MMP-3 (Matrix Metalloproteinase) ELISA

For the quantitative determination of MMP-3 in human serum

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 48-MP3HU-E01
Size: 96 wells
Preparation of Working Reagents:

<table>
<thead>
<tr>
<th>Working Reagents</th>
<th>Preparation</th>
<th>Stability after preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. MMP-3 Standards</td>
<td>Reconstitute 1, 2, 3, 4 and 5 with 1 mL of Assay Buffer, respectively.</td>
<td>1 month at 2-8°C</td>
</tr>
<tr>
<td>2. Anti-MMP-3 Conjugate Antibody</td>
<td>Add 1 mL of Anti-MMP-3 Conjugate Concentrate to 10 mL of Assay Buffer.</td>
<td>1 month at 2-8°C</td>
</tr>
<tr>
<td>3. Wash Buffer</td>
<td>Dilute all (50mL) of Wash Buffer Concentrate with 450 mL of deionized water.</td>
<td>1 month at 2-8°C</td>
</tr>
</tbody>
</table>

Other reagents: Use according to the directions for Assay Procedure. 12 months at 2-8°C

### Assay Procedures:

1. **Summary**

   - **Dilution of sample**
     - Microplate: 1 well
     - Assay Buffer: 160 µL
     - MMP-3 Standard: 40 µL

2. **Flow diagram of assay procedure**

   - **Dilution of sample or standard solution**
     - (1) Pipet 160 µL of the Assay Buffer into Microplate.
     - (2) Pipet 40 µL of sample or MMP-3 Standard and mix well.
     - (3) Pipet 120 µL of Anti-MMP-3 Coated Microplate.
     - (4) Pipet 40 µL of diluted MMP-3 Standard/Buffer Solution alone for a concentration of 0.0 ng/mL and sample into their respective microtiter (all measurements are done in duplicate), and mix thoroughly.
     - (5) Allow the plate to incubate at 20 - 30°C for exactly 90 min.
     - (6) Remove the reaction solution by aspirate, add 350 µL of Wash Buffer, and remove with aspiration. Repeat this operation three more times.
     - (7) Pipet 100 µL of Coloring Solution at regular intervals to stop the enzyme reaction. Allow to incubate at 20 - 30°C for exactly 30 min.
     - (8) Pipet 100 µL of Stop Solution at regular intervals to stop the enzyme reaction and read the absorbance at 450 nm.

### Reagents: One kit contains:

<table>
<thead>
<tr>
<th>No.</th>
<th>Reagent</th>
<th>Form</th>
<th>Specifications</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anti-MMP-3 Coated Microplate</td>
<td>Dried</td>
<td>1 plate x 96 wells</td>
<td>Mouse anti-MMP-3 antibody coated plate.</td>
</tr>
<tr>
<td>2</td>
<td>Anti-MMP-3 Antibody solution</td>
<td>Concentrated</td>
<td>1 vial x 2 mL.</td>
<td>A vial contains horseradish peroxidase-labeled mouse anti-MMP-3 antibodies.</td>
</tr>
<tr>
<td>3</td>
<td>Coloring Solution</td>
<td>Liquid</td>
<td>1 vial x 15 mL.</td>
<td>A vial contains TMB and H₂O₂.</td>
</tr>
<tr>
<td>4</td>
<td>Stop Solution</td>
<td>Liquid</td>
<td>1 vial x 15 mL.</td>
<td>1 mol/L sulfuric acid.</td>
</tr>
<tr>
<td>5</td>
<td>Assay Buffer</td>
<td>Liquid</td>
<td>2 vials x 25 mL.</td>
<td>Na-Phosphate Buffer.</td>
</tr>
<tr>
<td>6</td>
<td>MMP-3 Standard 1 (Lyophilized)</td>
<td>Lyophilized</td>
<td>1 vial x for 1 mL.</td>
<td>A vial contains 12.5 ng of MMP-3.</td>
</tr>
<tr>
<td>7</td>
<td>MMP-3 Standard 2 (Lyophilized)</td>
<td>Lyophilized</td>
<td>1 vial x for 1 mL.</td>
<td>A vial contains 50 ng of MMP-3.</td>
</tr>
<tr>
<td>8</td>
<td>MMP-3 Standard 3 (Lyophilized)</td>
<td>Lyophilized</td>
<td>1 vial x for 1 mL.</td>
<td>A vial contains 200 ng of MMP-3.</td>
</tr>
<tr>
<td>9</td>
<td>MMP-3 Standard 4 (Lyophilized)</td>
<td>Lyophilized</td>
<td>1 vial x for 1 mL.</td>
<td>A vial contains 400 ng of MMP-3.</td>
</tr>
<tr>
<td>10</td>
<td>MMP-3 Standard 5 (Lyophilized)</td>
<td>Lyophilized</td>
<td>1 vial x for 1 mL.</td>
<td>A vial contains 800 ng of MMP-3.</td>
</tr>
<tr>
<td>11</td>
<td>Wash Buffer Concentrate</td>
<td>Concentrated</td>
<td>2 vials x 50 mL.</td>
<td>Na-Phosphate Buffer.</td>
</tr>
</tbody>
</table>

