

HUMAN MATRIX METALLOPROTEINASE 3 ELISA

Cat. No.: PL1013

Enzyme Immunoassay for the quantitative determination of Matrix metalloproteinase 3 (MMP-3) in human serum and plasma.

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases, responsible for the integrity of the basement membrane (BM) via degradation of extracellular matrix and BM components. MMPs are presented in various types of cells, including cardiomyocytes, cerebral neurons, hepatocytes, and many others, thus influencing various processes.

MMP-3, also called stromelysin 1, is reported to be found in the cytosol of dopaminergic cells where its activation under stress conditions contributes to neuronal degradation.¹ In chondrocytes, nuclear MMP-3 is suggested to be involved in the development of inflammatory arthritic diseases.² Matrix metalloproteinase-3 (MMP-3) is implicated in the formation of atherosclerotic plaques, and the MMP-3 -1612 5A/6A polymorphism is associated with myocardial infarction (MI) and stable coronary artery disease (CAD).³

Principle of MMP-3 Elisa

The microtiter plate is coated with the antibody specifically binding the Matrix metalloproteinase 3. The human serum or plasma is incubated in the plate with the capture antibody.

The specimen is washed out and the specifically bound protein is incubated with biotin-labelled detection antibody. Following another washing step, Streptavidin-HRP conjugate is added into the well. Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).

The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of MMP-3 in the specimen. The concentration of MMP-3 in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.

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Add 100 µL of Standards, diluted QCs and Samples to the wells

Incubate for 1 hour at 25 °C, shaking at 300 rpm

3-times wash the wells (350 µL/well)

Add 100 µL of Biotin-labelled Antibody to the wells

Incubate for 1 hour at 25 °C, shaking at 300 rpm

3-times wash the wells (350 µL/well)

Add 100 µL of SAV-HRP to the wells

Incubate for 30 min at 25 °C, shaking at 300 rpm

3-times wash the wells (350 µL/well)

Add 100 µL of Substrate Solution to the wells

Incubate for 15 min in the dark at 25 °C, NO shaking

Add 100 µL of Stop Solution to the wells

Read the signal at 450 nm (450/630 nm) within 15 min

Kit contents

Item	Qty.
Antibody Coated Microtiter Plate	96 wells
Biotin-labelled Antibody	13 mL
Streptavidin-HRP Conjugate	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer	15 mL
Wash Buffer 15× conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

Material required but not supplied

1. Glassware and test tubes.
2. Microtiter plate washer.
3. Precision pipettes (various volumes) with tips.
4. Orbital shaker.
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation.

Warnings and precautions

1. For research use only.
2. For professional laboratory use.
3. The reagents with different lot numbers should not be mixed.

4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25 °C ±2 °C).

Storage conditions

1. The kit must be stored at 2–8 °C.
2. The opened components can be stored for one week at 2–8 °C.

Preparation of reagents

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25 °C ±2 °C.

Preparation of Standards

Reconstitute lyophilized Human MMP-3 Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min prior to use. The concentration of human MMP-3 in Master Standard is 10 ng/mL.

Prepare set of Standard solution as follows:

Use the Master Standard for serial dilution (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 10 ng/mL (lyophilized)	1000 µL	10 ng/mL
Std2	250 µL of Std1	250 µL	5 ng/mL
Std3	250 µL of Std2	375 µL	2 ng/mL
Std4	250 µL of Std3	250 µL	1 ng/mL
Std5	250 µL of Std4	250 µL	0.5 ng/mL
Std6	250 µL of Std5	375 µL	0.2 ng/mL
Blank		200 µL	0 ng/mL

Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls in deionized/distilled water, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min and dilute them 1:15 in Dilution Buffer, prior to use, see Preparation of samples.

Preparation of Wash Buffer 1*

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15* conc. to 700 mL of deionized/ distilled water (dH₂O). Mix well. Store at 4 °C for two weeks or at -20 °C for long term storage.

Preparation of samples

Human serum or plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70 °C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of samples is 1:15, i.e., for singlets 10 µL of sample + 140 µL of Dilution Buffer, for duplicates 20 µL of samples + 280 µL of Dilution Buffer, respectively.

Do not store the diluted samples.

