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Product Information

Tissue Inhibitor of Metalloproteinase-3, human recombinant, expressed in NSO cells

Catalog Number **T1327**
Storage Temperature –20 °C

Product Description

The tissue inhibitors of metalloproteinases (TIMPs) are naturally-occurring proteins that specifically inhibit matrix metalloproteinases (MMPs) 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 these 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-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.[1] The human homologue of TIMP-3 was originally detected as a serum inducible protein in WI-38 fibroblasts.[2]

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.[3,4] 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.[1]

TIMP-3 is unique among the TIMPs because of its high affinity for binding to the extracellular matrix.[5] TIMP-3 has been shown to promote the detachment of transformed cells from the ECM and to accelerate morphological changes associated with cell transformation.[6] 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. TIMP-3 is an efficient “sheddase” inhibitor. It is also an excellent inhibitor of human TACE (ADAM-17), with an IC_{50} values measured in the low nM range.

This recombinant, human Tissue Inhibitor of Metalloproteinase-3 (TIMP-3) product is from a DNA sequence encoding the human CD33 signal peptide and mature human TIMP-3 protein sequence (amino acid residues Cys^{24} to Pro^{211}).[7] It is expressed in a mouse myeloma cell line, NSO. By N-terminal sequencing, the mature protein starts at residue Cys^{24}.

The product is lyophilized from a 0.2 μm filtered solution of 25 mM Tris, pH 7.5, and 0.15 M sodium chloride.

Human TIMP-3 can be used as a positive control in enzymatic and other assays. The 188 amino acid residue recombinant protein has a predicted molecular mass of ~21.7 kDa. By SDS-PAGE, the apparent molecular mass of the glycosylated protein is ~30 kDa.

The specific activity is measured by its ability to inhibit human MMP-2 hydrolysis of a peptide substrate (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-(3-[2,4-dinitrophenyl]-L-2,3-diaminopropionyl)-Ala-Arg-NH_2. Recombinant human TIMP-3 has an IC_{50} value of ~3 nM under conditions in which MMP-2 is present at 2.8 nM and the substrate concentration is ~5 μM.

Purity: >95% (SDS-PAGE)

Endotoxin: <1.0 EU per 1 μg of protein
(LAL method)

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.

Preparation Instructions
Reconstitute with sterile distilled water to give a stock solution of desired concentration.

Storage/Stability
The protein should be stored at –20 °C to –70 °C upon receipt. Avoid repeated freeze-thaw cycles. Do not store in a frost-free freezer.

Upon reconstitution, this product may be stored in aliquots at –20 °C or below for up to 3 months.

References

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