ELISA Kit
Catalog #KAC1541

Human
MMP-3

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INTRODUCTION

Matrix Metalloproteinase-3 (MMP-3), also called stromelysin-1 is a member of the matrix metalloproteinase family. MMP-3 is capable of degrading many components of the connective tissue matrix including proteoglycan, link-protein, type II, IV, IX and XI collagens, laminin and fibronectin. In addition to its direct action, MMP-3 may indirectly affect the degradation of the extacellular matrix by activating procollagenase-1.

MMP-3 is secreted as a latent proenzyme of 57 kDa, which is N-glycosylated to a minor form of 59 kDa. MMP-3 is activated *in vivo* by limited proteolysis with tissue or plasma endopeptidases, and *in vitro* by mercurials (e.g., 4-aminophenylmercuricacetate, APMA) or proteinases (e.g., trypsin and plasmin).

MMP-3 activity is regulated at the level of gene expression and also post-translationally in the extracellular space. Active MMP-3 is inhibited by Tissue Inhibitors of Matrix metalloProteinases (TIMP) which interact with active MMP-3 with a 1:1 stoichiometry. MMP-3 activity can also be inhibited by α2-macroglobulin.

MMP-3 is thought to play a major role in natural processes of tissue remodeling and in pathological states, such as osteoarthritis and rheumatoid arthritis. The balance between MMP-3 and TIMPs is thought to be an important determinant of matrix breakdown.

Typically, MMP-3 is assayed by zymography or enzymatic methods using substrates such as casein or transferrin. These methods, however, have several disadvantages. They are not specific, require the activation of the pro-MMP-3, and do not recognize the enzyme-inhibitor complexes. Therefore, only the free active enzyme is detected. Several immunoassays for the determination of MMP-3 have now been described.

PURPOSE

The Invitrogen Human MMP-3 (Hu MMP-3) ELISA is to be used for the quantitative determination of Hu MMP-3 in human serum, heparinized plasma, synovial fluid, buffered solution or cell culture medium. The assay measures total Hu MMP-3 (pro-MMP-3, activated MMP-3 and MMP-3 in complex with TIMP-1 or TIMP-2) The assay also recognizes recombinant forms of Hu MMP-3.

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Read entire protocol before use.

PRINCIPLE OF THE METHOD

The Invitrogen Hu MMP-3 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Hu MMP-3 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Hu MMP-3 content, control specimens, and unknowns are pipetted into these wells followed by the addition of a second biotinylated monoclonal antibody.

During the first incubation, the Hu MMP-3 antigen binds to the immobilized (capture) antibody on one site and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Hu MMP-3 present in the original specimen.

REAGENTS PROVIDED

Note: *Store all reagents at 2 to 8°C.*

Reagents	***96 Test Kit***
Hu MMP-3 Standard, purified natural pro-MMP-3. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer. Contains 3.3 mM thymol; 25 mL per bottle.	2 bottles
Hu MMP-3 Antibody-Coated Wells, 96 wells per plate.	1 plate
Hu MMP-3 Biotin Conjugate, (Biotin-labeled anti-*Hu MMP-3*). Contains 3.3 mM thymol; 5.5 mL per bottle.	1 bottle
Incubation Buffer. Contains 3.3 mM thymol; 11 mL per bottle.	1 bottle
Streptavidin-Peroxidase (HRP), (100x) concentrate. Contains 3.3 mM thymol; 0.125 mL per bottle.	1 bottle
Streptavidin-Peroxidase (HRP) Diluent. Contains 3.3 mM thymol and 0.05% Proclin® 300; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25x); 100 mL per bottle.	1 bottle
Stabilized Chromogen, Tetramethylbenzidine (TMB); 25 mL per bottle.	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	3

Disposal Note: This kit contains materials with small quantities of Proclin® 300. Proclin® 300 is toxic. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
6. Glass or plastic tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.

3. The Hu MMP-3 ELISA kit may be used to measure Hu MMP-3 in serum, heparinized plasma, synovial fluid, buffered solutions and cell culture samples. **EDTA and citrate plasma are not recommended in this assay.**
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. Serum/plasma/synovial fluid and cell culture samples that are >20 ng/mL should be diluted with *Standard Diluent Buffer*. We recommend that each laboratory establish its own dilution range. For guidance, see **EXPECTED VALUES**.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. **Do not mix or interchange different reagent lots from various kit lots**.
11. Do not use reagents after the kit expiration date.
12. Read absorbances within 2 hours of assay completion.
13. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.

14. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
15. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also, avoid contact between *Stabilized Chromogen* and metal to prevent color development.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed.

