KAMIYA BIOMEDICAL COMPANY

Human MMP-3 ELISA

For the quantitative determination of MMP-3 in human serum.

Cat. No. KT-452

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** Human MMP-3 ELISA is for the quantitative determination of MMP-3 in human serum.

PRINCIPLE
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 metalloproteinases (TIMPs). This kit is based on a one-step sandwich enzyme immunoassay using two different mouse anti-MMP-3 monoclonal antibodies. MMP-3 in the specimen reacts 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 calibration curve.

COMPONENTS
- Anti-MMP-3 coated microplate: mouse anti-MMP-3 antibody coated plate, 96-wells
- HRP Conjugate Concentrate: horseradish peroxidase-labeled mouse anti-MMP-3 antibodies, 2 mL
- Coloring solution: TMB and H_2O_2, 15 mL
- Stop Solution: 1M Sulfuric Acid, 15 mL
- Assay Buffer: Na-Phosphate Buffer, 2 x 25 mL
- MMP-3 Calibrators: Lyophilized (12.5, 50, 200, 400, and 800 ng), 1 mL each
- Wash Buffer Concentrate: Na-Phosphate Buffer, 2 x 50 mL

Materials or Equipment required but not provided
- Microplate (U-bottom recommended)
- Graduated cylinders (1,000 mL)
- Micropipettes (40, 100, 120, 160, 1,000 µL)
- Pipettes (10 mL)
- Microplate washer
- Microtiter plate reader (450 nm)

PREPARATION OF REAGENTS
A. MMP-3 Calibrators
Reconstitute each of the 5 lyophilized calibrators with 1 mL of assay buffer. Stable for 1 month at 4℃.

B. HRP Conjugate
Add 1 mL of HRP Conjugate Concentrate to 10 mL of assay buffer. Stable for 1 month at 4℃.

C. Wash Buffer
Dilute the entire 50 mL of Wash Buffer Concentrate with 450 mL of de-ionized water. Stable for 1 month at 4℃.

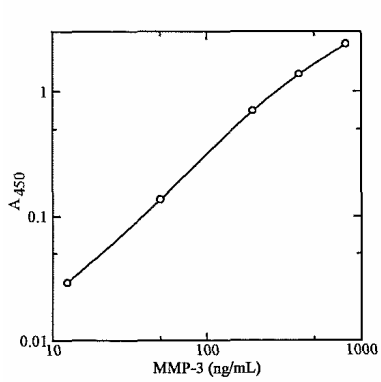
D. Other Reagents
Use according to the directions outlined in the procedure below. Stable for 12 months at 4℃.

PROCEDURE

1. Pipette 160 µL of the Assay Buffer into the necessary wells of the uncoated microplate.
2. Pipette 40 µL of specimen or MMP-3 Calibrator into the necessary wells of the uncoated microplate and mix well.
3. Pipette 120 µL of the HRP Conjugate into Anti-MMP-3 coated microplate.
4. Pipette 40 µL of diluted MMP-3 calibrator (Use buffer solution alone as 0 ng/mL calibrator) and specimen into their respective microwells. Mix thoroughly. It is recommended to do measurements in duplicate.
5. Allow the plate to incubate at 20-30℃ for 90 mins.
6. Remove the reaction solution by suction. Add 350 µL of wash buffer, and remove by suction. Repeat this step for a total of 4 times.
7. Pipette 100 µL of coloring solution at regular intervals.
8. Allow the plate to incubate at 20-30℃ for 30 mins.
9. Pipette 100 µL of stop solution at regular intervals to stop the enzyme reaction.
10. Read the absorbance at 450 nm.

CALCULATION OF RESULTS

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 the 0 ng/mL calibrator solution (mean of 2 measurements) from the values for the individual dilutions (mean of 2 measurements), against the MMP-3 concentration to prepare the calibration curve.
3. Using the net absorbance value for a specimen, determine the corresponding MMP-3 concentration from the calibration curve.



PERFORMANCE CHARACTERISTICS

1. **Sensitivity**: When determined from the calibration curve, the net absorbance obtained by subtracting the value for the 0 ng/mL calibrator solution from the value for individual dilution is 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 specimen is measured, the concentration is in the range of 100 ± 20% of its known value.
3. **Reproducibility**: When the 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

TECHNICAL HINTS

- Reactions may be somewhat influenced by times, temperature and other factors, therefore, a calibration curve should be prepared at each time of the assay.
- All measurements should be made in duplicate.
- Do no mix reagents from different kits or lot numbers.
- Use fresh serum as specimens.
- If it is impossible to carry out the assay immediately after the separation of serum, they are stable for 1 week when stored at 4℃ and for 1 year at -40℃.

- Avoid repeat cycles of freezing and thawing for specimens.
- All reagents must be allowed to equilibrate to the reaction temperature before use.
- When the upper limit of the determination range is exceeded, the specimen should be diluted with buffer solution and then retested.
- Possible Interferences:
  EDTA in specimens inhibits the immunoreaction.

STORAGE

Store at 4℃.

FOR RESEARCH USE ONLY

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