Quantikine® ELISA

Human Pro-MMP-13 Immunoassay

Catalog Number DM1300
SMP1300
PDM1300

For the quantitative determination of human Pro-Matrix Metalloproteinase-13 (Pro-MMP-13) concentrations in cell culture supernates.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Matrix metalloproteinases (MMPs), also called matrixins, constitute a family of zinc and calcium dependent endopeptidases that function in the breakdown of extracellular matrix (ECM). They play an important role in many normal physiological processes such as embryonic development, morphogenesis, reproduction and tissue remodeling (1). They also participate in many pathological processes such as arthritis, cancer and cardiovascular disease (2). While the amounts of newly synthesized MMPs are regulated mainly at the levels of transcription, the proteolytic activities of existing MMPs are controlled through both the activation of proenzymes or zymogens and the inhibition of active enzymes by endogenous inhibitors, α_2-macroglobulin and tissue inhibitors of metalloproteinases (TIMPs).

As the third collagenase found in humans after MMP-1 (collagenase 1) and MMP-8 (collagenase 2), MMP-13 (collagenase 3) has been proposed to participate in aggrecan degradation associated with osteoarthritis and cleavage of type II collagen in osteoarthritic cartilage explants, and in tumor progression and metastasis (3 - 6). Additionally, it can cleave type I, III, IV, IX, X and XIV collagens and fibronectin (4). MMP-13 is likely to play a crucial role in the modulation of extracellular matrix degradation and cell-matrix interactions (5). MMP-13 is expressed by several metastatic tumors including breast carcinomas, chondrosarcomas, head and neck carcinomas, and in degenerative bone diseases including rheumatoid arthritis (6). Wild type and mutant p53, a tumor suppressor gene, differentially regulate MMP-13 gene expression (7). Members of the activator protein-1 and core-binding factor families increase MMP-13 promoter activity in normal, differentiating osteoblasts (6).

MMP-13 is normally secreted as a proenzyme of 452 amino acids. The N-terminal pro-domain (84 residues) contains the cysteine switch motif conserved in MMPs that maintains MMP-13 in a latent state (8). The removal of the pro-domain can be initiated by other MMPs such as MMP-2 and MMP-14, and by plasmin (9). The resulting active enzyme consists of a catalytic domain with a zinc-binding motif conserved in metzincins (10, 11). A short hinge peptide links the catalytic domain to the C-terminal hemopexin-like domain. The C-terminal domain can also be removed from MMP-13 by incubation with plasmin, MMP-2, MMP-14 and *p*-aminophenylmercuric acetate (9).

The Quantikine® Human Pro-MMP-13 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Pro-MMP-13 in cell culture supernates. It contains NS0-expressed recombinant human Pro-MMP-13 and antibodies raised against the recombinant factor. The Quantikine® kit will not detect recombinant human active MMP-13 existing in either free or TIMP-bound form. Natural human Pro-MMP-13 showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine® kit standards. These results indicate that this kit can be used to determine relative levels of natural human Pro-MMP-13.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Pro-MMP-13 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Pro-MMP-13 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human Pro-MMP-13 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Pro-MMP-13 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DM1300	CATALOG # SMP1300	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Pro-MMP-13 Microplate	890884	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human MMP-13.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human Pro-MMP-13 Conjugate	890885	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human Pro-MMP-13 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Pro-MMP-13 Standard	890886	1 vial	6 vials	Recombinant human Pro-MMP-13 in a buffer with preservatives, lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-34	895265	1 vial	6 vials	11 mL/vial of a buffer with preservatives.	
Calibrator Diluent RD5-10	895266	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

DM1300 contains sufficient materials to run an ELISA on one 96 well plate.
SMP1300 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDM1300). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards.
- Human Pro-MMP-13 Controls (optional; R&D Systems®, Catalog # QC132).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

