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Product Information

Matrix Metalloproteinase-9, human, recombinant
buffered aqueous solution

Catalog Number **M8945**
Storage Temperature –70 °C

EC 3.4.24.35
Synonyms: MMP-9; Gelatinase-B; 95 kDa Gelatinase

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 pro-peptide, 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 (where X represents any amino acid) that binds zinc in the catalytic site, as well as another zinc molecule and two calcium molecules structurally. MMPs 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. Most MMPs 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 important roles in wound healing, apoptosis, bone elongation, embryo development, uterine involution, angiogenesis,[5] and tissue remodeling, and in diseases such as multiple sclerosis,[3,6] Alzheimer's,[3] malignant gliomas,[3] lupus, arthritis, periodontis, glomerulonephritis, athero-sclerosis, tissue ulceration, and in cancer cell invasion and metastasis.[7] Numerous studies have shown close associations between expression of various members of the MMP family by tumors and their proliferative and invasive behavior and metastatic potential.

Human Matrix Metalloproteinase-9 is a type IV collagenase that degrades a broad range of substrates including gelatin, type IV, V, and XIV collagens, α2-macroglobulin, elastin, vitronectin, and proteoglycan. Structurally, MMP-9 is divided into five distinct domains:

- a pro-domain which is cleaved upon activation
- a catalytic domain containing the zinc binding site
- a fibronectin-like domain that has a role in substrate targeting
- a collagen-like domain
- a carboxyl terminal (hemopexin-like) domain.

Expression of MMP-9 is more restricted than MMP-2. MMP-2 and MMP-9 are thought to play important roles in the final degradation of fibrillar collagens after initial cleavage by collagenases. Both MMP-2 and MMP-9 have been reported also to 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 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 are thought to play a more important role in the remodeling of collagenous ECM (extracellular matrix).

In general, inducers such as PMA, EGF, IL-1β, or TNF-α enhance MMP-9 production without altering MMP-2 levels; whereas, TGF-β, that down regulates most MMPs, enhances the expression of both MMP-2 and MMP-9.[10] MMP-9 is produced by keratinocytes and PMN leukocytes. Monocytes and macrophages also produce MMP-9.

This recombinant, human Matrix Metalloproteinase-9 (MMP-9) product is from a DNA sequence encoding the human MMP-9 enzyme, and is expressed in a Chinese Hamster Ovary (CHO) cell line. It is supplied as a 0.2 μm filtered solution of 50 mM Tris-HCl, pH 7.5, with 10 mM $CaCl_2$, 150 mM NaCl, and 0.05% (w/v) BRIJ 35.

Note: Centrifuge the vial before opening to recover the entire contents of the vial. Because of possible sublimation during storage, the buffer volume may decrease over time. However, the product is sold by mass and the amount of protein will remain constant. To ensure a quantitative recovery, it is suggested to prepare the stock solution in the original vial.

Human MMP-9 can be used as a positive control in enzymatic and other assays. This recombinant MMP-9 protein has a predicted molecular mass of ~77 kDa. By SDS-PAGE, the apparent molecular mass is ~93 kDa.

Purity: > 90% (SDS-PAGE, visualized by silver stain)

Procedure
To activate recombinant human MMP-9, prepare a *p*-aminophenylmercuric acetate (APMA) solution in DMSO. Add the APMA solution to the rhMMP-9v to a final APMA concentration of 1 mM. Incubate at 37 °C for 16−24 hours.

Specific activity: > 1,300 pmoles/min/µg

Specific activity is measured with 10 µM of the fluorogenic substrate (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-(3-[2,4-dinitrophenyl]-L-2,3-diaminopropionyl)-Ala-Arg-NH_2 (Mca-PLGL-Dpa-AR-NH_2) and 0.2 ng/µL of activated enzyme, at room temperature, in an aqueous buffer of 50 mM Tris-HCl, pH 7.5, with 10 mM $CaCl_2$, 150 mM NaCl, and 0.05% (w/v) BRIJ 35. Cleavage of Mca-PLGL-Dpa-AR-NH_2 can be measured at excitation and emission wavelengths of 320 nm and 405 nm, respectively.

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses.

Storage/Stability
The product ships on dry ice. Storage at −70 °C or below in aliquots is recommended. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. This product may be aliquoted and stored under sterile conditions at −70 °C in a manual defrost freezer.

References

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