



## Product Information Sheet

### Human MMP-2 ELISA Kit

<b>Catalog No.</b>	EK0459
<b>Size</b>	96T
<b>Range</b>	156pg/ml-10,000pg/ml
<b>Sensitivity</b>	< 10pg/ml

#### Specificity

No detectable cross-reactivity with any other cytokine.

#### Storage

Store at 4°C for frequent use, at -20°C for infrequent use.

Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

#### Expiration

Four months at 4°C and eight months at -20°C.

#### Application

For quantitative detection of human MMP-2 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

#### Principle

Human MMP-2 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human MMP-2 specific polyclonal antibodies were pre-coated onto 96-well plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human MMP-2 amount of sample captured in plate.

#### Kit Components

1. Lyophilized recombinant human MMP-2 standard: 10ng/tubex2.
2. One 96-well plate pre-coated with anti-human MMP-2 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti-human MMP-2 antibody : 130µl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC) : 130µl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

#### Material Required But Not Provided

1. Microplate reader in standard size and Automated plate washer.
2. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
3. Clean tubes and Eppendorf tubes.
4. Washing buffer (neutral PBS or TBS).

Preparation of 0.01M **TBS**: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H<sub>2</sub>O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M **PBS**: Add 8.5g sodium chloride, 1.4g Na<sub>2</sub>HPO<sub>4</sub> and 0.2g NaH<sub>2</sub>PO<sub>4</sub> to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

To reorder contact us at:

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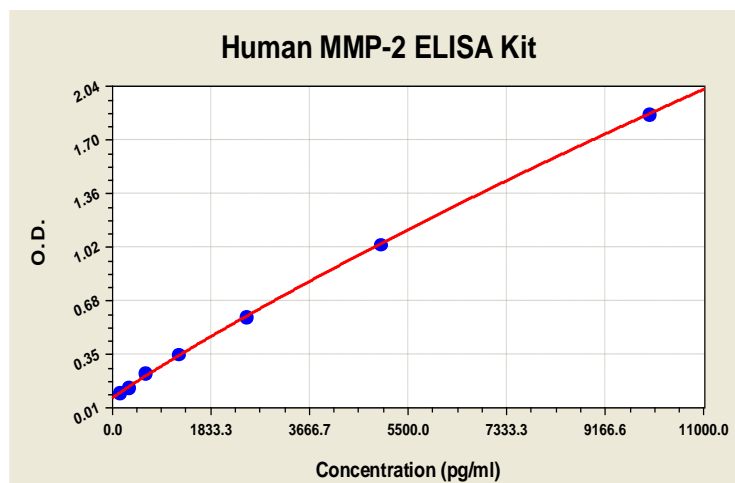
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## Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. In order to avoid marginal effect of plate incubation due to temperature difference ( reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

## Human MMP-2 ELISA Kit-1X96 Well Plate Image



## Background

Type IV collagenase, 72-kD, is officially designated matrix metalloproteinase-2 (MMP2). It is also known as gelatinase, 72-kD. MMP-2 plays an essential role in angiogenesis and arteriogenesis, two processes critical to restoration of tissue perfusion after ischemia. MMP-2 expression is increased in tissue ischemia, but the responsible mechanisms remain unknown.<sup>1</sup> Matrix metalloproteinases (MMPs) catalyze extracellular matrix degradation. Control of their activity is a promising target for therapy of diseases characterized by abnormal connective tissue turnover. MMPs are expressed as latent proenzymes that are activated by proteolytic cleavage that triggers a conformational change in the propeptide (cysteine switch). The structure of proMMP-2 reveals how the propeptide shields the catalytic cleft and that the cysteine switch may operate through cleavage of loops essential for propeptide stability.<sup>2</sup> The gene is localized to 16q21 using somatic cell hybrids and in situ hybridization.<sup>3</sup> The standard product used in this kit is recombinant human MMP-2, consisting of 631 amino acids with the molecular mass of 71KDa. The detected MMP-2 includes zymogen and active enzyme.

## Reference

1. Lee, J. G.; Dahi, S.; Mahimkar, R.; Tulloch, N. L.; Alfonso-Jaume, M. A.; Lovett, D. H.; Sarkar, R. Intronic regulation of matrix metalloproteinase-2 revealed by in vivo transcriptional analysis in ischemia. *Proc. Nat. Acad. Sci.* 102: 16345-16350, 2005.
2. Morgunova, E.; Tuuttila, A.; Bergmann, U.; Isupov, M.; Lindqvist, Y.; Schneider, G.; Tryggvason, K. Structure of human pro-matrix metalloproteinase-2: activation mechanism revealed. *Science* 284: 1667-1670, 1999.
3. Huhtala, P.; Eddy, R. L.; Fan, Y. S.; Byers, M. G.; Shows, T. B.; Tryggvason, K. Completion of the primary structure of the human type IV collagenase preproenzyme and assignment of the gene (CLG4) to the q21 region of chromosome 16. *Genomics* 6: 554-559, 1990.

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