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# ProductInformation

**Tissue Inhibitor of Metalloproteinase-3 (TIMP-3)**
**Human, recombinant**

Product Number **T 9197**
Storage Temperature –20 °C

## Product Description

Tissue Inhibitor of Metalloproteinase-3 (TIMP-3) is purified by substrate-affinity chromatography from BHK (baby hamster kidney) cells producing recombinant human TIMP-3. It is essentially free of matrix metalloproteinases and other known TIMPs.

Human TIMP-3 can be used as a positive control in enzymatic assays, ELISA assays, immunoblotting, and substrate gel analysis (reverse zymograms). TIMP-3 is a mixture of the glycosylated and unglycosylated forms, approximately 30 kDa and 23 kDa, respectively, using immunoblotting run 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 (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 function 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 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).

Tissue Inhibitor of Metalloproteinase-3 (TIMP-3) was first purified from chicken embryo fibroblasts and identified as ChIMP-3.[7] The human homologue of TIMP-3 was originally detected as a serum inducible protein in WI-38 fibroblasts.[8] The TIMP-3 localization differs from the other three TIMPs, and is thought to be primarily deposited into the extracellular matrix (ECM). TIMP-3 is insoluble and localizes to the ECM on a variety of cell types and is widely distributed throughout the body.[9,10] TIMP-3 has a more basic isoelectric point (pI) than the other TIMPs. The basic residues are thought to help anchor TIMP-3 into the ECM. TIMP-3 shows 30% amino acid homology with TIMP-1 and 38% homology with TIMP-2.[7]

TIMP-3 has been shown to promote the detachment of transformed cells from the ECM and to accelerate morphological changes associated with cell transformation.[11] Furthermore, up-regulation of TIMP-3 has been associated with blocking in the $G_1$ phase of the cell cycle during differentiation of HL-60 leukemia cells.[8] TIMP-3 is an efficient "sheddase" inhibitor, inhibiting ADAM-17 (TACE) at the low nanomolar levels that also produce MMP inhibition.

The human TIMP-3 gene has the chromosomal location of 22q12-22q13.[12]

**Reagent**

Human Tissue Inhibitor of Metalloproteinase-3 (TIMP-3) is supplied in active form in 20 mM MES, pH 5.0, with 200 mM sodium chloride and 0.02% Brij-35 (v/v).

**Storage/Stability**

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

**Product Profile**

Purity: >95% (SDS-PAGE, visualized by silver stain)

Note: TIMP-3 is produced in low (pg/ml) levels by most cell types, and appears to be preferentially secreted into the ECM. In immunoblotting and reverse zymogen analysis, an ECM preparation is most often used, but a total cell lysate can also be used.

**References**


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