Product Information

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Anti-Matrix Metalloproteinase-9, C-Terminal
produced in rabbit, affinity isolated antibody

Catalog Number **M5177**

Synonym: Anti- MMP-9

Product Description
Anti-Matrix Metalloproteinase-9, C-Terminal, is produced in rabbit using as immunogen a synthetic peptide corresponding to the C-terminal of human MMP-9 (gelatinase B). Affinity isolated antibody is obtained by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-MMP-9, C-Terminal specifically binds to gelatinase B and does not cross-react with other MMP family members (MMP-1, MMP-2, MMP-3, etc). By immunoblotting against the reduced protein, the antibody reacts with bands at 92 kDa and 88 kDa (the proform and active form). It does not react as well with non-reduced MMP-9. Therefore the antibody has limited use in immunoprecipitation, Immunohisto-chemistry and ELISA. When used on reduced samples from tissue culture media, the antibody may also recognize a band at 65 kDa, which may be removed by enriching the gelatinase on gelatin agarose. Higher antibody concentrations may be necessary for non-human samples.

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 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, periodontis, glumerulonephritis, 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 metastaic 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 TNFα and IL-1β. 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, 92 kDa type IV collagenase. Expression of MMP-9 is more restricted than MMP-2: it 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 ¾ and C-terminal ¼ 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 shown to cleave type I, II and V collagens in the N-terminal non-helical telopeptide.[9] It is therefore possible that due to their ability to initiate and continue degradation of fibrillar collagen of type I, MMP-2 and MMP-9 play a more important role in the remodeling of collagenous ECM than has been previously thought.

In general, inducers such as PMA, EGF, IL-1β, or TNFα enhance MMP-9 production without altering MMP-2 levels, and TGFβ, which downregulates most MMPs, enhances both MMP-2 and MMP-9 expression.[10] The human MMP-9 gene has the chromosomal location of 20q12-13.

Reagents
Supplied in phosphate buffered saline, pH 7.4, containing 50% glycerol and 0.05% sodium azide as preservative.

Protein concentration: ~1 mg/ml.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet 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 at –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
Immunoblotting: a working dilution of 1:1,000 is determined using a concentrated cell culture media from a stimulated human cell line.
Substrate: BCIP/NBT.

Note: MMP-9 is constitutively produced in some tumor cell lines (i.e., HT1080, HL60, 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.

Note: 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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