

Product Information

Tissue Inhibitor of Metalloproteinase-1, human recombinant, expressed in CHO cells

Product Number **T8947**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

EC 3.4.24.x

Synonym: TIMP-1

Product Description

Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) is purified by substrate-affinity chromatography from recombinant, human TIMP-1 expressed in CHO cells. It is essentially free of matrix metalloproteinases and other known TIMPs. TIMP-1 consists largely of the glycosylated form (~29 kDa).

Human TIMP-1 can be used as a positive control in enzymatic assays, ELISA assays, immunoblotting, and substrate gel analysis (reverse zymograms).

Note: TIMP-1 is produced in low (pg/ml) levels in most cell types. Treatment of cells with phorbol ester TPA stimulates production of TIMP-1 in some cell types, but the low protein levels produced often require concentration of cell culture media to visualize the bands by immunoblotting.

The matrix metalloproteinases (MMPs) are a family of at least eighteen secreted and membrane-bound zinc-endopeptidases. Collectively, these enzymes can degrade all the components of the extracellular matrix (ECM), including fibrillar and non-fibrillar collagens, fibronectin, laminin, and basement membrane glycoproteins. In general, a signal peptide, a propeptide, and a catalytic domain containing the highly conserved zinc-binding site characterize the structure of the MMPs. In addition, fibronectin-like repeats, a hinge region, and a carboxyl-terminal hemopexin-like domain allow categorization of MMPs into the collagenase, gelatinase, stomelysin, and membrane-type MMP subfamilies.¹⁻³ MMPs contain the motif His-Glu-X-X-His (X represents any amino acid) that binds zinc in the catalytic site, as well as another zinc molecule and two calcium molecules structurally. They fall within the matrixin subfamily (EC 3.4.24.x). This group also includes astacin, reprolysin, and serralysin, as well as other more divergent metalloproteinases. All MMPs are synthesized as proenzymes, and most of them are secreted from the cells as proenzymes. Thus, the activation of these proenzymes is a critical step that leads to extracellular matrix breakdown.

MMPs play an important role in wound healing, apoptosis, bone elongation, embryo development, uterine involution, angiogenesis,⁴ and tissue remodeling. They have a role in diseases such as multiple sclerosis,^{2,5} Alzheimer's,² malignant gliomas,² lupus, arthritis, periodontitis, glomerulonephritis, atherosclerosis, tissue ulceration, and in cancer cell invasion and metastasis.⁶ Numerous studies have shown that there is a close association between expression of various members of the MMP family by tumors and their proliferative and invasive behavior, and metastatic potential.

The tissue inhibitors of metalloproteinases (TIMPs) are naturally-occurring proteins that specifically inhibit matrix metalloproteinases and regulate extracellular matrix turnover and tissue remodeling by forming tightly bound inhibitory complexes with the MMPs. Thus, TIMPs maintain the balance between matrix destruction and formation. An imbalance between MMPs and the associated TIMPs may play a significant role in the invasive phenotype of malignant tumors.

TIMP proteins share several structural features including six loops held in place by six disulfide bonds arranged in three knotlike structures. The 12 cysteine residues that form the six disulfide bonds are located in conserved regions of the molecule and are essential for the formation of native conformations. The N-terminal region is necessary for inhibitory activities and contains a consensus sequence (VIRAK). Each TIMP is translated with a 29 amino acid leader sequence that is cleaved to produce the mature protein. The C-terminal regions are divergent, which may enhance the selectivity of inhibition and binding efficiency. Although the TIMP proteins share high homology, they may either be secreted extracellularly in soluble form (TIMP-1, TIMP-2, and TIMP-4) or bind to extracellular matrix components (TIMP-3).

The MMPs and TIMPs can be divided into two groups with respect to gene expression: the majority exhibit inducible expression and a small number are produced constitutively or are expressed at very low levels and are not inducible. Among agents that induce MMP and TIMP production are the inflammatory cytokines TNF- α and IL-1 β . A marked cell type specificity is a hallmark of both MMP and TIMP gene expression (i.e., only a limited number of cell types can be induced to make these proteins).

Human Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) was fully sequenced and cloned.⁷ TIMP-1 is a glycoprotein that plays an important role in modulating the activity of some of the metalloendoproteases of connective tissue origin including collagenase, gelatinase, and proteoglycanase.⁷ It is produced and secreted in a soluble form by a variety of cell types and is widely distributed throughout the body.⁸

TIMP-1 has a greater efficiency against MMP-9, MMP-1, and MMP-3 than the other MMPs. It inhibits the active forms of the MMPs⁹ and complexes with the proform of MMP-9.^{10,11} Like MMP-9, TIMP-1 expression is sensitive to many factors. Increased synthesis of TIMP-1 is induced by a wide variety of agents including TGF- β , EGF, PDGF, FGF- β , PMA, all-trans-retinoic acid (RA), IL-1, and IL-11.

The human TIMP-1 gene has the chromosomal location of Xp11.23-Xp11.4.¹²

Reagent

The product is supplied in 0.01 M sodium phosphate buffer, pH 7.3, and 0.15 mM sodium chloride.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The solution may be stored at -20 °C.

References

1. Borkakoti, N., Matrix metalloproteases: variations on a theme. *Prog. Biophys. Mol. Biol.*, **70**, 73-94 (1998).
2. Yong, V.W., *et al.*, Matrix metalloproteinases and diseases of the CNS. *Tr. Neuro.*, **21**, 75-80 (1998).
3. Kähäri, V.M., and Saarialho-Kere, U., Matrix metalloproteinases in skin. *Exp. Dermatol.*, **6**, 199-213 (1997).
4. Halpert, I., *et al.*, Matrilysin is expressed by lipid-laden macrophages at sites of potential rupture in atherosclerotic lesions and localizes to areas of versican deposition, a proteoglycan substrate for the enzyme. *Proc. Natl. Acad. Sci., USA*, **93**, 9748-9753 (1996).
5. Chandler, S., *et al.*, Matrix metalloproteinases, tumor necrosis factor and multiple sclerosis: an overview. *J. Neuroimmunol.*, **72**, 155-161 (1997).
6. Birkedal-Hansen, H., *et al.*, Matrix metalloproteinases: a review. *Crit. Rev. Oral Biol. Med.*, **4**, 197-250 (1993).
7. Carmichael, D.F., *et al.*, Primary structure and cDNA cloning of human fibroblast collagenase inhibitor. *Proc. Natl. Acad. Sci., USA*, **83**, 2407-2411 (1987).
8. Cawston, T.E., in *Proteinase Inhibitors*, Barret, A.J. and Salvesen, G., (eds.), Elsevier, (Amsterdam, The Netherlands: 1986) pp. 589-610).
9. Woessner, J.F., Jr., Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J.*, **5**, 2145-2154 (1991).
10. Goldberg, G.I., *et al.*, Interaction of 92-kDa type IV collagenase with the tissue inhibitor. *J. Biol. Chem.*, **267**, 4583-4591 (1992).
11. Kolkenbrock, H., *et al.*, Generation and activity of the ternary gelatinase B/TIMP-1/LMW-stromelysin-1 complex. *Biol. Chem. Hoppe-Seyler*, **376**, 495-500 (1995).
12. Willard, H.F., *et al.*, Regional localization of the TIMP gene on the human X chromosome. *Human Genetics*, **81**, 234-238 (1989).

KAA, RBG, MAM 06/07-1

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.