Assay procedure

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for 1 hour at 25 °C ±2 °C, shaking at 300 rpm.
3. Wash the wells 3-times with 1* Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of Biotin-labelled Antibody into each well. Incubate for 1 hour at 25 °C ±2 °C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL of Streptavidin-HRP into each well. Incubate for 30 min at 25 °C ±2 °C, shaking at 300 rpm.

7. Wash the wells as described in point 3.
8. Pipette 100 µL Substrate solution, incubate for 15 min, at 25 °C ±2 °C. Avoid exposure to the light during this step.
9. Pipette 100 µL of STOP solution.
10. Read the signal at 450 or 450/630 nm within 15 min.

Performance characteristics

Samples used in the tests were diluted 1:15 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human MMP-3 giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 0.05 ng/mL of sample.

2. Precision

Intra-assay

Sample	Mean (ng/mL)	SD	CV (%)
1	16.1	0.4	2
2	21.8	1.3	6

Inter-assay (Run – to – run)

Sample	Mean (ng/mL)	SD	CV (%)
1	20.5	1.2	6
2	6.0	0.1	2

3. Accuracy

Dilution linearity

Sample	Dilution	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1		16.0	-	-
	2*	8.0	8.0	100
	4*	3.7	4.0	93
	8*	1.9	2.0	96
2		21.1	-	-
	2*	10.6	10.5	101
	4*	5.0	5.3	94
	8*	2.2	2.6	84

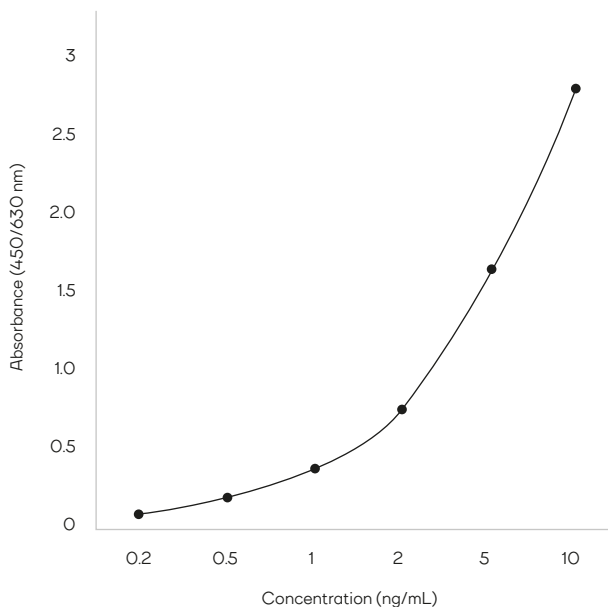
Spiking Recovery

Sample	Spike (ng/mL)	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1	-	15.8	-	-
	30.0	51.4	45.8	112
	15.0	31.0	30.8	101
	7.5	23.7	23.3	101

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.

Human MMP-3 standard curve



Resources

- 1 Choi DH, Kim EM, Son HJ, Joh TH, Kim YS, Kim D, Flint Beal M & Hwang O (2008) A novel intracellular role of matrix metalloproteinase-3 during apoptosis of dopaminergic cells. *J Neurochem* 106, 405–415.
- 2 Eguchi T, Kubota S, Kawata K, Mukudai Y, Uehara J, Ohgawara T, Ibaragi S, Sasaki A, Kuboki T & Takigawa M (2008) Novel transcription-factor-like function of human matrix metalloproteinase 3 regulating the CTGF/CCN2 gene. *Mol Cell Biol* 28, 2391–2413.3 Moolhuijsen LME, Visser JA. Anti-Müllerian Hormone and Ovarian Reserve: Update on Assessing Ovarian Function. *J Clin Endocrinol Metab.* 2020 Nov 1;105(11):3361–73. doi: 10.1210/clinem/dgaa513. PMID: 32770239; PMCID: PMC7486884.
- 3 Samnegård A, Silveira A, Lundman P, Boquist S, Odeberg J, Hulthe J, McPheat W, Tornvall P, Bergstrand L, Ericsson CG, Hamsten A, Eriksson P. Serum matrix metalloproteinase-3 concentration is influenced by MMP-3 -1612 5A/6A promoter genotype and associated with myocardial infarction. *J Intern Med.* 2005 Nov;258(5): 411-9. doi: 10.1111/j.1365-2796.2005.01561.x. PMID: 16238676.