In vitro Research Use Only

Reviesd on March, 2016

For Determination of Human MMP-3

MMP−3 ELISA Kit

Introduction:

Matrix metalloproteinase 3 (MMP-3, Stromelysin-1) plays an important role in degradation and reconstitution of extracellular matrix by degrading proteoglycan, fibronectin, type IV collagen, laminin and type IX collagen. MMP-3 is secreted as a latent form and activated out of membrane, which participate in metabolism of matrix tissue. Activity of MMP-3 is inhibited specifically by tissue inhibitors of metalloprotienases (TIMPs).

This MMP-3 plate kit is based on one-step sandwich enzyme immunoassay using two different mouse anti MMP-3 monoclonal antibodies.

Reagents; One kit contains:

No.	Reagent	Form	Specifications	Remarks
①	Anti-MMP-3 Coated Microplate	Dried	1plate × 96wells	Mouse anti-MMP-3 antibody coated plate.
②	Mab-HRP Conjugate Concentrate	Concentrated solution	1vial × 2mL	A vial contains horse-radish peroxidase-labeled mouse anti-MMP-3 antibodies.
③	Coloring Solution	Liquid	1vial × 15mL	A vial contains TMB and H_2O_2.
④	Stop Solution	Liquid	1vial × 15mL	1mol/L Sulfuric Acid
⑤	Assay Buffer	Liquid	2vails × 25mL	Na-Phosphate Buffer
⑥-1	MMP-3 Standard (Lyophilized)	Lyophilized	1vial × for 1mL	A vial contains 12.5 ng of MMP-3.
⑥-2	MMP-3 Standard (Lyophilized)	Lyophilized	1vial × for 1mL	A vial contains 50 ng of MMP-3.
⑥-3	MMP-3 Standard (Lyophilized)	Lyophilized	1vial × for 1mL	A vial contains 200 ng of MMP-3.
⑥-4	MMP-3 Standard (Lyophilized)	Lyophilized	1vial × for 1mL	A vial contains 400 ng of MMP-3.
⑥-5	MMP-3 Standard (Lyophilized)	Lyophilized	1vial × for 1mL	A vial contains 800 ng of MMP-3.
⑦	Wash Buffer Concentrate	Concentrated solution	2vials × 50mL	Na-Phosphate Buffer

Adaptation:

Determination of human MMP-3 in human serum.

Safety Warning and Handling Precautions:

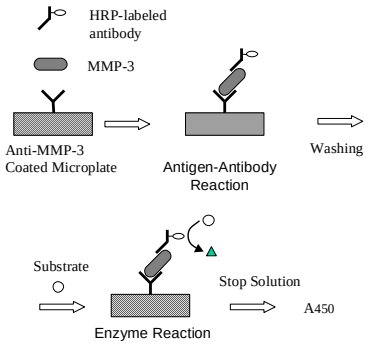
Warning: For research use only. Not recommended or intended for diagnosis of disease in human or animals. Do not use internally or externally in human or animals.

Warning: This kit contains Sulfuric acid. See a safety data sheet.

All chemicals should be considered as if they were potentially hazardous. All specimens used should be also handled as if they were capable of infecting diseases. Wear suitable protective clothing such as laboratory overall, safety glasses, gloves and shoes. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with copious amounts of water.

Principles of the Test:

This assay quantitates MMP-3 by a one-step sandwich EIA method. MMP-3 in the specimen react with anti-MMP-3 antibody coated wells (solid phase) and enzyme labeled antibody in the first reaction. MMP-3 molecule is sandwiched between solid phase and enzyme labeled antibody. After removing unbound enzyme labeled antibody, the plate is then incubated with enzyme substrate, resulting in the development of a color. The activity of peroxidase is proportional to the amount of antigen, so that concentration in specimens can be determined from the standard curve.



