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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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# Total MMP7 ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of Total MMP7

**Catalogue number LDG00011E**

**For Research Use or Further Manufacturing Only.**

RUO

96 wells

Store at 2-8℃

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# Leadgene® Total MMP7 ELISA Kit

## 1. Introduction

Matrix metalloproteinase7 (MMP7), also known as matrilysin, is a member of the matrix metalloproteinase family involved in extracellular matrix remodeling. MMP7 is synthesized as an inactive proenzyme and subsequently processed into its mature, active form, both of which are associated with tissue remodeling, inflammation, and cancer progression. Accurate and sensitive detection of total MMP7, including both pro and mature forms, is important for basic research and disease-related studies. Leadgene® Total MMP7 ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of total MMP7 levels in sample solution. The Total MMP7 ELISA Kit is for research use only (RUO). Not suitable for use in diagnostic or therapeutic procedures.

## 2. Test principle

Total MMP7 ELISA Kit is used to detect Total MMP7 in samples by sandwich ELISA method. This assay uses microplate pre-coated with mouse anti-Total MMP7 monoclonal antibody to the solid phase. Total MMP7 in the samples conjugates on solid phase and then react with the HRP conjugated mouse anti-Total MMP7 monoclonal antibody. Subsequent wash steps will residually unbound antibody. After incubation with substrate solution, the reaction is determined by the absorbance at 450 nm. Quantification of Total MMP7 level is accomplished by comparing the absorbance with standard curve.



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## 3. Reagents provided and reconstitution

| Reagents (Store at 2-8℃) | Quantity 1x96 well kit | Reconstitution |
|---|---|---|
| **Antibody-coated microplate** Stripwell microplate with 96 anti-Total MMP7 monoclonal antibodies coated wells | 96 wells (12 x 8-well strips) | **Ready** for use |
| **Standard** Lyophilized | 1 vial (Lyophilized form) | **Refer to the vial label for reconstitution volume.** Reconstitute by adding **Standard reconstitution buffer** to be a stock solution of 200 ng/mL. (see procedure, section 8.(2)) |
| **Standard reconstitution buffer** Buffered protein solution with preservatives | 1 vial (1.1 mL) | **Ready** for use |
| **Sample diluent buffer** Buffered solution with preservatives | 1 vial (15 mL) | **Ready** for use |
| **Antibody diluent buffer** Buffered solution with preservatives | 1 vial (12 mL) | **Ready** for use |
| **Assay buffer** Buffered solution with preservatives | 1 vial (12 mL) | **Ready** for use |
| **HRP-antibody conjugate** HRP conjugated anti-Total MMP7 monoclonal antibody in buffered protein solution with preservatives | 1 vial (65 µL) | **Dilute** 200 x with Antibody diluent buffer (see reagent preparation, section 5.A) |
| **10 X wash buffer** 10-fold concentrated solution of buffered surfactant with preservatives | 1 vial (30 mL) | **Dilute** 10 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** | 2 sheets | 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



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(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) Stripwell microplate spectrophotometer capable of reading at 450 nm
(13) Clean paper towels
(14) Disposable gloves
(15) 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.

(1) **Working HRP-antibody conjugate (1 X):** Dilute 1 volume of **HRP-antibody conjugate** with 199 volumes of **Antibody diluent buffer** and homogenize by vortex.
(2) **Working wash buffer (1 X):** Dilute 1 volume of **10 X wash buffer** with 9 volumes of distilled water and homogenize by using a magnetic stirrer.

## 6. Storage and expiration date of reagents

- Before opened, all kit reagents should be kept properly at 2-8℃. Please see the box front label for expiration date.
- Once opened, the kit should be used within 2 weeks, and the remaining reagents should be immediately returned to 2-8℃ after used, except the reconstituted standard, it must be stored at -80℃.
- Avoid multiple freeze-thaw cycles of the frozen standard, and if stored properly at -80℃, it should be valid for maximum 2 weeks.
- Unused strips must be stored at 2-8℃ in a sealed bag containing a desiccant and should be used as soon as possible.
- All working reagents, Working HRP-antibody conjugate (1 X) and Working wash buffer (1 X), should be prepared freshly and used on the same day.
- Alterations in physical appearance of kit components may indicate instability or deterioration.

## 7. Precautions & warnings

To obtain reproducible test results, the following rules should be strictly obeyed:

- All reagents and specimens should be considered as potentially hazardous. We therefore recommend that this product is handled by those people who have been properly trained.
- Wear suitable protective clothing and disposable gloves.
- Care should be taken to avoid reagents (especially TMB and Stop solution, which contains $H_2SO_4$) contacting with skin or eyes. If contacted, wash immediately and thoroughly with plenty of clean water.


