CHEMICON®
INTERNATIONAL
a Serologicals® Company

Human MMP-2 Immunoassay Kit

Cat. No. ECM492

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

USA & Canada
Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000 • Germany +49-6192-207300 • ISO Registered Worldwide
www.chemicon.com • custserv@chemicon.com • techserv@chemicon.com

Introduction

Matrix metalloproteinase 2 (72 kDa gelatinase, gelatinase A, MMP-2) has been identified in a number of tissues, cells and blood. The precursor form of MMP-2 (pro-MMP-2) is tightly associated with tissue inhibitor of metalloproteinases-2 (TIMP-2) and regulated autoactivation.

Pro-MMP-2 may be quantitated using this sensitive immunoassay against MMP-2

Test Principle

The MMP-2 immunoassay kit utilizes antibodies immobilized on a bead matrix, in combination with enzyme-labeled antibodies, directed against different antigenic sites on the same MMP-2 molecule. Upon addition of an MMP-2-containing specimen, the result is an MMP-2 molecule being sandwiched between the solid phase and enzyme labeled antibodies. After removing unbound enzyme-labeled antibody, the bead containing the sandwich is incubated with enzyme substrate and *o*-phenylenediamine, resulting in the development of color. The activity of peroxidase enzyme is proportional to the amount of antigen, MMP-2, so that MMP-2 concentration in specimens can be determined from a standard curve.

Application

The MMP-2 immunoassay kit is useful for the determination of MMP-2 (pro-MMP-2) levels in fresh human sera.

The values measured by using this kit represent pro-MMP-2 concentrations, because pro-MMP-2 is used as a standard in this kit. This system recognizes both free pro-MMP-2 and pro-MMP-2 complexed with TIMP-2. It does not recognize active MMP-2. Contents of this kit are sufficient for assay of 100 samples, including standard curve. Testing of samples in duplicate or triplicate is strongly recommended. This kit is intended for research use only; not for diagnostic or therapeutic applications.

Analytical Sensitivity and Detection Limits

Sensitivity:	6.3 ng/mL
Range of Detection:	6.3 ng/mL to 400 ng/mL
Typical values:	570 ng/mL ± 118 ng/mL.

Storage

Store kit at 2-8°C.

Kit Components

1. Anti-MMP-2 Coated Beads - One (1) bottle containing 100 anti-MMP-2 coated polystyrene beads.
2. Concentrated Enzyme Labeled Antibody Solution - One (1) 1.2 mL bottle containing MMP-2 antibody.
3. Color Reagent (Lyophilized) –Four (4) bottles *o*-Phenylenediamine (OPD), each sufficient to make one 12 mL bottle of color reagent.
4. Substrate Solution - One (1) 50 mL bottle 0.02% Hydrogen Peroxide.
5. Stop Solution - Two (2) bottles, sufficient to make 100 mL 1.3 N Sulfuric acid per bottle.
6. Buffer Reagent (Lyophilized) - One (1) 50 mL bottle BSA in sodium phosphate buffer.
7. MMP-2 Standard - Two (2) bottles, sufficient to make 0.5 mL Human pro-MMP-2 per bottle.
8. Concentrated Washing Solution - Two bottles, sufficient to make 1L Sodium phosphate buffer, pH 7.0, per bottle.

Materials Not Supplied

- Pipette (10 mL)
- Pipettors & tips capable of accurately measuring 50-1000 µL
- Dispensor (3 mL)
- Test tubes (internal diameter 10-13 mm; length 60-75 mm)
- Aspirator
- Graduated cylinder (50 mL, 1000 mL)
- Spectrophotometer (492 nm)

Precautions

- All specimens should be handled as potentially infectious.
- Do not freeze reagents
- Do not combine reagents from different lots.
- Stop solution (1.3 N Sulfuric acid) should be handled with caution.
- Do not allow Coloring or Stop Solution to come into contact with metal.
- Do not use sera which were frequently frozen and thawed.
- All reagents should be brought to ambient temperature before use. Gently stir each bottle of liquid reagent. Do not shake reagent bottles.
- Use pipette with disposable tips for pipetting standards and specimens.

Preparation of Reagents

1. Buffer Solution

   Add 50 mL of deionized water to Buffer Reagent. Stable for 1 month at 2-8°C.

2. Standard Solution

   Add 500 µL of Buffer Solution I to MMP-2 Standard to obtain a concentration of 400 ng/mL. At this concentration, solution is stable for 1 month at 2-8°C.