REAGENT PREPARATION AND STORAGE

A. Reconstitution and Dilution of Hu MMP-3 Standard

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute the standard to 200 ng/mL with *Standard Diluent Buffer*. Refer to the standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use the standard within 1 hour of reconstitution.
2. Add 0.05 mL of the reconstituted standard to a tube containing 0.450 mL *Standard Diluent Buffer*. Label as 20 ng/mL Hu MMP-3. Mix.
3. Add 0.200 mL of *Standard Diluent Buffer* to each of 5 tubes labeled 10, 5, 2.5, 1.25 and 0.62 ng/mL Hu MMP-3.
4. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of Hu MMP-3 Standard

Standard:	Add:	Into:
20 ng/mL	Prepare as described in Step 2.	
10 ng/mL	0.200 mL of the 20 ng/mL std.	0.200 mL of the Diluent Buffer
5 ng/mL	0.200mL of the 10 ng/mL std.	0.200 mL of the Diluent Buffer
2.5 ng/mL	0.200 mL of the 5 ng/mL std.	0.200 mL of the Diluent Buffer
1.25 ng/mL	0.200 mL of the 2.5 ng/mL std.	0.200 mL of the Diluent Buffer
0.62 ng/mL	0.200 mL of the 1.25 ng/mL std.	0.200 mL of the Diluent Buffer
0 ng/mL	0.200 mL of the Diluent Buffer	An empty tube

Discard all remaining reconstituted and diluted standards after completing the assay. Return the *Standard Diluent Buffer* to the refrigerator.

C. Storage and Final Dilution of Streptavidin-HRP

1. Dilute 10 µL of this 100x concentrated solution with 1 mL of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Streptavidin-HRP Concentrate	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused *Streptavidin-HRP* concentrate to the refrigerator.

D. Dilution of Wash Buffer

Allow the 25x concentrate to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the 25x wash buffer concentrate with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters; 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Dilute serum/plasma/synovial fluid and culture samples with *Standard Diluent Buffer*. We recommend that each laboratory establish its own dilution range. For guidance, see **EXPECTED VALUES**.
3. Add 50 µL of *Incubation Buffer* into each well of the microplate. Well(s) reserved for chromogen blank should be left empty.
4. Add 50 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
5. Add 50 µL of standards or samples to the appropriate microtiter wells. (See **REAGENT PREPARATION AND STORAGE**, Section B.)
6. Pipette 50 µL of biotinylated anti-Hu MMP-3 (*Biotin Conjugate*) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
7. Cover plate with a *plate cover* and incubate for **2 hours at room temperature**.

8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
9. Add 100 μL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE**, Section C.)
10. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
11. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
12. Add 100 μL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
13. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
14. Add 100 μL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.

15. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
16. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, a four parameter algorithm provides the best curve fit.
17. Read the Hu MMP-3 concentrations for unknown samples and controls from the standard curve plotted in step 16. (Samples producing signals greater than that of the highest standard (20 ng/mL) should be diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 20 ng/mL Hu MMP-3.

Standard Hu MMP-3 (ng/mL)	Optical Density (450 nm)
0	0.042
	0.038
0.62	0.081
	0.071
1.25	0.134
	0.142
2.5	0.278
	0.291
5	0.811
	0.840
10	1.693
	1.699
20	3.011
	3.080

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 20 ng/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >20 ng/mL with *Standard Diluent Buffer*, reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Hu MMP-3 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of Hu MMP-3 is <0.1 ng/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 24 times.

PRECISION

1. Intra-Assay Precision

Samples of known Hu MMP-3 concentration were assayed in replicates of 24 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	2.45	5.6	11.4
SD	0.12	0.16	0.46
%CV	4.9	2.8	4.0

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 40 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	3.9	8.4	16.3
SD	0.22	0.36	1.06
%CV	5.6	4.3	6.5

SD = Standard Deviation
CV = Coefficient of Variation

LINEARITY OF DILUTION

Human serum, heparinized plasma, synovial fluid and cell culture samples were serially diluted in *Standard Diluent Buffer* over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded an average correlation coefficient of 0.99.

	Heparinized plasma			**Serum**		
Dilution	**Measured (ng/mL)**	**Expected (ng/mL)**	**% Expected**	**Measured (ng/mL)**	**Expected (ng/mL)**	**% Expected**
neat	13.4	-	-	5.7	-	-
1/2	7	6.7	104	3.1	2.85	109
1/4	3.6	3.4	106	1.5	1.4	107
1/8	1.8	1.7	106	0.75	0.71	105
1/16	0.95	0.84	113			

	Synovial fluid		
Dilution	**Measured (ng/mL)**	**Expected (ng/mL)**	**% Expected**
1/120	17.2	-	-
1/240	8.4	8.6	97
1/480	4.1	4.3	95
1/960	2.3	2.15	106
1/1920	1.2	1.08	111
1/3840	0.52	0.54	96

Dilution	Cell Culture		
	Measured (ng/mL)	**Expected (ng/mL)**	**% Expected**
1/1	16.8	-	-
1/2	8.8	8.4	104
1/4	4.3	4.2	102
1/8	2.3	2.1	109
1/16	1.04	1.05	99

RECOVERY

The recovery of Hu MMP-3 added to human serum, heparinized plasma and synovial fluid averaged 105%, 97.6% and 96%, respectively. The recovery of Hu MMP-3 added to tissue culture medium containing both 1% and 10% fetal calf serum averaged 90%.

SPECIFICITY

Cross reactivity was determined by addition of different matrix metalloproteinases to 0, 5 or 10 ng/mL of pro-MMP-3 and the apparent pro-MMP-3 was measured.

