



3050 Spruce Street  
Saint Louis, Missouri 63103 USA  
Telephone (800) 325-5832 (314) 771-5765  
Fax (314) 286-7828  
email: [techserv@sial.com](mailto:techserv@sial.com)  
[sigma-aldrich.com](http://sigma-aldrich.com)

## Product Information

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

Product Number **T8062**

#### Product Description

Anti-Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) is developed in rabbit using a synthetic peptide corresponding to the first loop of the human TIMP-2 sequence as immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-TIMP-2 by immuno-specific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Rabbit Anti-TIMP-2, First Loop specifically binds to TIMP-2 and does not cross-react with other TIMP family members (TIMP-1, TIMP-3, TIMP-4). By immunoblotting against the reduced protein, the antibody reacts with a band at 21 kDa. It also reacts with non-reduced TIMP-2. The TIMP-2 sequence is well conserved, and the antibody also recognizes rat, mouse, porcine and bovine TIMP-2. Higher antibody concentrations may be necessary for non-human samples.

Rabbit Anti-TIMP-2 may be used for the detection and localization of TIMP-2 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, stromelysin and membrane-type MMP subfamilies.<sup>1,2,3</sup> 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, reprotoxin, 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,<sup>4</sup> and tissue remodeling, and in diseases such as multiple sclerosis,<sup>2,5</sup> Alzheimer's,<sup>2</sup> malignant gliomas,<sup>2</sup> lupus, arthritis, periodontitis, glomerulonephritis, atherosclerosis, tissue ulceration, and in cancer cell invasion and metastasis.<sup>6</sup> 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 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. 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 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 TNF- $\alpha$  and IL-1 $\beta$ . 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-2 (TIMP-2) was first described by three groups in 1989.<sup>7,8,9</sup> It is generally constitutively expressed.<sup>10</sup> Like MMP-2, which binds to TIMP-2, it shows little inducibility, and TIMP-2 message levels are unaffected by TGF- $\beta$ , IL-1 or TNF- $\alpha$ . It is produced and secreted in soluble form by a variety of cell types, with major sites in lung and liver. It is an unglycosylated protein with a molecular mass of 21 kD. TIMP-2 shows 40% amino acid identity with TIMP-1.<sup>7</sup>

TIMPs inhibit the active forms of MMPs. MMP-2 complexes with TIMP-2, but not TIMP-1.<sup>7,9</sup> The human TIMP-2 gene, about has the chromosomal location of 17q23-17q25.<sup>11</sup>

### Reagents

Rabbit Anti-TIMP-2, First Loop 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° 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-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 best results and assay sensitivity in different techniques and preparations we recommend determining optimum working dilutions by titration assay.

1. Borkakoti, N., *Prog. Biophys. Mol. Biol.*, 70, 73 (1998).
2. Yong, V.W., et al., *Trends in Neuroscience*, 21, 75 (1998).
3. Kähri, V.-M., and Saarialho-Kere, U., *Exp. Dermatol.*, 6, 199 (1997).
4. Halbert, I., et al., *Proc. Natl. Acad. Sci., USA*, 93, 9748 (1996).
5. Chandler, S., et al., *J. Neuroimmunol.*, 72, 155 (1997).
6. Birkedal-Hansen, H., et al., *Crit. Rev. Oral. Biol. Med.*, 4, 197 (1993).
7. Stetler-Stevenson, W.G., et al., *J. Biol. Chem.*, 264, 17374 (1989).
8. DeClerck, Y.A., et al., *J. Biol. Chem.*, 264, 17435 (1989).
9. Goldberg, G.I., et al., *Proc. Nat'l Acad. Sci., USA*, 86, 8207 (1989).
10. Overall, C.M., *Ann. NY Acad. Sci.*, 732, 51 (1994).
11. DeClerck, Y.A., et al., *Genomics*, 14, 782 (1992).

1pg 12/98

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.