

Product Information

ANTI-MATRIX METALLOPROTEINASE-20 (MMP-20), C-Terminal

Developed in Rabbit, Affinity Isolated Antibody

Product Number **M5809**

Product Description

Anti-Matrix Metalloproteinase-20 (MMP-20) is developed in rabbit using a synthetic peptide corresponding to the C-terminal of human MMP-20 (enamelysin) as immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-MMP-20 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-20, C-Terminal may be used for the detection and localization of MMP-20 by various immunochemical techniques including immunoblotting, immunoprecipitation, immunohistochemistry, and ELISA.

Rabbit Anti-MMP-20, C-Terminal specifically binds to MMP-20 and does not cross-react with the other MMP family members (MMP-1, MMP-2, MMP-3, MMP-9, etc). The antibody recognizes the pro-form and most of the active forms of MMP-20. The final active form of MMP-20 lacks the hemopexin domain and the antibody does not detect this form. By immunoblotting against the reduced protein, the antibody reacts with bands at 58 kDa (proenzyme), 45 kDa (active), and a series of further cleaved active forms.^{7,8,9} Anti-MMP-20, C-terminal, also recognizes non-reduced MMP-20.

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, stomelysin 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 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, 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-20 (MMP-20), also known as enamelysin, was first described in bovine dental pulp.¹⁰ It is a classical type-I collagenase, which is similar to MMP-1 in its ability to cleave intact type-I collagen at the same site in all three strands. Activated MMP-20 has a functional role in the initial cleavage^{11, 12} and degradation⁹ of amelogenin, a major protein component of the enamel matrix. Because MMP-20 plays an important role in the process of tooth enamel formation, it may also be involved in the pathogenesis of odontogenic tumors.¹³

Until recently, it was thought that MMP-20 expression was restricted to dental pulp (enamel). MMP-20 has been detected in other cell types, such as osteosarcoma cells, HeLa cells, and gingival fibroblasts.

The human MMP-20 gene has the chromosomal location of 11q22.⁹

Reagent

Rabbit Anti-MMP-20, 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 the substrate. Higher antibody concentrations may be necessary for non-human samples.

Note: Most cell types do not produce MMP-20; it has been found in osteosarcoma cells, HeLa cells, and gingival fibroblasts. MMP-20 levels in quiescent cells and tissues are minimal (with the exception of dental pulp). Mitogen stimulation or protein concentration is often needed to visualize the bands by immunoblotting. In addition, cell types differ greatly in the quantity of MMP-20 produced.

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**, 73-94 (1998).
2. Yong, V.W., et al., Matrix metalloproteinases and diseases of the CNS. *Trends in Neuroscience*, **21**, 75-80 (1998).
3. Kähäri, V.M., and Saarialho-Kere, U., Matrix metalloproteinases in skin. *Exp. Dermatol.*, **6**, 199-213 (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**, 9748-9753 (1996).
5. Chandler, S., et al., Matrix metalloproteinases, tumor necrosis factor and multiple sclerosis: an overview. *J. Neuroimmunol.*, **72**, 155-161 (1997).
6. Birkedal-Hansen, H., et al., Matrix metalloproteinases: a review. *Crit. Rev. Oral. Biol. Med.*, **4**, 197-250 (1993).
7. Bartlett, J.D., et al., Molecular cloning and mRNA tissue distribution of a novel matrix metalloproteinase isolated from porcine enamel organ. *Gene*, **183**, 123-128 (1996).
8. Grant, G.M., et al., Overview of expression of matrix metalloproteinases (MMP-17, MMP-18, and MMP-20) in cultured human cells. *Matrix Biol.*, **18**, 145-148 (1999).
9. Llano, E., et al., Identification and structural and functional characterization of human enamelysin (MMP-2). *Biochemistry*, **36**, 15101-15108 (1997).

10. Den Besten, P.K., et al., Purification and sequencing of a 21 kDa and 25 kDa bovine enamel metalloproteinase. *Eur. J. Oral Sci.*, **106**, 345-349 (1998).
11. Li, W., et al., Activation of recombinant bovine matrix metalloproteinase-20 and its hydrolysis of two amelogenin oligopeptides. *Eur. J. Oral Sci.*, **107**, 352-359 (1999).
12. Bartlett, J.D., and Simmer, J.P., Proteinases in developing dental enamel. *Crit. Rev. Oral Biol. Med.*, **10**, 425-441 (1999).
13. Takata, T. et al., Immunohistochemical detection and distribution of enamelysin (MMP-20) in human odontogenic tumors. *J. Dent. Res.*, **79**, 1608-1613 (2000).

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