Product Information Sheet

# Human MMP-8 ELISA Kit

**Catalog No.** EK0464

**Size** 96T

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

**Sensitivity** < 10pg/ml

**Specificity**

No detectable cross-reactivity with any other cytokine.

**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-8 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

**To reorder contact us at:**

**Antagene, Inc.**

**Toll Free: 1(866)964-2589**

**Tel: (650) 964-2589**

**Fax: (650) 964-2519**

**email: Info@antageneinc.com**

## *Principle*

Human MMP-8 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human MMP-8 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-8 amount of sample captured in plate.

## *Kit Components*

1. Lyophilized recombinant human MMP-8 standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- human MMP-8 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human MMP-8 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 $H_2O$ 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.

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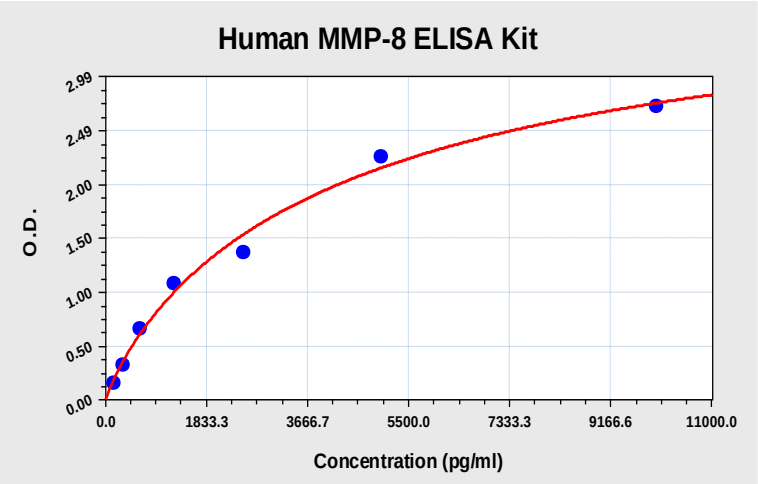
### *Notice for Application of Kit*

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℃ for 30 min before using.

### *Human MMP-8 ELISA Kit-1X96 Well Plate Image*




### *Background*

Matrix metalloproteinase 8 (MMP8) also called neutrophil collagenase. Neutrophil collagenase, a member of the family of matrix metalloproteinases, is distinct from the collagenase of skin fibroblasts and synovial cells in substrate specificity and immunologic crossreactivity. MMP8, an enzyme that degrades fibrillar collagens imparting strength to the fetal membranes, is expressed by leukocytes and chorionic cytotrophoblast cells.[1] The human neutrophil collagenase (HNC) cDNA clone has been sequenced and shown to encode a 467-residue protein.[2] Neutrophil collagenase has been found to possess 57% identity with the deduced protein sequence for fibroblast collagenase with 72% chemical similarity. Certain regions of the molecule, including the putative zinc-binding region, are highly conserved. When compared with the published sequence for fibroblast collagenase, neutrophil collagenase contains four additional sites for glycosylation.[3] The standard product used in this kit is natural, isolating from human MMP-8. The detected MMP-8 includes zymogen and active enzyme.

### *Reference*


1. Wang, H.; Parry, S.; Macones, G.; Sammel, M. D.; Ferrand, P. E.; Kuivaniemi, H.; Tromp, G.; Halder, I.; Shriver, M. D.; Romero, R.; Strauss, J. F., III Functionally significant SNP MMP8 promoter haplotypes and preterm premature rupture of membranes (PPROM). *Hum. Molec. Genet.* 13: 2659-2669, 2004.
2. Devarajan, P.; Mookhtiar, K.; Van Wart, H.; Berliner, N. Structure and expression of the cDNA encoding human neutrophil collagenase. *Blood* 77: 2731-2738, 1991.
3. Hasty, K. A.; Pourmotabbed, T. F.; Goldberg, G. I.; Thompson, J. P.; Spinella, D. G.; Stevens, R. M.; Mainardi, C. L. Human neutrophil collagenase: a distinct gene product with homology to other matrix metalloproteinases. *J. Biol. Chem.* 265: 11421-11424, 1990.