

Product Information

ANTI-MATRIX METALLOPROTEINASE-8 (MMP-8), N-TERMINAL OF ACTIVE ENZYME

Developed in Rabbit, Affinity Isolated Antibody

Product Number **M 8933**

Product Description

Anti-Matrix Metalloproteinase-8 (MMP-8) is developed in rabbit using a synthetic peptide corresponding to the aminoterminal end of the active enzyme site of human matrix metalloproteinase-8 (neutrophil collagenase, E.C. 3.4.24.34, collagenase-2) as immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-MMP-8 by immuno-specific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Rabbit Anti-MMP-8, N-Terminal of active enzyme specifically reacts against the reduced protein, the antibody reacts with bands at 64 kDa and 58 kDa and a series of further active forms, by immunoblotting. It also reacts with non-reduced MMP-8. The antibody does not cross-react with other MMP family members (MMP-1, MMP-2, MMP-3, MMP-9, etc.). Higher concentrations of antibody may be needed for non-human samples.

Rabbit Anti-MMP-8 may be used for the detection and localization of MMP-8 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, stomelysin and membrane-type MMP subfamilies.^{1,2,3} 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, reprolysin, 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,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-8 (MMP-8) is also known as neutrophil collagenase and collagenase-2. MMP-8 degrades fibrillar collagens types I, II, III, aggrecan, serpins and α_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 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.^{7,8} 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.^{9,10} MMP-8 is very similar to MMP-1, sharing 57% amino acid identity. Until recently it was thought to be produced exclusively by neutrophils, but recently it has been detected in other cell types. MMP-8 is heavily glycosylated, and the zymogen has a mass of 85 kDa. The human MMP-8 gene has the chromosomal location of 11q22.2-22.3.

Reagents

Rabbit Anti-MMP-8, N-Terminal of the active enzyme 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 pro-longed storage, clarify the solution by centrifugation before use.

Product Profile

Recommended working dilution of 1:1,000 is determined by immunoblotting using a conditioned media from human neutrophils. (Substrate: BCIP/NBT).

Note: Collagenase levels in quiescent cells and tissues is minimal, and stimulation or protein concentration is often needed to visualize the bands. In addition, cell types differ greatly in the quantity of collagenase 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., Prog. Biophys. Mol. Biol., **70**, 73 (1998).
2. Yong, V.W., et al., Trends in Neuroscience, **21**, 75 (1998).
3. K@h@ri, 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. Kn@uper, V., et al., J Biol. Chem., **271**, 1544 (1996).
10. Mitchell, P.G., et al., J. Clin. Invest., **97**, 761 (1996).

lpg 6/00

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.