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ProductInformation

MONOCLONAL ANTI -TISSUE INHIBITOR OF METALLOPROTEINASE-1 (TIMP-1), CLONE 2E7.1
Purified Mouse Immunoglobulin Fraction

Product Number **T8687**

Product Description
Monoclonal Anti-Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) (mouse IgG2a isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from mice immunized with native human tissue inhibitor of metalloproteinase-1 (TIMP-1) as immunogen. The immunoglobulin fraction of antibody to TIMP-1 is purified from ascites fluid using protein G affinity chromatography.

Monoclonal Anti-TIMP-1 specifically binds to TIMP-1 and does not cross-react with other TIMP family members (TIMP-2, TIMP-3, TIMP-4). By immunoblotting against the reduced protein, the antibody reacts with a band at 29 kDa. It also reacts with non-reduced TIMP-1. Higher antibody concentrations may be necessary for non-human samples.

Monoclonal Anti-TIMP-1 may be used for the detection and localization of TIMP-1 by various immunochemical techniques such as immunoblotting, immunoprecipitation, immunohistochemistry, and ELISA.

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, including fibrillar and non-fibrillar collagens, fibronectin, laminin, and basement membrane glycoproteins. In general, the structure of MMPs is characterized by a signal peptide, a propeptide, and a catalytic domain containing the highly conserved zinc-binding site. 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.[1,2,3] MMPs contain the motif His-Glu-Xaa-His 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 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 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, periodontis, glumerulonephritis, 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 metastaic 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. These include the 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. TheN-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).

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 TNFα and IL-1β. 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-1 (TIMP-1) was fully sequenced and clone by Carmichael, et al.[7] It is produced and secreted in soluble form by a variety of cell types and is widely distributed throughout the body.[8] It is an extensively glycosylated protein with a molecular mass of 28.5 kDa.[9]

TIMP-1 inhibits the active forms of MMPs,[10] and complexes with the proform of MMP-9.[11-12] Like MMP-9, TIMP-1 expression is sensitive to many factors. Increased synthesis of TIMP-1 is caused by a wide variety of reagents that include: TGF-β, EGF, PDGF, FGFb, PMA, all-trans-retinoic acid (RA), IL-1 and IL-11. The human TIMP-1 gene, about 0.9 kb, has the chromosomal location of Xp11.23-11.4.[13]

Reagents
Monoclonal Anti-TIMP-1 is supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 50% glycerol and 15 mM sodium azide as preservative.

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 2-8 °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 a stimulated human cell line. (Substrate: BCIP/NBT).
Control: MMP Control-1, Product No. M2928.

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.

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

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