MMP1 Human ELISA Kit

Cat. No.:DEIA1577
Pkg.Size:96T

Intended use

For the quantitative measurement of Human MMP1 pro and active forms in serum, plasma, cell culture supernatants and urine.

General Description

Interstitial collagenase also known as matrix metalloproteinase-1 (MMP-1) and fibroblast collagenase is an enzyme that in humans is encoded by the MMP1 gene. Human Fibroblast Collagenase (MMP-1) was the first vertebrate collagenase both purified to homogeneity as a protein, and cloned as a cDNA.

Principle Of The Test

This assay employs an antibody specific for Human MMP1 coated on a 96-well plate. Standards and samples are pipetted into the wells and MMP1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Human MMP1 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of MMP1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents And Materials Provided

• MMP1 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-Human MMP1.
• Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution
• Standards (Item C): 2 vials, recombinant Human MMP1.
• Assay Diluent (Item E): 15 ml of 5x concentrated buffered. For Standard/Sample (serum/plasma/cell culture medium/urine) diluent.
• Detection Antibody MMP1 (Item F): 2 vial of biotinylated anti- Human MMP1 (each vial is enough to assay half microplate).
• HRP-Streptavidin Concentrate (Item G): 200 µl 440x concentrated HRP-conjugated streptavidin.
• TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3’,5,5’- tetramethylbenzidine (TMB) in buffered solution.
• Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.

Materials Required But Not Supplied

• 1 Microplate reader capable of measuring absorbance at 450nm.
• Precision pipettes to deliver 2 µl to 1 ml volumes.
• Adjustable 1-25 ml pipettes for reagent preparation.
• 100 ml and 1 liter graduated cylinders.
• Absorbent paper.
• Distilled or deionized water.
• Log-log graph paper or computer and software for ELISA data analysis.
• 8 Tubes to prepare standard or sample dilutions.

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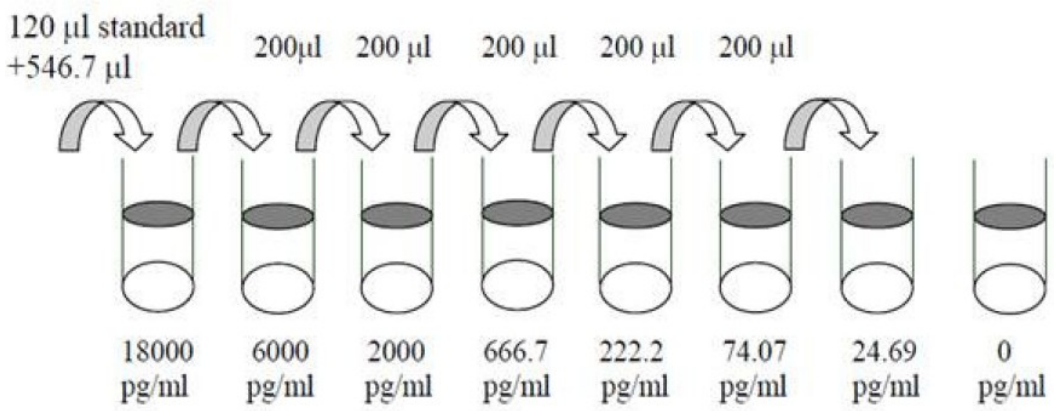
Storage

This kit may be stored for up to 6 months at 2 to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at –80°C) after reconstitution. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Sample dilution: If your samples need to be diluted, Assay Diluent (Item E) is used for dilution of serum/plasma/culture supernatants/urine.
Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.
3. Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use.
4. Preparation of standard: Briefly spin the vial of Item C and then add 400 µl 1x Assay Diluent (Item E) into Item C vial to prepare a 0.1 µg/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 120 µl MMP1 standard from the vial of item C, into a tube with 546.7 µl 1x Assay Diluent Buffer (for serum/plasma/cell culture medium/urine) to prepare an 18000 pg/ml stock standard solution. Pipette 400µl 1x Assay Diluent into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Gently vortex to mix. 1x Assay Diluent serves as the zero standard (0 pg/ml).
table 1.



5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent and used in step 4 of Part 7 Assay Method.
7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 440-fold with 1x Assay Diluent.
For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 25 µl of HRP-Streptavidin concentrate into a tube with 11 ml 1x Assay Diluent to prepare a final 440 fold diluted HRP-Streptavidin solution.

