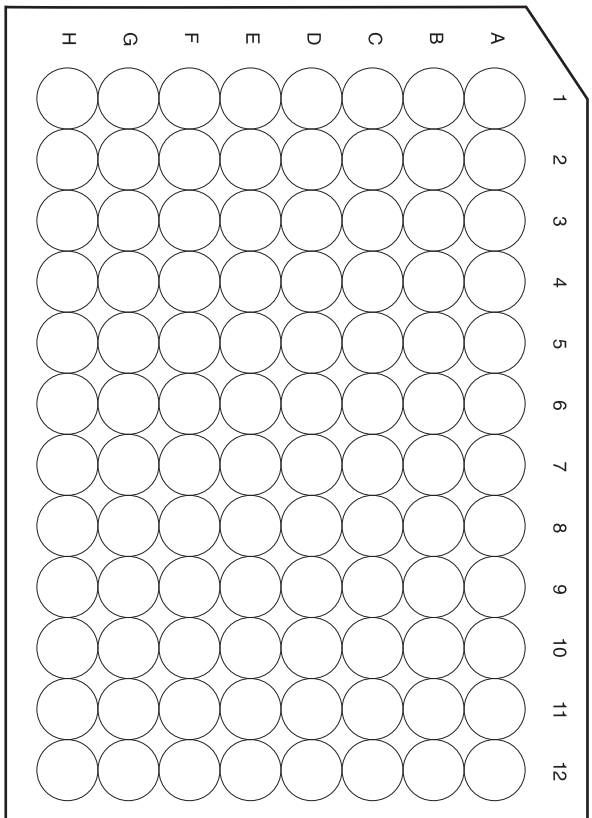

Rat MMP-2 Immunoassay

Catalog Number: SEKR-0066

For the quantitative determination of rat MMP-2 concentrations in cell culture supernates, serum, and plasma.

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

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LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of MMP-2 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	94	97
	Range(%)	85-103	89-109
1:4	Average% of Expected	96	96
	Range(%)	87-109	90-112
1:8	Average% of Expected	92	97
	Range(%)	84-104	88-111
1:16	Average% of Expected	93	95
	Range(%)	86-103	87-113

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 78pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant rat MMP-2. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant rat	Recombinant human	Recombinant Porcine
MMP-9	MMP-1	
MMP-3		
MMP-1		
IFN-γ		
IL-1β		
IL-2		
IL-4		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY: The recovery of MMP-2 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of MMP-2 in two matrices

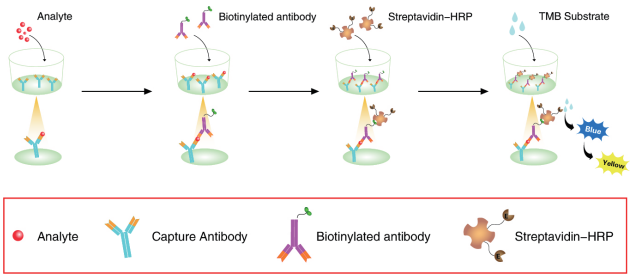
Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	93	97-110
Cell culture supernatants	95	89-109

BACKGROUND

Type IV collagenase, 72-kD, is officially designated matrix metalloproteinase-2 (MMP2). It is also known as gelatinase, 72-kD. MMP-2 plays an essential role in angiogenesis and arteriogenesis, two processes critical to restoration of tissue perfusion after ischemia. MMP-2 expression is increased in tissue ischemia, but the responsible mechanisms remain unknown. Matrix metalloproteinases (MMPs) catalyze extracellular matrix degradation. Control of their activity is a promising target for therapy of diseases characterized by abnormal connective tissue turnover. MMPs are expressed as latent proenzymes that are activated by proteolytic cleavage that triggers a conformational change in the propeptide (cysteine switch). The structure of proMMP-2 reveals how the propeptide shields the catalytic cleft and that the cysteine switch may operate through cleavage of loops essential for propeptide stability. The gene is localized to 16q21 using somatic cell hybrids and in situ hybridization.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for MMP-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MMP-2 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for MMP-2 is added to detect the captured MMP-2 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

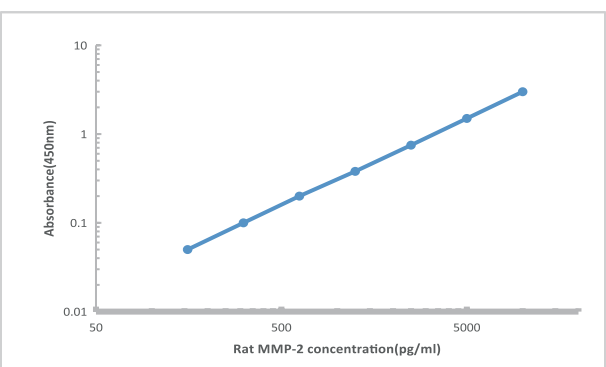
PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the MMP-2 ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.048	0.042	0.045	-
156.25	0.142	0.142	0.142	0.097
312.5	0.207	0.206	0.206	0.161
625	0.307	0.307	0.307	0.262
1250	0.488	0.487	0.487	0.442
2500	0.792	0.791	0.792	0.746
5000	1.290	1.287	1.289	1.244
10000	2.102	2.098	2.100	2.055



Representative standard curve for MMP–2 ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100μl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-rat MMP-2 antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature (25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 30 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100μl Substrate solution to each well, incubate 5-20 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the MMP-2 concentrations versus the log of the O.D. and the best fit line can be determined by

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,10000 pg/ml upon reconstitution	2 vials	Store at 2-8°C **for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120ul/vial	1 vial	Store at 2-8°C **for six months
Standard /Sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

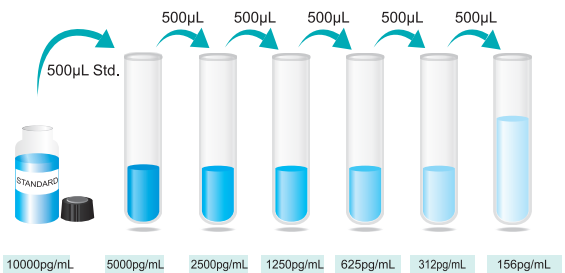
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard\Sample** -Reconstitute the Standard with 1mL of Standard /Sample Diluent. This reconstitution produces a stock solution of 10000

pg /mL.Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500uL of Standard/Sample Diluent into the 5000 pg/mL tube and the remaining tubes. Use the stock solution of 10000 pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 10000 pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).



Preparation of MMP–2 standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-rat MMP-2 antibody**:Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**
5. **Working solution of Streptavidin-HRP**:Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**