

REFERENCES

1. Nagase, H. and J.F. Woessner, Jr. (1999) J. Biol. Chem. 274:2191.
2. Parks, W.C. and R.P. Mecham (1998) in Matrix Metalloproteinases, Academic Press, San Diego.
3. Tschesche, H. and M. Pieper (1998) in Handbook of Proteolytic Enzymes, Barrett, A.J. et al. eds., Academic Press, San Diego, pp1162-1167.
4. Van Wart, H.E. and H. Birkedal-Hansen (1990) Proc. Natl. Acad. Sci. USA 87:5578.
5. Jiang, W. and J.S. Bond (1992) FEBS Lett. 312:110.
5. Bode, W. et al. (1993) FEBS Lett. 331:134.

HumanTotal MMP-8 Immunoassay

Catalog Number: SEKH-0256

For the quantitative determination of humanTotal MMP-8 concentrations in cellculture supernates, serum, and plasma.

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

MANUFACTURED AND DISTRIBUTED BY:

Country | Company: China | Beijing Solarbio Science & Technology Co.,Ltd
Address:NO.85A, Liandong U Valley, Tongzhou District, Beijing, P.R.China.
Tel: 86-10-56371241 Fax: 86-10-56371282 E-mail: service@solarbio.com

TABLE OF CONTENTS

SECTION	PAGE
BACKGROUND.....	01
PRINCIPLE OF THE ASSAY.....	01
TECHNICAL HINTS AND LIMITATIONS.....	02
PRECAUTIONS.....	02
KIT COMPONENTS& STORAGE CONDITIONS.....	03
OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED.....	04
SPECIMEN COLLECTION & STORAGE.....	04
REAGENTS PREPARATION.....	04
ASSAY PROCEDURE.....	06
CALCULATION OF RESULTS.....	06
PERFORMANCE CHARACTERISTICS.....	08
REFERENCES.....	10

LINEARITY:To assess the linearity of the assay, three samples were spiked with high concentrations of Total MMP-8 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	98	103
	Range (%)	94–112	97–106
1:4	Average% of Expected	105	101
	Range (%)	97–113	91–110
1:8	Average% of Expected	97	96
	Range (%)	92–102	87–105
1:16	Average% of Expected	101	102
	Range (%)	97–105	91–108

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 31pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human Total MMP-8. 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 human	Recombinant mouse	Recombinant porcine
ADAM8		
ADAM9		
ADAM10		
ADAM12		
ADAM15		
ADAM19		
ADAM22		
ADAM23		
ADAM28		
ADAM33		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY: The recovery of Total MMP-8 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of Total MMP-8 in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	96	86–98
Cell culture supernatants	105	96–113

BACKGROUND

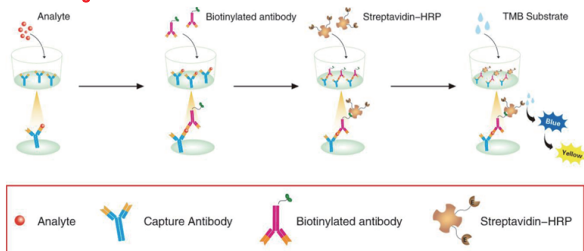
Matrix metalloproteinases (MMPs), also called matrixins, constitute a family of zinc and calcium dependent endopeptidases that function in the breakdown of extracellular matrix (ECM). They play an important role in many normal physiological processes such as embryonic development, morphogenesis, reproduction and tissue remodeling. They also participate in many pathological processes such as arthritis, cancer and cardiovascular disease. While the amounts of newly synthesized MMPs are regulated mainly at the levels of transcription, the proteolytic activities of existing MMPs are controlled through both the activation of proenzymes and the inhibition of active enzymes by endogenous inhibitors, α 2-macroglobulins and tissue inhibitors of metalloproteinases (TIMPs). MMP-8 (also referred to as neutrophil collagenase and collagenase 2) is stored intracellularly as a latent proenzyme in the specific granules of polymorphonuclear leukocytes. PMNs play an essential role in phagocytosis and also have a high capacity to infiltrate connective tissue. Various agents, such as IL-1 and IL-8, TNF- α , and GM-CSF, stimulate the release of MMP-8 from neutrophils, a key enzyme initiating the breakdown of the ECM, especially during pathological processes such as inflammation, rheumatoid arthritis or osteoarthritis.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Total MMP-8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Total MMP-8 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for Total MMP-8 is added to detect the captured Total MMP-8 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.

DESCRIPTION

Schematic diagram:



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.

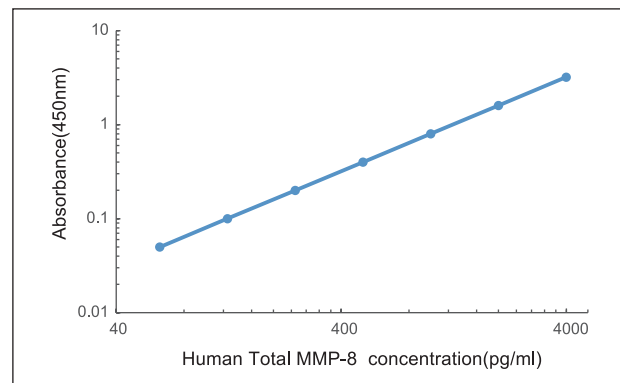
DESCRIPTION

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 Total MMP-8 ELISA

Standard(pg/ml)	OD.	OD.	Average	Corrected
0	0.087	0.079	0.083	---
62.5	0.180	0.175	0.178	0.095
125	0.241	0.240	0.241	0.158
250	0.354	0.351	0.353	0.270
500	0.578	0.573	0.576	0.493
1000	1.004	0.998	1.001	0.918
2000	1.604	1.601	1.603	1.520
4000	2.519	2.517	2.518	2.435



Representative standard curve for Total MMP-8 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, incubate 90 minutes, 37°C.



Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human Total MMP-8 antibody to each well, incubate 60 minutes, 37°C.



Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes, 37°C.



Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 15 minutes, 37°C.
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 Total MMP-8 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an

KIT COMPONENTS & STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate-antibody coated 96-well Microplate (8 wells x12 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°C**
Standard - lyophilized, 4000 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°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) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent - 16ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent- 16ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent - 16ml/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. Squirrt 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 $\leq -20^{\circ}\text{C}$. 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 $2-8^{\circ}\text{C}$. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

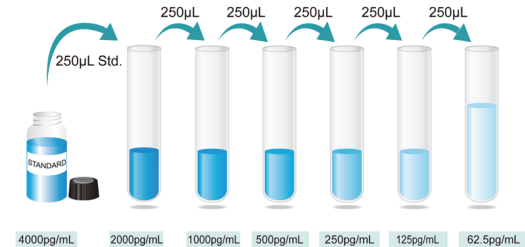
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature ($20-25^{\circ}\text{C}$) before use.
2. **Wash Buffer** - Dilute 30 mL of Wash Buffer Concentrate with 570 mL of deionized or distilled water to prepare 600 mL 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 0.5 mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 μL of Standard/Sample Diluent into 2000 pg/mL tube and the remaining tubes. Use the stock solution of 4000 pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 4000 pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).

**Preparation of Total MMP-8 standard dilutions**

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

4. **Working solution of Biotin-Conjugate anti-human Total MMP-8 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.**