Added pro-MMPs (1000 ng/mL) to Pro-MMP-3 samples	Observed Values for 0 ng/mL Pro-MMP-3	Observed Values for 5 ng/mL Pro-MMP-3	Observed Values for 10 ng/mL Pro-MMP-3
Hu. Pro-MMP-1	0	4.8	10.4
Hu. Pro-MMP-2	0	4.6	10.2
Hu. Pro-MMP-9	0	4.9	10.5

The reactivities of free active MMP-3 and active MMP-3 complexed with TIMP1, TIMP2 or alpha-2-macroglobulin (α2-macro) are tabulated below:

MMP-3	Inhibitor			
	None	TIMP1	TIMP2	α2-macro
Pro-MMP-3	100%	100%	100%	100%
Active MMP-3 by trypsin	75-90%	75-90%	75-90%	3%

HIGH DOSE HOOK EFFECT

A sample spiked with 10 µg/mL of Hu MMP-3 gave a response higher than that obtained for the last standard point.

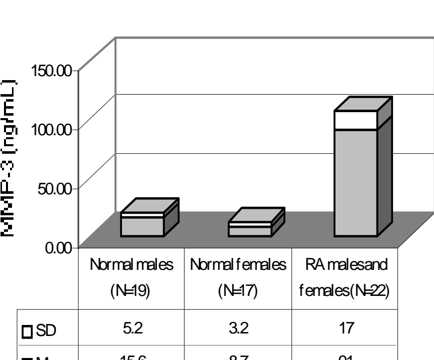
EXPECTED VALUES

We recommend that each laboratory establish its own normal values. For guidance, see the following information.

Sera and plasma

The mean of 20 normal sera was 10.7 ng/mL (SD=7.8), ranging between 2 and 28.8 ng/mL. The mean of 20 normal heparinized plasma was 9.3 ng/mL (SD=6.3), ranging between 1.6 and 24.2 ng/mL.

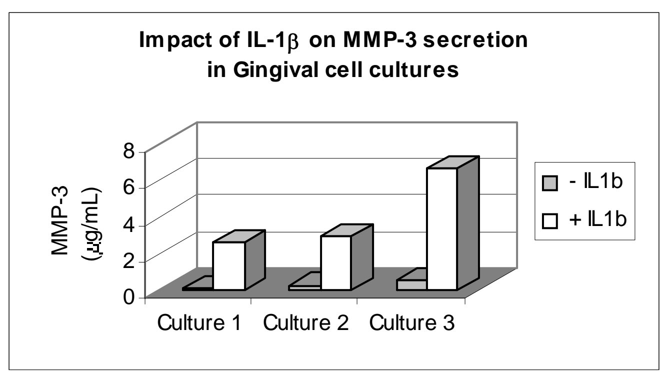
The MMP-3 concentrations were significantly higher in normal male sera than in normal female sera. As compared to these groups, the MMP-3 concentrations were significantly higher in Rheumatoid Arthritis (RA) sera.



	Normal males (N=19)	Normal females (N=17)	RA males and females (N=22)
SD	5.2	3.2	17
Mean	15.6	8.7	91

Culture supernatants

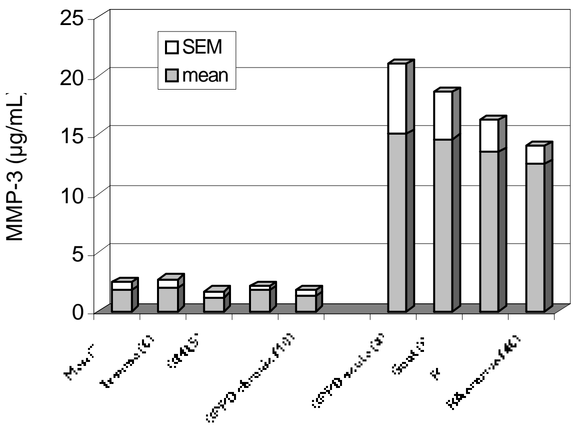
Human gingival fibroblasts were cultured in DMEM supplemented or not with IL-1β (10^{-10}M). The detected MMP-3 concentrations ranged between 0.08 and 6.6 μg/mL, and the production of MMP-3 was greatly stimulated by IL-1β.



Synovial fluids

The MMP-3 concentrations were measured in 146 synovial fluids of patients with meniscus (Men), trauma, chondromalacia (CM), osteoarthritis (OA), chronic crystal pyrophosphate disease (CPPD), acute crystal pyrophosphate disease (CPPD acute), gout, reactive arthritis (ReA), and erosive rheumatoid arthritis (RA erosive). The MMP-3 concentrations ranged between 0.2 and 63 µg/mL. Similar concentrations were detected in the Men, trauma, CM, OA and CPPD chronic synovial fluid. As compared to these groups, MMP-3 concentrations were significantly higher in the inflammatory arthropathies.

MMP-3 concentrations in synovial fluids



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Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
Protect from Light	Protect from light		Consult accompanying documents
i	Directs the user to consult instructions for use (IFU), accompanying the product.		

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Human MMP-3 Assay Summary