Total MMP2 ELISA Kit

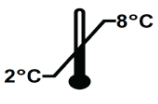
Enzyme-linked immunosorbent assay for quantitative detection of Matrix metallopeptidase 2 (MMP-2)

Catalogue Number: LDG00015E

Research Use or Further Manufacturing Only.

RUO

96 wells



Leadgene® Total MMP2 ELISA Kit

1. Introduction

Matrix metallopeptidase 2 (MMP-2), also known as gelatinase A and 72 kDa type IV collagenase, is a member of the zinc-dependent endopeptidase family that degrades components of the extracellular matrix (ECM), particularly type IV collagen. MMP-2 is synthesized as an inactive pro-enzyme (pro-MMP-2) and is activated extracellularly by proteolytic cleavage or intracellularly via S-glutathionylation. Through ECM remodeling, MMP-2 plays essential roles in physiological processes such as angiogenesis, tissue repair, and embryonic development, while its dysregulated expression is closely associated with tumor invasion and metastasis, cardiovascular diseases, and chronic inflammation. Additionally, mutations in the MMP-2 gene have been associated with Winchester syndrome and nodulosis-arthropathy-osteolysis (NAO) syndrome, and two transcript variants encoding different isoforms of MMP-2 have been identified.

2. Test principle

The MMP-2 ELISA kit is designed for the quantitative detection of natural and recombinant human MMP-2 in serum, plasma, and cell culture supernatants using the sandwich ELISA method. In this assay, a microplate is pre-coated with a mouse anti-MMP-2 monoclonal capture antibody. Standards and samples are added to the wells, allowing MMP-2 to bind to the immobilized antibody on the solid phase, followed by reaction with an HRP-conjugated mouse anti-MMP-2 monoclonal detection antibody. After washing away unbound substances, a substrate solution is added for color development, which is then terminated by a stop solution. The optical density (OD) is measured at 450 nm, and MMP-2 concentrations are determined by comparing the sample absorbance against a standard curve.

3. Reagents provided and reconstitution

Reagents (Store at 2-8℃)	Quantity 1x96 well kit	Reconstitution
MMP2 ELISA plate Stripwell microplate with 96 anti-MMP2 monoclonal antibodies coated wells	96 wells (12 x 8-well strips)	**Ready** for use
Standard Human MMP2 lyophilized from buffered protein solution with preservatives	1 vial (lyophilized form)	**Refer to the vial label for reconstitution volume.** Reconstitute by adding **Standard reconstitution buffer** to be a stock solution of 100 ng/mL. (see procedure, section 7.(2))
Standard reconstitution buffer Buffered protein solution with preservatives	1 vial (1.1 mL)	**Ready** for use
HRP-antibody conjugate HRP conjugated anti-Human MMP2 monoclonal antibody in buffered protein solution with preservatives	1 vial (70 μL)	**Dilute** 200 x with **Antibody diluent buffer** (see reagent preparation, section 5.A)
Assay buffer Buffered protein solution with preservatives	1 vial (12 mL)	**Ready** for use
Sample diluent buffer Buffered protein solution with preservatives	1 vial (15 mL)	**Ready** for use
Antibody diluent buffer Buffered protein solution with preservatives	1 vial (12 mL)	**Ready** for use
20 X wash buffer 20-fold concentrated solution of buffered surfactant with preservatives	1 vial (15 mL)	**Dilute** 20 x with distilled water (see reagent preparation, section 5.B)
TMB Chromogenic substrate (tetramethylbenzidine) for HRP	1 vial (12 mL)	**Ready** for use
Stop solution H_2SO_4 solution	1 vial (6 mL)	**Ready** for use
Microplate sealing film	1 sheet	N/A

4. Materials required but not provided

(1) High-quality distilled water

(2) 10 mL graduated pipettes

(3) 5 μL to 1000 μL adjustable single-channel micropipettes with disposable tips

(4) 50 μL to 300 μL adjustable multi-channel micropipettes with disposable tips

(5) Multi-channel micropipette reservoir
(6) Disposable microcentrifuge tubes
(7) Beakers, flasks, cylinders necessary for preparation of reagents
(8) Timer
(9) Magnetic stirrer
(10) Vortex mixer
(11) Washer for microplates
(12) Incubator capable of maintaining temperature at 37±1°C
(13) Stripwell microplate spectrophotometer capable of reading at 450 nm
(14) Clean paper towels
(15) Disposable gloves
(16) Discard container for bio-medical waste

5. Reagent preparation

All the working reagents should be prepared with adequate volume and discarded at the end of the day.

A. **Working HRP-antibody conjugate (1 X):** Dilute 1 volume of **HRP-antibody conjugate** with 199 volumes of **Antibody diluent buffer** and homogenize by vortex.
B. **Working wash buffer (1 X):** Dilute 1 volume of **20 X wash buffer** with 19 volumes of distilled water and homogenize by using a magnetic stirrer.

