3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Matrix Metalloproteinase-9, human recombinant, expressed in transfected cells

Catalog Number **M4809**
Storage Temperature –70 °C

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

Product Description

Human Matrix Metalloproteinase-9 (MMP-9) is a matrix metalloproteinase that has been substrate-affinity purified from transfected cells (pro and active forms).

Matrix Metalloproteinase-9 (MMP-9) may be used in various immunochemical techniques such as immunoblotting, ELISA, enzyme kinetics assays, and substrate assays. This preparation consists primarilay of the proenzyme form, with a small amount of active enzyme and TIMP present. The following bands may be detected:

MMP-9 Proenzyme (>85%) at ~88 kDa non-reduced and 92 kDa reduced
minor band (MMP-9 dimer) at ~180 kDa
intermediate active form (very small amounts) at ~84 kDa non-reduced
mature active form (very small amounts) at 82 kDa non-reduced
TIMP-1 (~10%) at 28 kDa

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,[5] and tissue remodeling, and in diseases such as multiple sclerosis,[3,6] Alzheimer's,[3] malignant gliomas,[3] lupus, arthritis, periodontis, glomerulonephritis, atherosclerosis, tissue ulceration, and in cancer cell invasion and metastasis.[7] 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-1β. 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).

Human Matrix Metalloproteinase-9 is a type IV collagenase that degrades a broad range of substrates including gelatin, type IV, V, and XIV collagens, α_2-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.[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. The human MMP-9 gene has the chromosomal location of 20q12-13.

This MMP-9 product is supplied in a solution of 5 mM Tris-HCl, pH 7.5, containing 0.1 mM calcium chloride and 0.005% BRIJ® 35. TIMP-1, an endogenous inhibitor of MMP-9, is often complexed with this enzyme *in vivo* and may be present in low quantities (~10%). The zymogen can be activated by incubation at 37 °C for 2–6 hours with the organomercurial compound APMA at 1 mM.[1]

Purity: >85% (SDS-PAGE)

Note: MMP-9 is constitutively produced in some tumor cell lines (i.e., HT1080 fibrosarcoma cells, HL60 myeloid leukemia cells, and U937 monoblastoid cells) and transformed cells, but not in most quiescent cells and tissues. Treatment of cells with the phorbol ester TPA stimulates production of MMP-9 in some cell types, but the low protein levels produced (pg/ml) often require concentration of the cell culture medium to visualize the bands by immunoblotting. MMP-2 and MMP-9 may be enriched from conditioned cell culture medium by binding to gelatin-agarose and eluting with 10% DMSO.

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.

References

1. Seifert, W.F., *et al*., Gut, **39**, 114-119 (1996).
2. Borkakoti, N., Prog. Biophys. Mol. Biol., **70**, 73 (1998).
3. Yong, V.W., *et al*., Trends Neurosci., **21**, 75 (1998).
4. Kähäri, V.M., and Saarialho-Kere, U., Exp. Dermatol., **6**, 199 (1997).
5. Halbert, I., *et al*., Proc. Natl. Acad. Sci., USA, **93**, 9748 (1996).
6. Chandler, S., *et al*., J. Neuroimmunol., **72**, 155 (1997).
7. Birkedal-Hansen, H., *et al*., Crit. Rev. Oral. Biol. Med., **4**, 197 (1993).
8. Aimes, R.T., and Quigley, J.P., J.Biol Chem., **270**, 5872 (1995).
9. Okada, Y., et al., Lab. Invest., **72**, 311 (1995).
10. Borden, P., and Heller, R.A., