



ANTI-MATRIX METALLOPROTEINASE-8 (MMP-8), C-TERMINAL

Developed in Rabbit, Affinity Isolated Antibody

Product Number **M 8305**

Product Description

Anti-Matrix Metalloproteinase-8 (MMP-8) is developed in rabbit using a synthetic peptide corresponding to the C-terminal of human MMP-8 (neutrophil collagenase) as immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-MMP-8 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-8, C-terminal may be used for the detection and localization of MMP-8 by various immunochemical techniques such as immunoblotting, immunoprecipitation, immunohistochemistry and ELISA.

Rabbit Anti-MMP-8, C-terminal specifically binds to neutrophil collagenase (MMP-8) and does not cross-react with other MMP family members (MMP-1, MMP-2, MMP-3, MMP-9, etc). The zymogen of MMP-8 is heavily glycosylated and has a mass of 85 kDa. By immunoblotting against the reduced protein, the antibody detects bands at 64 kDa, 58 kDa and a series of further cleaved active forms. It will also react with non-reduced MMP-8. The antibody does not detect the smallest active form of MMP-8 since the hemopexin domain is cleaved off.

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

Product Information

are EC designated 3.4.24.x. This group also contains astacin, reprotolysin, 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. Most cell types do not produce MMP-8. Until recently, it was thought that MMP-8 was produced exclusively by neutrophils, but it has also been detected in other cell types including arthritic chondrocytes and gingival fibroblasts. The human MMP-8 gene has the chromosomal location of 11q22.2-22.3.

Reagent

Rabbit Anti-MMP-8, 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: Collagenase levels in quiescent cells and tissues are minimal, and stimulation of 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

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