### Adaptation:

Determination of human MMP-3 in human serum.

### Safety Warning and Handling Precautions:

**Warning:** Not recommended or intended for diagnosis of disease in human or animals. Do not use internally or externally in human or animals. All chemicals should be handled as if they were potentially hazardous. All samples used should be handled as if they were potentially infectious. Wear suitable protective clothing such as laboratory coat, 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 generous amounts of water.

### Principles of the Test:

This test quantitates MMP-3 by a one-step sandwich ELISA method. MMP-3 in the sample reacts with anti-MMP-3 antibody coated wells (solid phase) and enzyme-labeled antibody in the first reaction. MMP-3 molecule is sandwiched with 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; the concentration of samples can be determined from the standard curve.

### Materials required but not provided:

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

### 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 IV collagen. MMP-3 is secreted in a latent form and activated out of membrane vesicles to participate in metabolism of matrix tissue. Activity of MMP-3 is inhibited specifically by tissue inhibitors of metalloproteases (TIMPs). This MMP-3 plate kit is based on one-step sandwich enzyme immunoassay using two different mouse anti-MMP-3 monoclonal antibodies.
Calculation of MMP-3 Concentration:
(1) The MMP-3 concentration (ng/mL) is plotted as abscissa and the absorbance as ordinate, on graph paper.
(2) Plot the net absorbance value for each dilution level, obtained by subtracting the value for 0 ng/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 sample, 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 numbers.
(4) Use fresh sera for samples.
(5) If it is impossible to carry out assay immediately after the separation of sera, keep samples 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 repeated freezing and thawing of samples.
(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, the sample should be diluted with Assay Buffer and then retested.
(9) Possible interferences: EDTA in samples inhibits the immunoreaction.

Specific Performance Characteristics:
This test kit, when used by the prescribed Assay Procedure, exhibits the following specific performance characteristics.

(1) Sensitivity
When determined from the standard curve, the net absorbance obtained by subtracting the value for 0 ng/mL standard solution from the value for individual dilution was from 0.010 to 0.075 for 12.5 ng/mL and from 1.4 to 3.8 for 800 ng/mL.

(2) Specificity
When a control sample was measured, the concentration was was the range of 100 ± 20 % of its known value.

(3) Reproducibility
When the same sample was measured simultaneously 4 times in duplicate, the coefficient of variance for the measured values was less than 10%.

(4) Assay Range:
12.5 to 800 ng/mL.

Storage:
The kit should be stored at 2-8°C.

Expiration Date:
The kit must be used within 12 months after manufacture (see the expiry date given on the box and labels).

References:

Material Safety Data Sheet Information:
Product name: sulfamic acid CAS No. 7664-93-9
Composition: sulfamic acid solution (Stop Solution in kit is 1 mol/L)
Hazard identification: Harmful if vapour inhaled for long time; if contact occurs with eyes or skin, or if 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 coat, safety glasses, 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. LD₅₀: 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.
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.