studies suggest a role in tumor-associated tissue remodeling. [provided by RefSeq, Jul 2008]

MMP9 may play an essential role in local proteolysis of the extracellular matrix and in leukocyte migration. Could play a role in bone osteoclastic resorption. Cleaves KISS1 at a Gly-| -Leu bond. Cleaves type IV and type V collagen into large C-terminal three quarter fragments and shorter N-terminal one quarter fragments. Degrades fibronectin but not laminin or Pz-peptide. [UniProt]

### PRINCIPLE OF THE ASSAY

This assay kit provides a simple, specific and precise quantitative determination of Human MMP9 in the active or pro-form in biological samples. An antibody specific for MMP9 is pre-coated onto a microtiter plate. The standards (human pro-MMP9) or samples are pipetted into the wells and any MMP9 present is bound by the immobilized antibody. After washing away any unbound substances, APMA is added in the standard wells and the wells used to determine total MMP9 activity, but not the wells used to detect endogenous MMP9 activity. The pro-MMP9 in samples or standards is activated by APMA.

## Human MMP9 Assay kit ARG82631

---

The pro-enzyme in detection reagent is then added into the well and then it in turn to active form of enzyme by activated MMP9 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 MMP9 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 should be stored at -70°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 wells X 12 strips	-20°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (640 ng/ml)	50 µl	-70°C
Detection enzyme	600 µl	-20°C or lower
Assay buffer	100 ml (Ready-to-Use)	-20°C
Substrate	1 ml	-20°C or lower
APMA (17.5 mg)	1 vial	-20°C
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.

## Human MMP9 Assay kit ARG82631

---

- Dimethyl Sulphoxide (DMSO)
- 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 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.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- 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.
- Change pipette tips between the addition of different reagent or samples.
- Avoid using reagents from different batches.

## Human MMP9 Assay kit ARG82631

---

- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.

### **SAMPLE COLLECTION & STORAGE INFORMATION**

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

**Serum**- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and rapidly freeze the samples by dry ice, liquid nitrogen or a cold bath is recommended. Store the aliquoted samples at -20°C or lower. Avoid repeated freeze-thaw cycles. Dilution of the serum with Assay buffer (30 fold or more) might be required for a good recovery.

**Plasma** - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes 1,000 x g at 4°C. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and rapidly freeze the samples by dry ice, liquid nitrogen or a cold bath is recommended. Store the aliquoted samples at -20°C or lower. Avoid repeated freeze-thaw cycles. Dilution of the plasma with Assay buffer (30 fold or more) might be required for a good recovery.

**Cell Culture Supernatants** - Remove particulates by centrifugation for at least 10 min at 10,000 x g at 4°C. Collect the supernatants and assay immediately or aliquot and rapidly freeze and store samples at -20°C or lower. Avoid repeated freeze-thaw cycles.

**Urine**- Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation 10,000 x g at 4°C for at least 10 min. Collect the

## Human MMP9 Assay kit ARG82631

---

supernatants and assay immediately or aliquot and rapidly freeze and store samples at -20°C or lower. (Note: Creatinine assay may be used for results normalization.)

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

Cut the tissues in small pieces, homogenize with Tris-HCl buffer (50 mM, pH 7-8) containing a non-ionic detergent e.g. 0.1% (v/v) by Potter homogenizer or other mechanical device (depending on the tissue) on ice. Centrifuge samples 10000 X g at 4 °C for at least 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay. Assay immediately or aliquot and rapidly freeze and store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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.
- c) Do not use haemolytic, icteric or lipaemic specimens.

### REAGENT PREPARATION

- **1X Wash Buffer:** It is recommended to store concentrated wash buffer at  $-20^{\circ}\text{C}$  and dilute only the 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 a few days at  $2^{\circ}\text{C}$  to  $8^{\circ}\text{C}$ . For long-term storage, the diluted Wash buffer can be stored at  $-20^{\circ}\text{C}$  for a few months. Keep the 1X wash buffer at RT prior to use.
- **Assay buffer:** Thaw the assay buffer and store at  $2-8^{\circ}\text{C}$  prior to use. Assay buffer is stable at  $2-8^{\circ}\text{C}$  for a few days only, and can be stored at  $-20^{\circ}\text{C}$  for a few months. It is recommended to aliquot in a small volume and store at  $-20^{\circ}\text{C}$ .
- **APMA:** Reconstitute the APMA with  $50\ \mu\text{l}$  of DMSO to yield a stock concentration of 1M. Replace the cap and vortex until the solution is clear. The 1M APMA stock solution should be aliquoted and stored at  $-20^{\circ}\text{C}$ . Do not thaw the 1M APMA stock solution for more than once. Prior to use, add  $10\ \mu\text{l}$  of 1M APMA stock solution into 10 ml of assay buffer at room temperature and mix well to yield a 1 mM APMA working solution.
- **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.



## Human MMP9 Assay kit ARG82631

---

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

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

Add 950  $\mu$ l of assay buffer to the standard vial, gently mix, to yield a stock concentration of 32,000 pg/ml. Aliquot and store this stock standard at  $-70^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles. The assay buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with assay 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, 62.5 pg/ml, 31.25 pg/ml and 15.625 pg/ml.