Materials required but not provided:

Microplate (U-bottom recommended)
Graduated cylinders (1000mL)
Micropipettes (40, 100, 120, 160, 1000μL)
Pipettes (10mL)
Microplate washer
Microplate reader (450nm)

Preparation of Working Reagents:

Working Reagents	Preparation	Stability after preparation
1. MMP-3 Standards	Reconstitute ⑥-1, 2, 3, 4 and 5 with 1mL of ⑤ Assay Buffer, respectively.	1month at 2-8°C
2. Mab-HRP Conjugate	Add 1mL of ②Mab-HRP Conjugate Concentrate to 10 mL of ⑤Assay Buffer.	1month at 2-8°C
3. Wash Buffer	Dilute the all (50mL) of ⑦Wash Buffer Concentrate with 450mL of deionized water.	1month at 2-8°C
4. Other reagents	Use according to the directions for Assay Procedure.	12months at 2-8°C

Assay Procedures :

(1) Summary

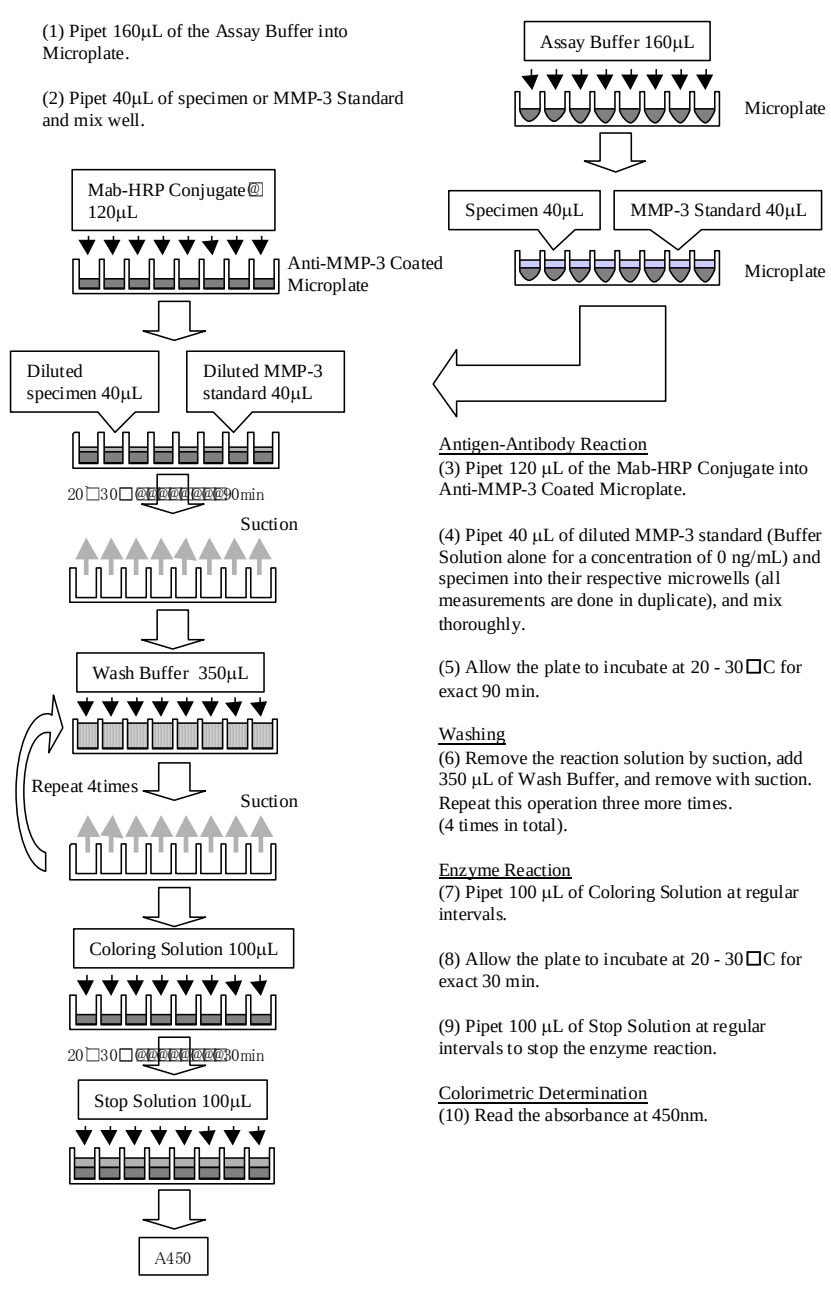
Dilution of specimen

	Specimen	Standard
Microplate	1well	1well
Assay Buffer	160 μL	160 μL
Specimen or MMP-3 Standard	40 μL	40 μL

Assay

	Specimen	Standard
Anti-MMP-3 Coated Microplate	1well	1well
Mab-HRP Conjugate	120 μL	120 μL
Diluted Specimen or MMP-3 Standard	40 μL	40 μL
[Antigen-antibody reaction] (1st reaction)	After mixing, allow to incubate at 20 – 30 °C for exact 90 min.	
[Washing]	Suck the solution and wash 4 times with 350 μL Wash Buffer	
Coloring Solution	100 μL	100 μL
[Enzyme reaction] (2nd reaction)	Allow to incubate at 20 – 30 °C for exact 30 min.	
Stop Solution	100 μL	100 μL
[Determination]	Read the absorbance at 450nm	

(2) Flow diagram of assay procedure



Antigen-Antibody Reaction

(3) Pipet 120 μL of the Mab-HRP Conjugate into Anti-MMP-3 Coated Microplate.