3. Labeling of Standard Curve Tubes

   Label 8 tubes with the following values: 400 (Standard Solution in original vial), 200, 100, 50, 25, 12.5, 6.3, and 0, to represent MMP-2 ng/mL concentrations for the MMP-2 standard curve.

4. Serial Dilutions for Standard Curve

   a) Fill tubes 200, 100, 50, 25, 12.5, 6.3 and 0 with 150 µL of Buffer Solution in each tube.

   b) Remove 150 µL of 400 ng/mL MMP-2 solution from Standard Solution Vial and place into tube "200" which already contains 150 µL Buffer Solution. Mix briefly by vortexing.

   c) Remove 150 µL of 200 ng/mL MMP-2 solution from "200" tube and place into tube "100". Mix briefly by vortexing.

   d) Remove 150 µL of 100 ng/mL MMP-2 solution from "100" tube and place into tube "50". Mix briefly by vortexing.

   e) Remove 150 µL of 50 ng/mL MMP-2 solution from "50" tube and place into tube "25". Mix briefly by vortexing.

   f) Remove 150 µL of 25 ng/mL MMP-2 solution from "25" tube and place into tube "12.5". Mix briefly by vortexing.

   g) Remove 150 µL of 12.5 ng/mL MMP-2 solution from "12.5" tube and place into tube "6.3". Mix briefly by vortexing.

   Important Note: *Tube "0" will contain only buffer solution. Do not transfer any quantity of MMP-2 into tube "0".*

5. Enzyme-Labeled Antibody Solution
   Add 1 mL Concentrated Enzyme-Labeled Antibody Solution to 35 mL Buffer Solution. This solution is stable for 1 month at 2-8°C.

6. Coloring Solution

   Add 12 mL Substrate Solution to Color Reagent. This solution is stable for 3 hours 2-8°C.

7. Washing Solution

   Add 100 mL Concentrated Washing solution to 900 mL deionized water. Total volume will be 1000 mL. This solution is stable for 1 month at 2-8°C.

Assay Instructions

1. Pipette 50 µL of each Standard Curve Solution or Specimen into the bottom of a test tube. If necessary, dilute specimens with Washing Solution. If using human serum as a specimen, dilute samples 1:9 with Washing Solution before adding 50 µL into a test tube.
2. Pipette 300 µL Enzyme Labeled Antibody Solution into each specimen- or standard-containing tube.
3. Using clean forceps, remove a bead out of its bottle, allowing the excess solution to drain back into the bottle. Place one anti-MMP-2 coated bead into each tube at regularly timed intervals.

   Note*:* *Anti-MMP-2 beads must be combined as above within 60 minutes after mixing specimen and Enzyme Labeled Antibody Solution.*

4. Incubate the tubes at room temperature for 1 hour.
5. Stop the reaction by adding 3.0 mL Washing Solution to each tube (at the same regularly timed intervals).
6. Aspirate the solution and dispense 3.0 mL Washing Solution to each tube. Repeat this washing step 3 times.
7. Transfer each washed bead into a clean fresh tube.
8. Pipette 300 µL Coloring Solution into each tube at regularly timed intervals.
9. Incubate the tubes at room temperature for 30 min.
10. Stop the enzyme reaction by adding 1.5 mL of Stop Solution to each tube.

11. Using deionized water as a blank, read the absorbance at 492 nm (A_{492}) for the Standard Curve Solutions and Specimens.

Calculation of Results

1) Plot the net absorbance value for each MMP-2 concentration, subtracting the value for the buffer solution alone (0 ng/mL) from the value for individual dilutions. Plot net absorbance values as the ordinate versus the MMP-2 concentration (from 6.3 to 400 ng/mL) as abscissa on graph paper.
2) Draw a smooth curve that fits the 7 plotted points.
3) Using the net absorbance value for a specimen, determine the MMP-2 concentration from the standard curve, and multiply the ng/mL value by any additional dilution of the specimen.

Reference

Fujimoto, N., et al. *Clin Chim Acta.* 1993;221, 91-103.

Manufactured by Fuji Chemical Industries, Ltd. Not available for sale in Japan.

Warranty

These products are warranted to perform as described in their labeling and in CHEMICON® literature when used in accordance with their instructions. THERE ARE NO WARRANTIES, WHICH EXTEND BEYOND THIS EXPRESSED WARRANTY AND CHEMICON® DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CHEMICON®'*s* sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CHEMICON®, to repair or replace the products. In no event shall CHEMICON® be liable for any proximate, incidental or consequential damages in connection with the products.

Cat No. ECM 492

October 2004
Revision D: 40734