

**ANTI-MATRIX METALLOPROTEINASE-12 (MMP-12),
C-TERMINAL**

Developed in Rabbit, Affinity Isolated Antibody

Product Number **M 0807**

Product Description

Anti-Matrix Metalloproteinase-12 (MMP-12) is developed in rabbit using a synthetic peptide corresponding to the C-terminal of human MMP-12 (macrophage elastase) as immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-MMP-12 antiserum by immuno-specific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Rabbit Anti-MMP-12, C-Terminal may be used for the detection and localization of MMP-12 by immunoblotting.

Rabbit Anti-MMP-12, C-Terminal specifically binds to MMP-12 and does not cross-react with the other MMP family members (MMP-1, MMP-2, MMP-3, MMP-9, etc). The antibody recognizes most active forms of MMP-12. The hemopexin domain is cleaved in the final form of MMP-12, thus the antibody does not detect this form. By immunoblotting against the reduced protein, the antibody reacts with bands at 54 kDa (zymogen), 45 kDa, and a cascade of other activated 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 characterizes 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, 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 matrixin subfamily and are EC designated 3.4.24.x. This group also contains astacin, reprolysin, and serralyisin, as well as other more divergent metalloproteinases. All MMPs are synthesized as proenzymes, and most of them are

Product Information

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, 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 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- α and IL-1 β . 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-12 (MMP-12, macrophage elastase) was first described in murine macrophages, later in human macrophages, and recently in other cell types. MMP-12 is also known as metalloelastase and EC 3.4.24.65. MMP-12 degrades elastin, entactin, laminin-1, fibronectin, type IV collagen as well as insulin B-chain and casein. Structurally, human MMP-12 is similar to the classical MMPs (MMP-1, MMP-3), sharing 49 % amino acid identity. MMP-12 contains a

propeptide with an autoinhibitory cysteine switch site, a well-conserved zinc site, a hinge region and a hemopexin. MMP-12 lacks a transmembrane domain and a furin cleavage site. The zymogen for MMP-12 is approximately 54 kDa and is quickly activated to the 45 kDa form, which breaks down to a cascade of active forms, ending with a 22 kDa form.

MMP-12 levels in quiescent cells and tissue are minimal (with the exception of macrophages).

Stimulated macrophages produce MMP-12. MMP-12 has also been detected in osteosarcoma cells, synovial fibroblasts and lung fibroblasts. MMP-12 plays a central role in the pathogenesis of pulmonary emphysema⁷ and atherosclerotic lesions.⁸ In addition, MMP-12 may have beneficial functions such as modulating macrophage influx to inflammatory sites and limiting growth of tumor metastases.⁹

The human MMP-12 gene has the chromosomal location of 11q22.2/22.3.¹⁰

Reagent

Rabbit Anti-MMP-12, C-Terminal is supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 50 % glycerol and 0.1 % 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 °C to 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, an alkaline phosphatase conjugated secondary antibody, and BCIP/NBT as substrate. Higher antibody concentrations may be necessary for non-human samples.

Note: Since cell types differ greatly in the quantity of MMP-12 produced, the conditioned media may require protein concentration or mitogen stimulation 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.

References

1. Borkakoti, N., Matrix metalloproteases: variations on a theme. *Prog. Biophys. Mol. Biol.*, **70**, 73 (1998).
2. Yong, V.W., et al., Matrix metalloproteinases and diseases of the CNS. *Trends in Neuroscience*, **21**, 75 (1998).
3. Kähäri, V.M., and Saarialho-Kere, U., Matrix metalloproteinases in skin. *Exp. Dermatol.*, **6**, 199 (1997).
4. Halpert, I., et al., Matrilysin is expressed by lipid-laden macrophages at sites of potential rupture in atherosclerotic lesions and localizes to areas of versican deposition, a proteoglycan substrate for the enzyme. *Proc. Natl. Acad. Sci., USA*, **93**, 9748 (1996).
5. Chandler, S., et al., Matrix metalloproteinases, tumor necrosis factor and multiple sclerosis: an overview. *J. Neuroimmunol.*, **72**, 155 (1997).
6. Birkedal-Hansen, H., et al., Matrix metalloproteinases: a review. *Crit. Rev. Oral. Biol. Med.*, **4**, 197 (1993).
7. Hautamaki, R.D., et al., Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. *Science*, **277**, 2002-2004 (1997).
8. Carmeleit, O., et al., Plasmin predisposes to atherosclerotic aneurysm formation by activation of matrix metalloproteinases. *Nature Genetics*, **17**, 439-444 (1997).
9. Dong, Z., et al., Macrophage-derived metallo-elastase is responsible for the generation of angiostatin in Lewis lung carcinoma. *Cell*, **88**, 801-810 (1997).
10. Belaouaj, A., et al., Human macrophage metallo-elastase. Genomic organization, chromosomal location, gene linkage, and tissue-specific expression. *J. Biol. Chem.*, **270**, 14568-14575 (1995).

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