(4) Pipet 40 μL of diluted MMP-3 standard (Buffer Solution alone for a concentration of 0 ng/mL) and specimen into their respective microwells (all measurements are done in duplicate), and mix thoroughly.

(5) Allow the plate to incubate at 20 - 30□C for exact 90 min.

Washing

(6) Remove the reaction solution by suction, add 350 μL of Wash Buffer, and remove with suction. Repeat this operation three more times. (4 times in total).

Enzyme Reaction

(7) Pipet 100 μL of Coloring Solution at regular intervals.

(8) Allow the plate to incubate at 20 - 30□C for exact 30 min.

(9) Pipet 100 μL of Stop Solution at regular intervals to stop the enzyme reaction.

Colorimetric Determination

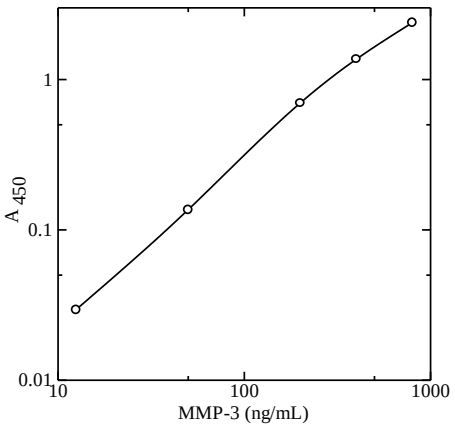
(10) Read the absorbance at 450nm.

Calculation of MMP-3 Concentration:

(1) The MMP-3 concentration (ng/mL) is plotted as abscissa and the absorbance as ordinate, on a graph paper.

(2) Plot the net absorbance value for each dilution level, obtained by subtracting the value for 0ng/mL standard solution (mean of 2 measurements) from the values for individual dilutions (mean of 2 measurements), against the MMP-3 concentration to prepare the standard curve.

(3) Using the net absorbance value for a specimen, determine the corresponding MMP-3 concentration from the standard curve.



Reference Intervals:

Reference Intervals of serum MMP-3 levels in normal subjects

Male: 36.9 - 121 ng/mL (n = 285)

Female: 17.3 - 59.7 ng/mL (n = 241)

Operational Precautions:

(1) Reactions may be somewhat influenced by times, temperature and other factors, therefore, a standard curve should be prepared at each time of assay.

