



# **Product Information Sheet**

# **Human TIMP-2 ELISA Kit**

Catalog No. EK0522

Size 96T

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

**Sensitivity** < 2 pg/ml

## **Specificity**

No detectable cross-reactivity with any other cytokine.

#### **Storage**

Store at  $4^{\circ}C$  for frequent use, at  $-20^{\circ}C$  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-2 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

**Principle** 

Human TIMP-2 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human TIMP-2 specific-specific monoclonal 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 washing with **PBS TBS** 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-2 amount of sample captured in plate.

#### Kit Components

- 1. Lyophilized recombinant human TIMP-2 standard: 10ng/tubex2.
- 2. One 96-well plate precoated with anti- human TIMP-2 antibody.
- 3. Sample diluent buffer: 30 ml
- 4. Biotinylated anti- human TIMP-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.
- 2. Automated plate washer.
- 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; 450 $\mu$ l of purified acetic acid or 700 $\mu$ l 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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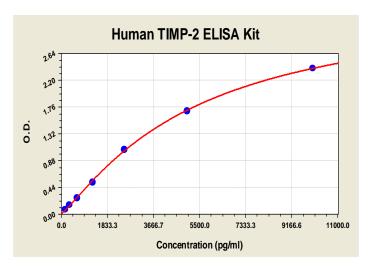
and 0.2g NaH<sub>2</sub>PO<sub>4</sub> to 1000ml distilled water and adjust pH to

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-2 ELISA Kit-1X96 Well Plate Image



## Background

TIMP2 gen is encoded by 5 exons spanning 83 kb of genomic DNA. TIMP2 is 83 kilobase pairs (kb) long with exon-intron splicing sites located in preserved positions among the three members of the TIMP family. The gene for tissue inhibitor of metalloproteinases-2 is localized on human chromosome arm 17q25. TIMP-2 abrogates angiogenic factor-induced endothelial cell proliferation in vitro and angiogenesis in vivo independent of MMP inhibition. The standard product used in this kit is recombinant human TIMP-2 with the molecular mass of 22Kda and 194 amino acid.

## Reference

- De Clerck, Y.; Szpirer, C.; Aly, M. S.; Cassiman, J.-J.; Eeckhout, Y.; Rousseau, G.: The gene for tissue inhibitor of metalloproteinases-2 is localized on human chromosome arm 17q25. Genomics 14: 782-784, 1992.
- 2. Hammani, K.; Blakis, A.; Morsette, D.; Bowcock, A. M.; Schmutte, C.; Henriet, P.; DeClerck, Y. A.: Structure and characterization of the human tissue inhibitor of metalloproteinases-2 gene. J. Biol. Chem. 271: 25498-25505, 1996.
- 3. Seo, D.-W.; Li, H.; Guedez, L.; Wingfield, P. T.; Diaz, T.; Salloum, R.; Wei, B.; Stetler-Stevenson, W. G.: TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism. Cell 114: 171-180, 2003.