



Product Information Sheet

Human MMP13 ELISA Kit

Catalog No. EK0468
Size 96T
Range 78pg/ml→5000pg/ml
Sensitivity < 5pg/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 MMP13 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle

Human MMP13 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human MMP13 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 MMP13 amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant human MMP13 standard: 5ng/tubex2.
2. One 96-well plate precoated with anti- human MMP13 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human MMP13 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 H₂O 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 Na₂HPO₄ and 0.2g NaH₂PO₄ to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

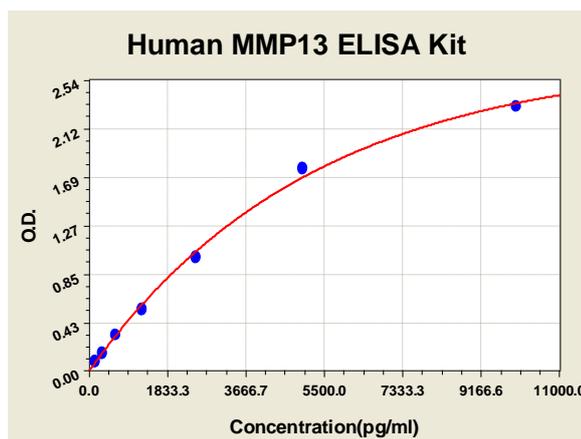
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Notice for Application of Kit

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 MMP13 ELISA Kit-1X96 Well Plate Image



Background

Collagenase 3 is an enzyme that in humans is encoded by the *MMP13* gene.^{[1][2]} Most MMP's are secreted as inactive propeptides which are activated when cleaved by extracellular proteinases. The protein encoded by this gene cleaves type II collagen more efficiently than types I and III.^[3] Mitchell et al. (1996) concluded that the expression of MMP13 in osteoarthritic cartilage and its activity against type II collagen indicates that the enzyme plays a significant role in cartilage collagen degradation and must, therefore, form part of a complex target for proposed therapeutic interventions based on collagenase inhibition. Reboul et al. (1996) likewise presented data on collagenase-3 expression and synthesis in human cartilage cells and suggested its involvement in human osteoarthritis cartilage pathophysiology. It may be involved in articular cartilage turnover and cartilage pathophysiology associated with osteoarthritis. The gene is part of a cluster of MMP genes which localize to chromosome 11q22.3.

Reference

1. ^ Freije JM, Diez-Itza I, Balbin M, Sanchez LM, Blasco R, Tolivia J, Lopez-Otin C (Jul 1994). "Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas". *J Biol Chem* 269 (24): 16766–73.
2. ^ "Entrez Gene: MMP13 matrix metalloproteinase 13 (collagenase 3)".
<http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=4322>.
3. ^ "Entrez Gene: MMP13 matrix metalloproteinase 13 (collagenase 3)".
<http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=4322>.