Matrix Metalloproteinase-9 
human, recombinant 
expressed in mouse NSO cells

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 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.

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, and tissue remodeling, and in diseases such as multiple sclerosis, Alzheimer’s, malignant gliomas, lupus, arthritis, periodontitis, glomerulonephritis, atherosclerosis, tissue ulceration, and in cancer cell invasion and metastasis. 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.

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, and a carboxyl terminal (hemopexin-like) domain.

The expression of MMP-9 is more restricted than MMP-2. 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 MMP-2 and MMP-9 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. 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. 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. 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 expressed in a mouse myeloma cell line, NSO. It is supplied in a 0.2 µm filtered solution of 50 mM Tris-HCl, pH 7.5, with 10 mM calcium chloride, 150 mM sodium chloride, and 0.05% BRIJ® 35.

Note: Centrifuge the vial before opening to recover the entire contents of the vial. Due to 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. The 688 amino acid residue recombinant protein has a predicted molecular mass of ~77 kDa. By SDS-PAGE, the apparent molecular mass is ~93 kDa.

Purity: >95% (SDS-PAGE, visualized by silver stain)

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.

The specific activity is measured with 10 µM of substrate and 20 ng of activated enzyme in 100 µL of buffer (50 mM Tris-HCl, pH 7.5, with 10 mM calcium chloride, 150 mM sodium chloride, and 0.05% BRIJ 35) at room temperature. The fluorogenic substrate is (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-(3-[2,4-dinitrophenyl]-L-2,3-diaminopropionyl)-Ala-Arg-NH₂. Cleavage of the substrate can be measured at excitation and emission wavelengths of 320 nm and 405 nm, respectively.

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
The product ships on dry ice and 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
11. For a recent review, see Collier, I.E., et al., Gelatinase B Handbook of Proteolytic Enzymes (San Diego, CA:1998), 1205-1210."

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