Dilute MMP9 standard as according to the table below:

Standard	MMP9 Conc. (pg/ml)	$\mu$ l of Assay buffer	$\mu$ l of standard
S11	16000	300	300 (32,000 pg/ml Stock)
S10	8000	300	300 (S11)
S9	4000	300	300 (S10)
S8	2000	300	300 (S9)
S7	1000	300	300 (S8)
S6	500	300	300 (S7)
S5	250	300	300 (S6)
S4	125	300	300 (S5)
S3	62.5	300	300 (S4)
S2	31.25	300	300 (S3)
S1	15.625	300	300 (S2)
S0	0	300	0

## **ASSAY PROCEDURE**

Standard MMP9 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 **standards, samples and zero controls** (S0, assay buffer) into wells. Cover the plate with foil and incubate for **overnight at 2-8°C in a moist environment (to prevent evaporation)**.
3. Prepare (or thaw) 1 mM APMA solution just before next wash step.
4. 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.
5. Add **50 µl** of **1 mM APMA working solution** into standard wells and the wells containing **samples** in which **total MMP9** are to be measured.
6. Add **50 µl** of **Assay buffer** into the wells containing **samples** in which **endogenous levels of active MMP9** are to be measured.
7. Cover the plate with foil and incubate **at 37°C for 1.5 hours in a moist environment (to prevent evaporation)**.
8. Prepare detection reagent just before the end of APMA incubation step.
9. Add **50 µl** of the **detection reagent** into all wells.

## Human MMP9 Assay kit ARG82631

---

10. **Shake** the plate for **20 seconds**. Read the plate at **405 nm** to obtain a **t = 0 value**.
11. Cover the plate with foil and incubate **at 37°C for 1 hour** in a moist environment (to prevent evaporation).
12. **Shake** the plate for **20 seconds**. Read the plate at **405 nm** to obtain a **t = 1 hour value**.
13. Cover the plate with foil and incubate **at 37°C for another 3 hours** in a moist environment (to prevent evaporation).
14. **Shake** the plate for **20 seconds**. Read the plate at **405 nm** to obtain a **t = 4 hour value**.

### Summary

	Standard	Total MMP9	Endogenous levels of active MMP9
<b>Standard</b>	100 µl	-	-
<b>Samples</b>	-	100 µl	100 µl
Cover the plate with foil and incubate for <b>overnight at 2-8°C</b> .			
Wash the wells for total 4 washes.			
<b>1 mM APMA</b>	50 µl	50 µl	-
<b>Assay buffer</b>	-	-	50 µl
Cover the plate with foil and incubate <b>at 37°C for 1.5 hours</b>			
<b>Detection reagent</b>	50 µl	50 µl	50 µl
<b>Shake</b> the plate for <b>20S</b> . Read the plate at <b>405 nm</b> to obtain a <b>t = 0 value</b> .			
Cover the plate with foil and incubate <b>at 37°C for 1 hour</b> .			
<b>Shake</b> the plate for <b>20S</b> . Read the plate at <b>405 nm</b> to obtain a <b>t = 1 value</b> .			

Cover the plate with foil and incubate <b>at 37°C for another 3 hour.</b>
---

<b>Shake</b> the plate for <b>20S</b> . Read the plate at <b>405 nm</b> to obtain a <b>t = 4 value.</b>
---

## **CALCULATION OF RESULTS**

1. Calculate the  $\Delta A$  for each well (samples and blanks) after 1h and 4h incubation by subtracting the A at t=0 hour from the A at t=1 hour and t=4 hour.
2. Average the  $\Delta A$  values of multiple blanks to obtain an average blank  $\Delta A$  value for t=1 hour and t=4 hour incubation.
3. Subtract the average blank  $\Delta A$  at t=1 hour from the  $\Delta A$  of the various samples at t=1 hour and subtract the average blank  $\Delta A$  at t=4 hour from the  $\Delta A$  of the various samples at t=4 hour.
4. Create a “high level” standard curve from the t=1 hour data by plotting the blank subtracted  $\Delta A$  values at t=1 hour against the MMP9 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=4 hour data by plotting the blank subtracted  $\Delta A$  values at t=4 hour against the MMP9 standard concentration. Use the OD of zero and standard concentrations of 15.625-1,000 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

## Human MMP9 Assay kit ARG82631

---

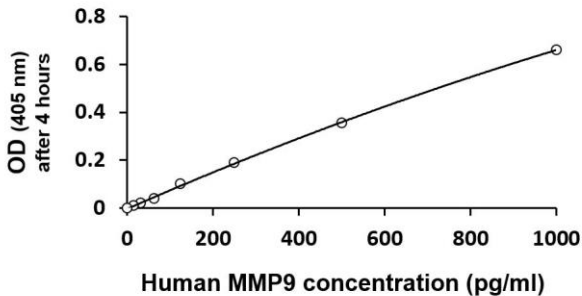
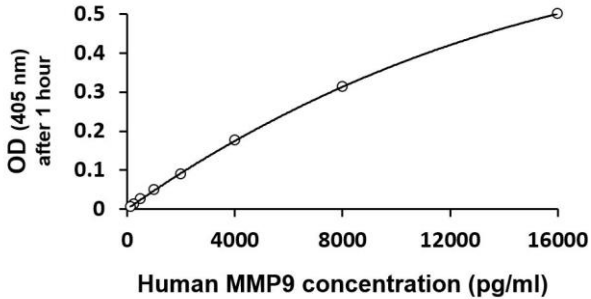
Logistics is the preferred method. Other data reduction functions may give slightly different results.

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 MMP9 ranged from 16- 16000 pg/ml. The mean MDD was  
500 pg/ml for 1 hour incubation  
10 pg/ml for 4 hours incubation.

### **Specificity**

Measures endogenous active MMP9 (naturally occurring) or total active MMP9 (following activation with APMA).