

Product Information

Matrix Metalloproteinase-12, Catalytic Domain human, recombinant, expressed in *E. coli*

Catalog Number **M9695**

Storage Temperature -70°C

EC 3.4.24.65

Synonyms: MMP-12, metalloelastase, macrophage elastase

Product Description

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.²⁻⁴ 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 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, 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.

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, but 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 ~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 α -1 proteinase inhibitor,¹² α -2 antiplasmin, and plasminogen activator inhibitor-2, but not interstitial collagens. MMP-12 also processes angiotensin¹¹ 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.

This recombinant, human Matrix Metalloproteinase-12, Catalytic Domain product is produced from human DNA expressed in *E. coli*. The enzyme consists of the catalytic domain of human MMP-12 (amino acid residues 84-255) with a C-terminal purification tag. It is supplied in a solution of 50 mM Tris, pH 9.5, with 5 mM calcium chloride, 500 mM sodium chloride, 20 μ M zinc chloride, 0.5% Brij® L23, and 30% glycerol.

The enzyme may be used to study enzyme kinetics, cleave target substrates, and screen for inhibitors.

Molecular mass: 20.3 kDa (calculated)

Purity: \geq 95% (SDS-PAGE).

The enzyme runs as a doublet (~20 kDa). The higher band represents the polypeptide and spontaneous cleavage of the tag results in the lower band. Both possess identical enzymatic activities.

Activity (One unit = 100 pmole/minute at 37 °C) is determined using the colorimetric substrate Ac-Pro-Leu-Gly-S-Leu-Leu-Gly-OEt.

Preincubation of MMP-12 catalytic domain (4 nM) with the inhibitor NNGGH (20 nM) or with the broad spectrum inhibitor GM6001 (5 nM) for 1 hour completely inhibits enzymatic activity.

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

Store the product at -70 °C.

The enzyme remains active on ice for several hours. However, it is recommended that thawing and dilution of the enzyme be done just prior to the start of the assay. After initial defrost, aliquot and refreeze at -70 °C. Avoid repeated freeze/thaw cycles.

References

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