

Product Information

Anti-Matrix Metalloproteinase-2

produced in rabbit, IgG fraction of antiserum

Catalog Number **M4065**

Product Description

Anti-Matrix Metalloproteinase-2 (MMP-2) is produced in rabbit using as immunogen a synthetic peptide from the second half of human MMP-2 (Gelatinase-A, 72 KDa Gelatinase). The immunogen is synthesized as a 631 amino acid proenzyme that is activated by cleavage of the first 80 amino acids. Whole antiserum is fractionated and then further purified by ion-exchange chromatography to provide the IgG fraction of antiserum that is essentially free of other rabbit serum proteins.

Anti-Matrix Metalloproteinase-2 specifically binds to gelatinase-A and does not cross-react with other MMP family members (MMP-1, MMP-3, MMP-9, etc). The antibody recognizes MMP-2 (cytoplasmic localization) from human, mouse, rat, and bovine. Anti-MMP-2 may be used for the detection and localization of MMP-2 by various immunochemical techniques such as immunoblotting (~72 kDa) and immunohistochemistry.

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-2 (MMP-2) is also known as gelatinase A, MMP-2, or 72 kDa type IV collagenase. MMP-2 is constitutively expressed in several types of cells in culture (i.e., epidermal keratinocytes, dermal fibroblasts). MMP-2 degrades gelatin, type IV, V, VII, X, and XI collagens, fibronectin, elastin, laminin, vitronectin, tenascin, 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, 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 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 had 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, and TGF β , which down-regulates most MMPs, enhances both MMP-2 and MMP-9 expression.¹⁰ The human MMP-2 gene has the chromosomal location of 16q13.

Reagent

Supplied in 10 mM phosphate buffered saline, pH 7.4, containing 0.2% bovine serum albumin and 0.09% sodium azide.

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 12 months. For extended storage, the solution may be aliquoted and stored at -20 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

Immunoblotting: a working concentration of 5-10 μ g/mL is recommended.

Immunohistochemistry a working concentration of 10-20 μ g/mL is recommended. No special pretreatment is required for staining formalin-fixed paraffin-embedded sections.

Controls: MMP Control-1, Catalog Number M2928.

Also, conditioned serum-free medium from TPA-treated human fetal lung (HFL-1) cells. Also, placenta cells or bladder, breast, and ovarian carcinoma cells may be used.

Note: Gelatinase-A is constitutively produced in quiescent cells and tissues, and the enzyme has a high specific activity against denatured collagen. 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.

Although the sequence homology for this portion of MMP-2 is well conserved, higher antibody concentrations may be necessary for non-human samples.

Note: In order to obtain best results and assay sensitivity in various techniques and preparations, we recommend determining optimum working dilutions by titration assay.

References

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