(2) All measurements should be made in duplicate.

(3) Do not intermix reagents from kits with different lot number.

(4) Use fresh sera as specimens.

(5) If it is impossible to carry out assay immediately after the separation of sera, keep them in a refrigerator (5°C) or in a freezer (-40°C); they are stable for 1 week for the former and 1 year for the latter.

(6) Avoid a repetition of freezing and thawing for specimens.

(7) All reagents must be allowed to equilibrate to the reaction temperature before use.

(8) When the upper limit of the determination range is exceeded, a specimen should be diluted with Buffer Solution and then retested.

(9) Possible interferences

EDTA in specimens inhibits the immunoreaction.

Specific Performance Characteristics:

This test kit, when used by the prescribed Assay Procedures, exhibits the following specific performance characteristics.

(1) Sensitivity

When determined from the standard curve, the net absorbance obtained by subtracting the value for 0ng/mL standard solution from the value for individual dilution is from 0.010 to 0.075 for12.5ng/mL and from 1.4 to 3.8 for 800ng/mL.

(2) Specificity

When control specimen is measured, the concentration is in the range of 100 ± 20 % of its known value.

(3) Reproducibility

When same specimen is measured simultaneously 4 times in duplicate, the coefficient of variance for the measured values is less than 10%.

(4) Assay Range

12.5 to 800 ng/mL

Storage:

The kit should be preserved at 2-8°C.

Expiration Date:

The kit must be used within 12 months after manufacture (see the indication given on the box and labels).

References:

(1) Y. Okada *et al.* : J. Biol. Chem., 261, 14245-14255, 1986.

(2) K. Obata *et al.* : Clin. Chim. Acta, 211, 59-72, 1992.

(3) J. Martel-Peletier *et al.* : Lab. Invest., 70, 807-815, 1994.

(4) S. Sasaki et al.: Clin. Rheum., 13, 228-233,1994.

(5) Y. Yoshihara et al.: Arthritis. Rheum., 38, 969-975,1995.

(6) K. Yokouchi et al.: J. New Rem. & Clin., 50, 215-221, 2001.

Safety Data Sheets:

Product name:	Sulfuric acid CAS No. 7664-93-9
Composition:	Sulfuric acid solution.
Hazards identification:	Harmful if inhaled the vapour for long time, contacted with eyes and skin and swallowed.
First aid measures:	In case of contact, immediately flush eyes or skin with copious amounts of water for at least 15 min while removing contaminated clothing and shoes and seek medical attention. If inhaled, remove to fresh air and seek medical attention. If swallowed, immediately wash mouth with copious amounts of water and seek medical attention as soon as possible.
Fire fighting measures:	Water, dry chemical powder and foam.
Accidental release:	Recover as much as possible. In treatment, wear suitable protective equipment; laboratory coats, safety glasses, cap, gloves and shoes.
Handling and storage:	Wear suitable protective clothing including laboratory overalls, safety glasses, cap, gloves and shoes. Avoid contact with eyes, skin or clothing. Do not breathe the vapour. Wash thoroughly after handling. Store without other reagents and organic solutions. Keep well closed and protected from light.
Physical and chemical properties:	Boiling point: 279 °C (93.19%). Density: 1.833 (92.97%) Melting point: -32.0 °C (93.10%). Freezing point: -29.4 °C (93.19%).
Stability and reactivity:	Sulfur dioxide is generated by heat. The solution temperature is increased when mixed with water.
Toxicological information:	Corrosive to all body tissues. Inhalation of concentrated vapour may cause serious lung damage. Contact with eyes may result in total loss of vision. LD_{50}: 2,140mg/kg oral, rat.
Ecological information:	Not applicable.
Disposal consideration:	Neutralize with slaked lime, sodium bicarbonate or sodium hydroxide etc.
Transport information:	No special considerations applicable.
Regulatory information:	The information contained in this safety data sheet is based on published sources and is believed to be correct. It should be used as a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.

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