



## Product Information

### ANTI-MATRIX METALLOPROTEINASE-1 (MMP-1)

Developed in Rabbit, IgG Fraction of Antiserum

Product Number **M 3940**

#### Product Description

Anti-Matrix Metalloproteinase-1 (MMP-1) is developed in rabbit using native human MMP-1 (interstitial collagenase) as immunogen. Whole antiserum is fractionated and then further purified by affinity chromatography on Protein G matrix to provide the IgG fraction of antiserum that is essentially free of other rabbit serum proteins.

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

Rabbit Anti-MMP-1 specifically binds to collagenase and does not cross-react with other MMP family members (MMP-2, MMP-3, MMP-9, etc). It reacts with both the reduced and non-reduced protein. By immunoblotting against the reduced protein, the antibody reacts with bands at 53 kDa and 51 kDa (the pro-form) as well as the initial active forms.

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, 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 C-terminal hemopexin-like domain allow categorization of MMPs into the collagenase, gelatinase, stomelysin and membrane-type MMP subfamilies.<sup>1-3</sup> 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 and are EC designated 3.4.24.x.

This group also contains astacin, reprotin, and serralyisin, 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. 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).

Matrix Metalloproteinase-1 (MMP-1), also known as interstitial collagenase and collagenase-1, degrades fibrillar collagens types I, II, III, VII, VIII, X, aggrecan, serpins, and  $\alpha_2$ -macroglobulin. All collagenases cleave fibrillar collagens at one specific site resulting in generation of N-terminal  $\frac{3}{4}$  and C-terminal  $\frac{1}{4}$  fragments, which then denature to gelatin at body temperature. The substrate specificity of the collagenases is variable: MMP-1 degrades type III collagen more efficiently than type I or type II collagen, whereas MMP-8 is more potent in degrading type I collagen than type III or type II collagen.<sup>7,8</sup> MMP-13, in turn degrades type II collagen 6-fold more efficiently than type I and type II collagens and displays almost 50-fold stronger gelatinolytic activity than MMP-1 and MMP-8.<sup>9,10</sup> Increased synthesis of MMP-1 is caused by a wide variety of agents that include: TNF,<sup>11,12</sup> IL-1,<sup>13</sup> serum, EGF and TGF- $\beta$ ,<sup>14-16</sup> phorbol ester tumor promoter, PMA,<sup>17</sup> ECM (extracellular matrix) components,<sup>18</sup> and polyoma and RSV infections. In contrast to these activators, several well-known antagonists, dexamethasone and all-trans-retinoic acid (RA) block the induced gene expression.<sup>19</sup> MMP-1 is expressed by fibroblasts, keratinocytes, endothelial cells, monocytes, and macrophages.

The human MMP-1 gene, about 17 kb, has the chromosomal location of 11q22.2-22.3.

### Reagent

Rabbit Anti-MMP-1 is supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 50% glycerol, and 0.05% sodium azide. 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 in 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 substrate. For chemiluminescent substrates, a starting dilution of 1:5,000 is recommended.

Control: MMP Control-1, Product Code M 2928.

Note: Collagenase levels in quiescent cells and tissues are minimal, so mitogen stimulation (TPA or TNF- $\alpha$ ) of protein synthesis is often needed to visualize the bands. In addition, cell types differ greatly in the quantity of collagenase produced.

Although the sequence of MMP-1 is well conserved, higher antibody concentrations may be necessary for non-human samples. MMP-1 has not been found in rodent species.

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

## References

1. Borkakoti, N., Prog. Biophys. Mol. Biol., **70**, 73 (1998).
2. Yong, V.W., et al., Trends in Neuroscience, **21**, 75 (1998).
3. Kähäri, 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. Hasty, K.A., et al., J. Exp. Med., **159**, 1455 (1984).
8. Mallya, S.K., et al., Biochemistry, **29**, 10628 (1990).
9. Knäuper, V., et al., J Biol. Chem., **271**, 1544 (1996).
10. Mitchell, P.G., et al., J. Clin. Invest., **97**, 761 (1996).
11. Brenner, D.A., et al., Nature, **337**, 661 (1989).
12. Sciavolina, P.J., et al., J. Biol. Chem., **269**, 21627 (1994).
13. Dayer, J.M., et al., J. Clin. Invest., **77**, 645 (1986).
14. Delany, A.M., and Brickerhoff, C.E., J. Cell Biochem., **50**, 400 (1992).
15. Edwards, D.R., et al., EMBO J., **6**, 1899 (1987).
16. Partridge, N.C., et al., Endocrinology, **120**, 1956 (1987).
17. Brickerhoff, C.E., et al., Biochemistry, **21**, 2674 (1982).
18. Shapiro, S.D., et al., J. Biol. Chem., **268**, 8170 (1993).
19. Brickerhoff, C.E., et al., N. Engl. J. Med., **303**, 432 (1980).

kaa 11/01

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.