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- This product is intended for *Research use only* and is not for use in diagnostic and therapeutic procedures.
- This product is designed for a single, one-time use only.
- The assay should be performed as outlined in this manual, and in accordance with all instructions.
- Do not use expired or damaged products.
- Do not mix or substitute reagents with those from different lots or other sources.
- Bring all the reagents and specimens to 15-30℃ prior to use.
- Thoroughly and gently mix all the reagents and specimens prior to use.
- Do not expose all the reagents to strong light during storage or incubation.
- Avoid contact of TMB with metal to prevent color development. The color of TMB should be colorless. If a blue color develops before use, indicating it is unusable, it must be discarded.
- Use disposable graduated pipettes and tips to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test.
- After use, all the reagents and specimens should be regarded as medical waste with risk of biological infection and properly disposed of in accordance with national regulations.

## 8. Procedure

(1) Evaluate the number of stripwell required to test the samples. 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 the reconstitution volume.** Reconstitute the lyophilized standard with Standard Reconstitution Buffer to a final concentration of 200 ng/mL. Vortex for 1 min and incubate for at least 10 minutes. Aliquot and store the standards at -20°C
- Add 900 µL Sample diluent buffer to 100 µL of 200 ng/mL standard to make a 20 ng/mL standard (Tube 1).
- Adding 400 µL of Sample diluent buffer to 400 µL of 100 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 contains Sample diluent buffer.

• **Sample preparation:**

- 100 µL Sample. If the initial assay found samples contain Total MMP7 higher than the highest standard, the samples can be diluted with Sample diluent buffer and then re-assay the samples.
- Add 100 µL of standards, blanks or samples into Total MMP7 ELISA stripwell microplates (see Table 1), then add 100 µL Assay buffer immediately. Cover with microplate sealing film and incubate sealed plate at room temperature for 2 hours.
- 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.
- Add 100 µL of Working HRP-antibody conjugate into each well. Cover with microplate sealing film and incubate



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sealed plate at room temperature for 1.5 hours in the dark.

- Remove the sealing film, aspirate the liquid from each well and then wash the plate six times with 300 µL Working wash buffer per well. After the last wash, tap stripwells on clean absorbent paper to remove excess wash buffer.
- Add 100 µL of TMB into each well. Incubate room temperature for 10 minutes in the dark.
- Add 50 µL Stop solution into each well.
- Read the absorbencies immediately at 450 nm after the Stop solution is added.

**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 |

## 9. Internal quality control

- The average absorbance of Blank: ≦ 0.15
- The average absorbance of highest concentration of standard (20 ng/mL): ≧ 1.2

## 10. Calculation of results

- The standard curve is generated by plotting the average absorbance of standards (linear, y-axis) against the corresponding standard concentrations (linear, x-axis) using four-parameter logistic (4-PL) curve fit.
- The Total MMP7 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 Antibody diluent buffer and re-assay again.



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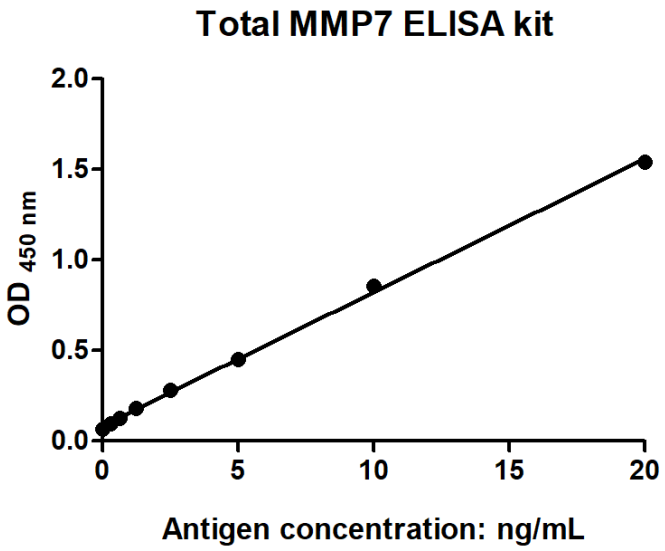
**Typical data**

The following data are for demonstration only

| Standard | Total MMP7 (ng/mL) | OD 450 nm | |
|---|---|---|---|
| 1 | 20 | 1.560 | 1.519 |
| 2 | 10 | 0.851 | 0.861 |
| 3 | 5 | 0.481 | 0.425 |
| 4 | 2.5 | 0.277 | 0.284 |
| 5 | 1.25 | 0.179 | 0.184 |
| 6 | 0.625 | 0.122 | 0.128 |
| 7 | 0.3125 | 0.096 | 0.099 |
| Blank | 0 | 0.071 | 0.061 |




## 11. Assay limitations

- Sample should be centrifuged to remove debris.

## 12. Performance characteristics

**Sensitivity**

- The limit of detection (LoD) of this Total MMP7 ELISA Kit is 0.079 ng/mL.
- The limit of quantification (LoQ) of this Total MMP7 ELISA Kit is 0.768 ng/mL.



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