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Product Information

ANTI-MATRIX METALLOPROTEINASE-9 (MMP-9), PROPEPTIDE REGION

Developed in Rabbit, Affinity Isolated Antibody

Product Number **M 0306**

Product Description

Anti-Matrix Metalloproteinase-9 (MMP-9) is developed in rabbit using a synthetic peptide corresponding to the propeptide region of human MMP-9 (gelatinase-B) as immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-MMP-9 antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Rabbit Anti-MMP-9, Propeptide Region may be used for the detection and localization of MMP-9 by various immunochemical techniques such as immunoblotting, immunoprecipitation, immunohistochemistry and ELISA.

Rabbit Anti-MMP-9, Propeptide Region specifically binds to gelatinase-B (MMP-9) and does not cross-react with other MMP family members (MMP-1, MMP-2, MMP-3, MMP-8, etc). Anti-MMP-9, propeptide region recognizes both native and reduced forms of MMP-9. By immunoblotting against the reduced protein, the antibody detects a band at 92 kDa (zymogen). The propeptide region is shed after activation; therefore, the antibody recognizes only the latent form of MMP-9 and not any of the active forms, thus this antibody can be used to differentiate latent from activated MMP-9.

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 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-9 (MMP-9) is also known as gelatinase B and 92 kDa type IV collagenase. The expression of MMP-9 is more restricted than MMP-2. MMP-9 is produced by keratinocytes and stored in the granules of neutrophils and eosinophils, but not expressed by dermal fibroblasts. MMP-9 degrades gelatin, type IV, V and XIV collagens, α_2 -macroglobulin, elastin, vitronectin and proteoglycans. MMP-2 and MMP-9 are thought to play an important role in the final degradation of fibrillar collagens after initial cleavage by collagenases. Interestingly, recent reports provide evidence that both gelatinases also possess collagenolytic activity. MMP-2 cleaves native type I collagen to N-terminal $\frac{3}{4}$ and C-terminal $\frac{1}{4}$ fragments identical to those generated by collagenases.⁸ In addition, MMP-9, which is expressed specifically by osteoclasts during murine fetal development and in adult human bone, has been shown to cleave type I, II and V collagens in the N-terminal non-helical telopeptide.⁹

Because of their ability to initiate and continue degradation of fibrillar collagen type I, MMP-2 and MMP-9 play an important role in the remodeling of collagenous ECM (extracellular matrix) than has been previously thought. In general, inducers such as PMA, EGF, IL-1 β , or TNF α enhance MMP-9 production without altering MMP-2 levels, whereas TGF β , which down-regulates most MMPs, enhances the expression of both MMP-2 and MMP-9.¹⁰ The human MMP-9 gene has the chromosomal location of 20q12-13.

Reagent

Rabbit Anti-MMP-9, Propeptide Region 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.

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

Note: MMP-9 is constitutively produced in some tumor cell lines (i.e., HT1080, HL60, and U937) but not in most quiescent cells and tissues. Treatment of cells with the phorbol ester TPA stimulates production of MMP-9 in some cell types, but the low protein levels produced (pg/ml) often require concentration of cell culture media to visualize the bands by immunoblotting. MMP-2 and MMP-9 may be enriched from conditioned cell culture media by binding to gelatin-agarose, and eluting with 10 % DMSO.

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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