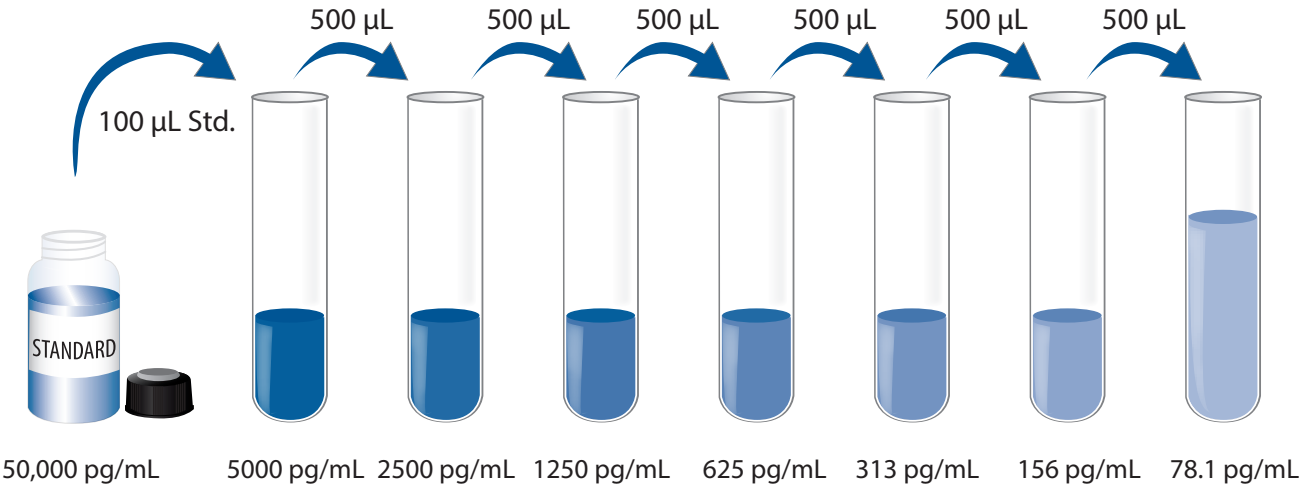
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human Pro-MMP-13 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Pro-MMP-13 Standard with deionized or distilled water. This reconstitution produces a stock solution of 50,000 pg/mL. Mix the standard to ensure complete reconstitution, and allow the standard to sit for a minimum of 30 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-10 into the 5,000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5,000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-10 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-34 to each well.
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, 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.
6. Add 200 µL of Human Pro-MMP-13 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

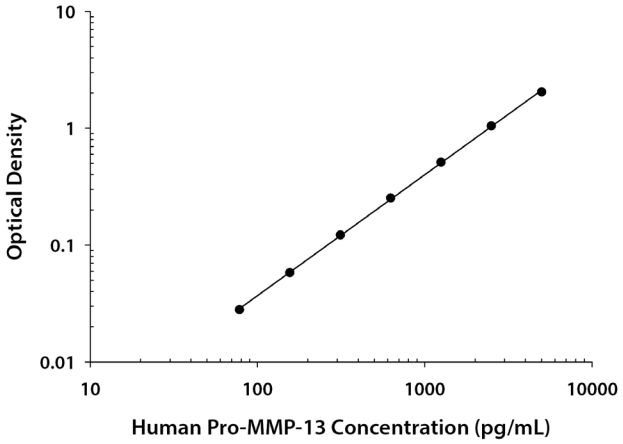
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human Pro-MMP-13 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.024 0.027	0.026	—
78.1	0.054 0.053	0.054	0.028
156	0.084 0.083	0.084	0.058
313	0.147 0.148	0.148	0.122
625	0.276 0.282	0.279	0.253
1250	0.542 0.532	0.537	0.511
2500	1.070 1.071	1.070	1.044
5000	2.095 2.045	2.070	2.044

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	485	1462	3521	533	1561	3823
Standard deviation	18.2	64.0	93.0	27.9	56.1	143
CV (%)	3.8	4.4	2.6	5.2	3.6	3.7

RECOVERY

The recovery of human Pro-MMP-13 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=3)	98	85-111%
Cell culture media (n=2)	101	86-112%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Pro-MMP-13 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=2)	Cell culture media (n=7)
1:2	Average % of Expected	101	105
	Range (%)	96-108	101-109
1:4	Average % of Expected	98	105
	Range (%)	92-107	95-112
1:8	Average % of Expected	96	107
	Range (%)	93-102	103-112
1:16	Average % of Expected	95	103
	Range (%)	84-104	99-106

SENSITIVITY

Thirty-eight assays were evaluated and the minimum detectable dose (MDD) of human Pro-MMP-13 ranged from 3.1-21.3 pg/mL. The mean MDD was 7.7 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human Pro-MMP-13 produced at R&D Systems®.

SAMPLE VALUES

Cell Culture Supernates - U2OS human osteosarcoma cells were grown to 100% confluency in McCoy's 5a, 15% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Aliquots of the cell culture supernates were removed and assayed for levels of human Pro-MMP-13.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Cell culture supernates (n=3)	428	181-674

SPECIFICITY

This assay recognizes natural and recombinant human Pro-MMP-13.

The factors listed below were prepared at 200 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range recombinant human pro-MMP-13 standard were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

MMP-1
MMP-2
MMP-3
MMP-7
MMP-8
MMP-9
MMP-10
TIMP-1
TIMP-2

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