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ProductInformation

TISSUE INHIBITOR OF METALLOPROTEINASE-4 (TIMP-4)
Human, Recombinant
Expressed in human kidney cells

Product Number **T9322**

Product Description
Tissue Inhibitor of Metalloproteinase-4 (TIMP-4) is purified by substrate-affinity chromatography from human kidney 293 cell line. It is essentially free of matrix metalloproteinases and other known TIMPs.

Human TIMP-4 can be used as a positive control in enzymatic assays, ELISA assays, immunoblotting, and substrate gel analysis (reverse zymograms). TIMP-4 is a mixture of the glycosylated and unglycosylated forms, approximately 29 and 24 kDa, respectively, by immunoblotting under reducing conditions.

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 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 with the EC designation 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, 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 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 amino-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 carboxyl-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-1β. 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-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] It is secreted extracellularly by a wide range of tissues, particularly brain, heart, ovary, and skeletal muscle.[7]

TIMP-4 may function in a tissue-specific fashion in extracellular matrix homeostasis. It has a tumor suppressive effect against Wilm's tumor, exhibits negative correlation with glioma maligancy,[8] and is found in breast carcinoma cells.[9] 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[10,11] TIMP-4 is a potent inhibitor of most MMPs, but is not an effective inhibitor of ADAMs, such as TACE.[10]

The human TIMP-4 gene has the chromosomal location of 3p25.[12]

Reagent
Human Tissue Inhibitor of Metalloproteinase-4 (TIMP-4) is supplied in latent form in 0.01 M phosphate buffered saline, pH 7.4, containing 150 mM sodium chloride and 50% glycerol (v/v).

Storage/Stability
Store at –20 °C. Do not store below –22 °C.

Product Profile
Purity: >95% as determined by SDS-PAGE, visualized by silver stain.

Note: TIMP-4 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.

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