ANTI-TISSUE INHIBITOR OF METALLOPROTEINASE-2 (TIMP-2), SECOND LOOP
Developed in Rabbit, Affinity Isolated Antibody

Product Number T8072

Product Description
Rabbit Anti-TIMP-2, Second Loop, is developed in rabbit using a synthetic peptide corresponding to the second loop of human TIMP-2 as immunogen. Affinity-isolated antigen-specific antibody is obtained from rabbit anti-TIMP-2 antiserum by immuno-specific purification that removes essentially all rabbit serum proteins, including immunoglobulins that do not specifically bind to the peptide.

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

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 characterize the structure of the MMPs. In addition, fibronectin-like repeats, a hinge region, and a carboxy-terminal hemopexin-like domain allow categorization of MMPs into the collagenase, gelatinase, stromelysin 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 matrix 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, in diseases such as multiple sclerosis,\(^2,3,5\) 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 N-terminal region is necessary for inhibitory activity. 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 to produce the mature protein. The C-terminal regions are divergent and enhance the inhibition selectivity and binding efficiency. Although the TIMP proteins share high homology, following secretion they are localized extracellularly either in soluble form (TIMP-1, TIMP-2, and TIMP-4) or bound 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., a limited number of cell types can be induced to make these proteins).

Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) was first described by three groups in 1989.\textsuperscript{7-9} It is an unglycosylated protein that shows a 40% amino acid identity with TIMP-1.\textsuperscript{7} TIMP-2 inhibits the active forms of the MMPs and complexes with the proform of MMP-2.\textsuperscript{7,9} Like MMP-2, which binds to TIMP-2, TIMP-2 shows little inducibility, and its message levels are unaffected by TGF-β, IL-1, or TNF-α.

TIMP-2 is constitutively produced and secreted in a soluble form by a variety of cell types, with major sites in the lung and liver. The human TIMP-2 gene has the chromosomal location of 17q23-17q25.\textsuperscript{10}

Reagent
Rabbit Anti-TIMP-2, Second Loop, is supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 50% glycerol and 0.05% 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 2-8 °C for up to six months. For extended storage, the solution may be stored at 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, an alkaline phosphatase conjugated secondary antibody and BCIP/NBT as the substrate. For immunoblotting with chemiluminescent substrates, a starting dilution of 1:5,000 is recommended. Higher antibody concentrations may be necessary for non-human samples.

Note: TIMP-2 is constitutively produced in low (pg/ml) levels in most cell types in tissue culture. The low protein levels produced often require concentration of the cell culture media to visualize the bands by immunoblotting.

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

References