

Product Information

ANTI-TISSUE INHIBITOR OF METALLOPROTEINASE-4 (TIMP-4), THIRD LOOP Developed in Rabbit, Affinity Isolated Antibody

Product Number **T9289**

Product Description

Rabbit Anti-TIMP-4, Third Loop is developed in rabbit using a synthetic peptide corresponding to the third loop of human TIMP-4 as immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-TIMP-4 antiserum by immuno-specific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Rabbit Anti-TIMP-4, Third Loop specifically binds to TIMP-4 and does not cross-react with the other TIMP family members (TIMP-1, TIMP-2, and TIMP-3). This antibody may be used for the detection and localization of human TIMP-4. By immunoblotting against the reduced protein, the antibody identifies bands at 23 kDa (unglycosylated) and 29 kDa (glycosylated).

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 characterizes the structure of the MMPs. In addition, fibronectin-like repeats, a hinge region, and a C-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 and are EC designated 3.4.24.x. This group also contains astacin, reprolysin, and serralyisin, 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 are considered to play an important role in wound healing, apoptosis, bone elongation, embryo development, uterine involution, angiogenesis,⁴ and tissue remodeling, and 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 tight-binding 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.

The TIMP proteins share several structural features including six loops held in place by six disulfide bonds arranged in three knotlike structures. These proteins also contain twelve cysteine residues in conserved regions of the molecule that form six disulfide bonds, essential for the formation of native conformations, and the N-terminal region that is necessary for inhibitory activities. The N-terminus of each TIMP contains a consensus sequence (VIRAK) and each TIMP is translated with a 29 amino acid leader sequence that is cleaved off 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., a limited number of cell types can be induced to make these proteins).

Tissue Inhibitor of Metalloproteinases-4 (TIMP-4) was identified by molecular cloning.⁷ TIMP-4 shows 37 % amino acid identity with TIMP-1 and 51 % homology with TIMP-2 and TIMP-3.⁷

TIMP-4 is secreted extracellularly, predominantly in heart and brain tissue. It may function in a tissue-specific fashion in extracellular matrix homeostasis.⁷ TIMP-4 has a strong inhibitory effect on the invasion of human breast cancer cells across reconstituted basement membranes suggesting that TIMP-4 may have an important role in inhibiting primary tumor growth and progression.^{8,9} The human TIMP-4 gene has the chromosomal location of 3p25.¹⁰

Reagent

Rabbit Anti-TIMP-4, Third Loop is supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 50 % glycerol and 0.1 % sodium azide. The protein concentration is approximately 1 mg/ml.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C to 8 °C for up to six months. For extended storage, the solution may be stored 0 °C to -20 °C. The antibody is supplied with 50 % glycerol to prevent freezing. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

A working dilution of 1:1,000 is determined by immunoblotting using a concentrated cell culture media from stimulated human endothelial cells, an alkaline phosphatase conjugated secondary antibody and BCIP/NBT as the substrate. Higher antibody concentrations may be necessary for non-human samples.

Note: TIMP-4 is reported in heart tissue, and is produced by some fibroblasts in cell culture conditions. Treatment of cells with the phorbol ester TPA stimulates production of TIMP-4 in some cell types, but the low protein levels produced often require concentration of the cell culture media to visualize the bands by immunoblotting.

In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimum working dilutions by titration assay.

References

1. Borkakoti, N., Matrix metalloproteinases: 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. *Trends in Neuroscience*, **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. Greene, J., et al., Molecular cloning and characterization of human tissue inhibitor of metalloproteinase 4. *J. Biol. Chem.*, **271**, 30375-30380 (1996).
8. Liu, Y.E., et al., Preparation and characterization of recombinant tissue inhibitor of metalloproteinase 4 (TIMP-4). *J. Biol. Chem.*, **272**, 20479-20483 (1997).
9. Wang, M., et al., Inhibition of tumor growth and metastasis of human breast cancer cells transfected with tissue inhibitor of metalloproteinase 4. *Oncogene*, **14**, 2767-2774 (1997).
10. Olson, T.M., et al., Cloning of the human tissue inhibitor of metalloproteinase-4 gene (TIMP4) and localization of the TIMP4 and Timp4 genes to human chromosome 3p25 and mouse chromosome 6, respectively. *Genomics*, **51**, 148-151 (1998).

kaa 11/00

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.