

Product Information

Matrix Metalloproteinase-12 (MMP-12)

from human fibroblast cells

Product Number **M 7442**

Storage Temperature $-70\text{ }^{\circ}\text{C}$

Synonyms: Macrophage Elastase, Metalloelastase;
EC 3.4.24.65

Product Description

Human Matrix Metalloproteinase-12 (MMP-12) is a matrix metalloproteinase that has been substrate-affinity purified from human fibroblasts. MMP-12 is essentially free of other matrix metalloproteinases and tissue inhibitors of metalloproteinases (TIMPs).

Matrix Metalloproteinase-12 (MMP-12) may be used as a control for immunoblotting and ELISA as well as for enzyme kinetics assays, and substrate assays. This product is a mixture of zymogen and active enzyme. By immunoblotting, bands are detected at approximately 54 kDa (zymogen) and 45 kDa (active). The purity is >95% by SDS-PAGE visualized by silver staining.

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 characterize 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.¹⁻⁴ 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 ion and two calcium ions structurally. They fall within the matrixin subfamily and are EC designated 3.4.24.x. This group also contains astacin, reprolysin, and serralsin, 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 tightly bound inhibitory complexes with the MMPs.⁴ 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-12 was first discovered in media conditioned by activated macrophages.⁸ It is also known as Macrophage Elastase, due to structural similarities. MMP-12 was originally thought to be macrophage specific, and is now known to be expressed by a wider range of cells. Like the "classical" secreted MMPs, MMP-12 is secreted as an inactive enzyme. The zymogen is activated by a proteolytic

cascade, which removes the propeptide domain after the cysteine switch motif. Macrophage elastase is further processed by cleavage of the hemopexin domain, leaving the catalytic domain. The 54 kDa zymogen is reduced to 45 kDa by enzymatic cleavage after the cysteine switch sequence, and then to approximately 22 kDa by removal of the hemopexin domain.⁸

MMP-12 is primarily expressed at low levels in normal macrophages, and at much higher levels in smoke-induced macrophages.¹⁰ A mouse model for cigarette smoke-induced emphysema was developed in a MMP-12 knockout line.¹⁰ In the MMP-12 emphysema model, mice deficient in MMP-12 did not develop emphysema after being subjected to cigarette smoke, while wild type mice did develop emphysema.

MMP-12 is not constitutively produced by most other tissues, but rather, its synthesis is induced in specific tissues. MMP-12 substrate specificity is similar to other secreted MMPs. It degrades elastin, collagen IV, laminin, fibronectin, serpins such as alpha-1 proteinase inhibitor,¹² alpha-2 antiplasmin and plasminogen activator inhibitor-2, but not interstitial collagens. MMP-12 also processes angiostatin¹¹ and TNF- α . When MMP-12 cleaves α -1 proteinase inhibitor, the cleaved protein becomes a chemoattractant for neutrophils.¹² MMP-12 is up regulated by the tumor promoter phorbol 12-myristate 13-acetate (PMA), TNF- α , EGF, and IL-1.

The gene for human MMP-12 is located on chromosome 11q22.2 and mouse MMP-12 is located on chromosome 9.

Reagent

Matrix Metalloproteinase-12 (MMP-12) is supplied in a buffer, containing 10 mM MES, pH 6.5, 0.10 M sodium chloride, 5 mM calcium chloride, 0.025% Brij-35, 50% glycerol (v/v), with 0.01% sodium azide as preservative. Each vial contains approximately 5 μ g of human MMP-12.

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

Store at -70°C in aliquots. Repeated freezing and thawing is not recommended. Storage in frost-free freezers is not recommended.

References

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