



## Product Information Sheet

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### Human TIMP-3 ELISA Kit

<b>Catalog No.</b>	EK0523
<b>Size</b>	96T
<b>Range</b>	156pg/ml-10,000pg/ml
<b>Sensitivity</b>	< 2 pg/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 TIMP-3 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

#### **Principle**

Human TIMP-3 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human TIMP-3 specific-specific monoclonal antibodies were precoated onto 96-well plates. The human specific detection monoclonal 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 TIMP-3 amount of sample captured in plate.

#### **Kit Components**

1. Lyophilized recombinant human TIMP-3 standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- human TIMP-3 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human TIMP-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<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>

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**Antagene, Inc.**  
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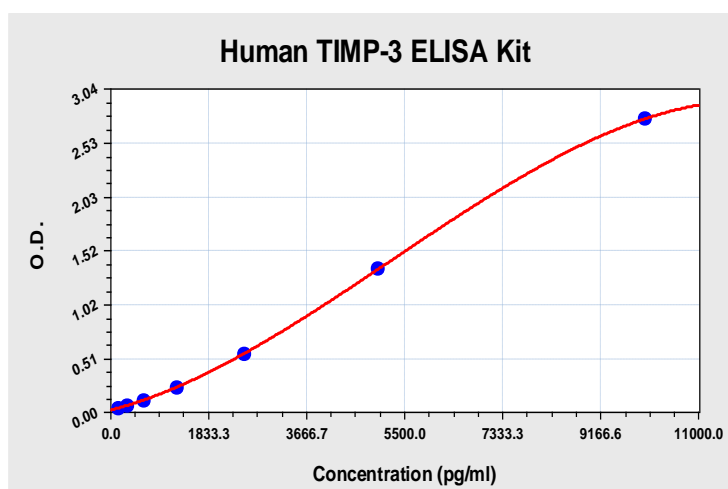
and 0.2g NaH<sub>2</sub>PO<sub>4</sub> to 1000ml  
distilled water and adjust pH to

7.2-7.6. Finally, adjust the total volume to 1L.

### **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 TIMP-3 ELISA Kit-1X96 Well Plate Image**



### **Background**

The tissue inhibitors of metalloproteinases (TIMPs) are natural inhibitors of the matrix metalloproteinases, a group of zinc-binding endopeptidases involved in the degradation of the extracellular matrix. The TIMP3 gene is expressed in many tissues, with highest expression in the placenta. TIMP3 encodes a potent angiogenesis inhibitor and is mutated in Sorsby fundus dystrophy, a macular degenerative disease with submacular choroidal neovascularization. TIMP3 gene is mapped to 22q12.1-q13.2. Mutations in TIMP3 cause the autosomal dominant disorder Sorsby's fundus dystrophy (SFD).

### **Reference**

1. Apte, S. S.; Mattei, M.-G.; Olsen, B. R.: Cloning of the cDNA encoding human tissue inhibitor of metalloproteinases-3 (TIMP-3) and mapping of the TIMP3 gene to chromosome 22. *Genomics* 19: 86-90, 1994.
2. Stohr, H.; Roomp, K.; Felbor, U.; Weber, B. H. F.: Genomic organization of the human tissue inhibitor of metalloproteinases-3 (TIMP3). *Genome Res.* 5: 483-487, 1995.

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