6. Procedure

(1) Evaluate the number of stripwell required to test the samples (serum, standards and blanks). Put the stripwells at room temperature (15-30℃) before use. The unused strips should be resealed in the bag and stored at 2-8℃. Each standard, blank, and sample should be assayed in duplicate.
(2) Standard and sample preparation:

- **Standard preparation (in microcentrifuge tubes):**

- **Refer to the vial label for reconstitution volume.** Reconstitute the lyophilized standard with Standard reconstitution buffer to the concentration of 100 ng/mL. Vortex for 1 min and incubate for at least 10 minutes. Aliquot and store the standards at -80°C.
- Add 400 µL Sample & antibody diluent buffer to 100 µL of 100 ng/mL standard to make a 20 ng/mL standard (Tube 1).
- Adding 200 µL of Sample & antibody diluent buffer to 200 µL of 20 ng/mL standard to make a 10 ng/mL standard (Tube 2).
- Repeat the above procedure to make serial diluted standards (Tube 3-7).
- Tube 8 is blank which only containing Sample & antibody diluent buffer.

- **Sample preparation:**

Each sample should use Sample diluent buffer to make a 100 µL volume.

(3) Add 100 µL of standards, blanks or samples into MMP2 ELISA stripwell microplates (see Table 1), then add 100 µL of Assay buffer immediately. Cover with microplate sealing film and incubate sealed plate at room temperature (15-30℃) for 2 hours.
(4) Remove the sealing film, aspirate the liquid from each well and then wash the plate three times with 300 µL Working

wash buffer per well. After the last wash, tap stripwells on clean absorbent paper to remove excess wash buffer.

(5) Add 100 µL of Working HRP-antibody conjugate into each well. Cover with microplate sealing film and incubate sealed plate at room temperature (15-30℃) for 1 hour in the dark.

(6) Remove the sealing film, aspirate the liquid from each well and then wash the plate four times with 300 µL Working wash buffer per well. After the last wash, tap stripwells on clean absorbent paper to remove excessive wash buffer.

(7) Add 100 µL of TMB into each well and incubate for 10 minutes at room temperature (15-30℃) in the dark.

(8) Add 50 µL Stop solution into each well.

(9) Read the absorbencies immediately at 450 nm.

Table 1 An example of orientation of standards, blanks and samples in the stripwells microplate

	1	2	3	4
A	Standard 1 (20 ng/mL)	Standard 1 (20 ng/mL)	Sample 1	Sample 5
B	Standard 2 (10 ng/mL)	Standard 2 (10 ng/mL)	Sample 1	Sample 5
C	Standard 3 (5 ng/mL)	Standard 3 (5 ng/mL)	Sample 2	Sample 6
D	Standard 4 (2.5 ng/mL)	Standard 4 (2.5 ng/mL)	Sample 2	Sample 6
E	Standard 5 (1.25 ng/mL)	Standard 5 (1.25 ng/mL)	Sample 3	Sample 7
F	Standard 6 (0.625 ng/mL)	Standard 6 (0.625 ng/mL)	Sample 3	Sample 7
G	Standard 7 (0.3125 ng/mL)	Standard 7 (0.3125 ng/mL)	Sample 4	Sample 8
H	Blank	Blank	Sample 4	Sample 8

7. Internal quality control

- The average absorbance of Blank: ≦ 0.15.
- The average absorbance of highest concentration of standard (20 ng/mL): ≧ 1.1.

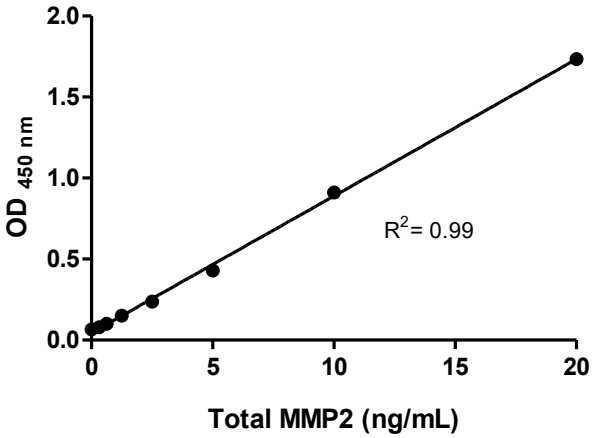
8. Calculation of results

- The linear regression of standard curve is generated by plotting the average absorbance of standards (linear, y-axis) against the corresponding standard concentrations (linear, x-axis).
- The MMP2 concentrations of samples are determined by interpolation on the calibration curve.
- If the assay concentrations of samples are higher than 20 ng/mL, the samples should be diluted with Sample diluent buffer and re-assay again.

Example of MMP2 standard curve

The following data are for demonstration only

Standard	Total MMP2 (ng/mL)	OD $_{450\ nm}$
1	20	1.734
2	10	0.911
3	5	0.428
4	2.5	0.237
5	1.25	0.151
6	0.625	0.100
7	0.3125	0.08
Blank	Blank	0.065



9. Assay limitations

- Hemolysis and hyperlipidemia might affect the test result.
- Sample should be centrifuged to remove debris.

10. Sensitivity

- The limit of detection (LoD) of this MMP2 ELISA kit is 0.22 ng/mL.
- The limit of quantification (LoQ) of this MMP2 ELISA kit is 0.66 ng/mL.

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