Product Information Sheet

Human MMP-3 ELISA Kit

Catalog No. EK0461

Size 96T

Range 156pg/ml-10,000pg/ml

Sensitivity < 10pg/ml

Specificity

Cross-reactivates with MMP-10 approximately 2%, and no detectable cross-reactivity with other MMPs.

Storage

Store at 4℃ for frequent use, at -20℃ for infrequent use.

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

Expiration

Four months at 4℃ and eight months at -20℃.

Application

For quantitative detection of human MMP-3 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

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Principle

Human MMP-3 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human MMP-3 specific-specific polyclonal antibodies were precoated 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-3 amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant human MMP-3 standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- human MMP-3 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human MMP-3 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.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. 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_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

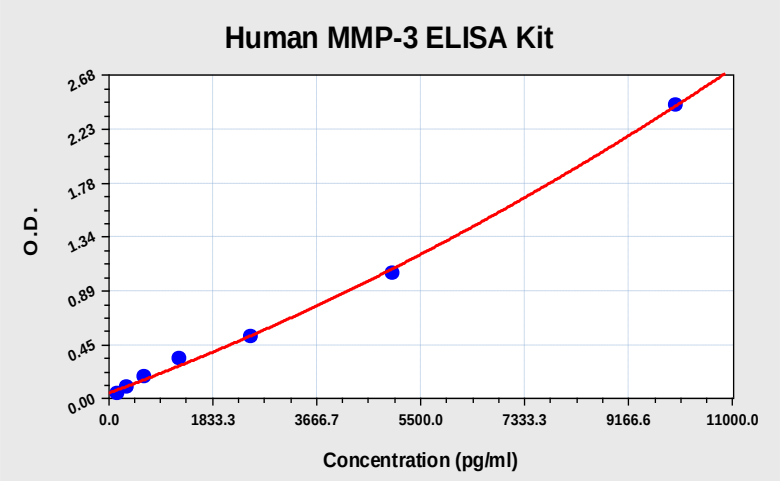
   Preparation of 0.01 M **PBS:** Add 8.5g sodium chloride, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6.

Finally, adjust the total volume to 1L.

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℃ for 30 min before using.

Human MMP-3 ELISA Kit-1X96 Well Plate Image



Background

Matrix metalloproteinase-3 (MMP-3) also called stromelysin or transin, is a proteoglycanase closely related to collagenase (MMP1) with a wide range of substrate specificities. The complete primary structure for human MMP-3, which has 477 residues including a 17-residue signal peptide. MMP-3 and collagenase are 54% identical in sequence, suggesting a common origin for the evolution of the two proteinases. MMP-3 and collagenase expression are coordinately modulated in synovial fibroblast cultures.[1] MMP-3 is a secreted metalloprotease produced predominantly by connective tissue cells. Together with other metalloproteases, it can synergistically degrade the major components of the extracellular matrix. It is capable of degrading proteoglycan, fibronectin, laminin, and type IV collagen, but not interstitial type I collagen. MMP-3 genotype may be an important determinant of vascular remodeling and age-related arterial stiffening, with the heterozygote having the optimal balance between matrix accumulation and deposition.[2] The standard product used in this kit is recombinant human MMP-3, consisting of 460 amino acids with the molecular mass of 52KDa. The detected MMP-3 includes zymogen and active enzyme.

Reference

1. Saus, J.; Quinones, S.; Otani, Y.; Nagase, H.; Harris, E. D., Jr.; Kurkinen, M. The complete primary structure of human matrix metalloproteinase-3: identity with stromelysin. *J. Biol. Chem.* 263: 6742-6745, 1988.
2. Medley, T. L.; Kingwell, B. A.; Gatzka, C. D.; Pillay, P.; Cole, T. J. Matrix metalloproteinase-3 genotype contributes to age-related aortic stiffening through modulation of gene and protein expression. *Circ. Res.* 92: 1254-1261, 2003.