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Assay Steps

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard (see Preparation of Reagents step 4) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of 1x prepared biotinylated antibody (see Preparation of Reagents step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µl of prepared Streptavidin solution (see Preparation of Reagents step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Calculation

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Standard Curve

These standard curves are for demonstration only. A standard curve must be run with each assay.

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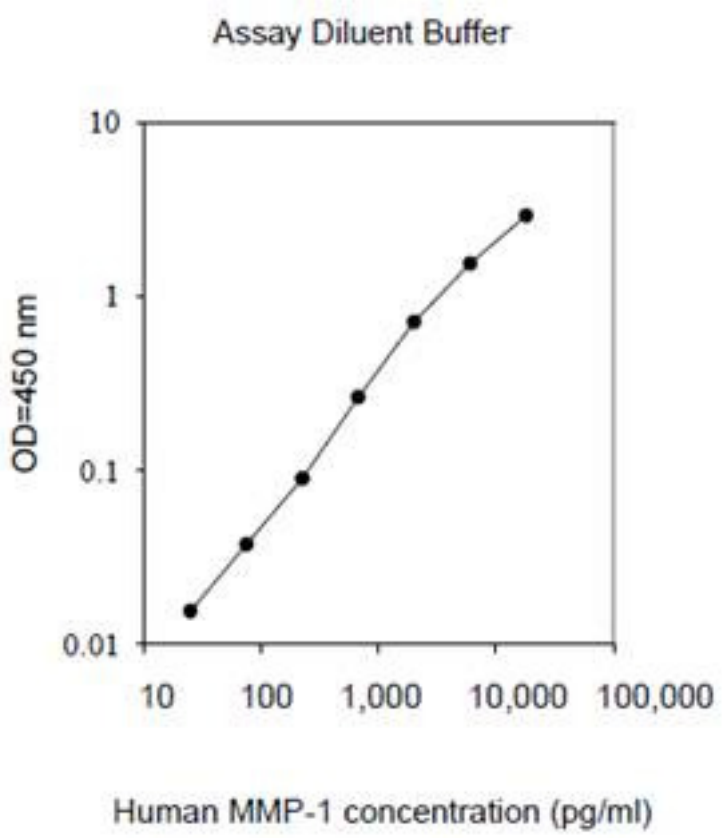

Detection Range

24.69 pg/ml - 18000 pg/ml

Sensitivity

The minimum detectable dose of MMP1 is typically less than 8 pg/ml.

Specificity

No detectable cross-reactivity with any other cytokine.

Linearity

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Sample	Type	Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	98	99	98
	Range (%)	90-106	89-106	89-107
1:4	Average % of Expected	97	95	96
	Range (%)	90-106	89-107	90-107
1:8	Average % of Expected	95	96	94
	Range (%)	88-108	90-106	90-109

Recovery

Recovery was determined by spiking various levels of Human MMP1 into Human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	97.78	88-106
Plasma	99.59	90-107
Cell culture media	98.66	90-106

Reproducibility

Intra-Assay: CV<10%
Inter-Assay: CV<12%

Analyte Gene Information

Gene Name MMP1 matrix metallopeptidase 1 (interstitial collagenase) [Homo sapiens]

Official Symbol MMP1

Synonyms MMP1; matrix metallopeptidase 1 (interstitial collagenase); CLG, matrix metalloproteinase 1 (interstitial collagenase); interstitial collagenase; fibroblast collagenase; matrix metalloprotease 1; matrix metalloproteinase 1; CLG; CLGN;

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GeneID	4312
mRNA Refseq	NM_001145938
Protein Refseq	NP_001139410
UniProt ID	P03956
Chromosome Location	11q21-q22
Pathway	Activation of Matrix Metalloproteinases, organism-specific biosystem; Androgen Receptor Signaling Pathway, organism-specific biosystem; Basigin interactions, organism-specific biosystem; Bladder cancer, organism-specific biosystem; Bladder cancer, conserved biosystem; Cell surface interactions at the vascular wall, organism-specific biosystem; Degradation of the extracellular matrix, organism-specific biosystem;
Function	calcium ion binding; metalloendopeptidase activity; peptidase activity; zinc ion binding;

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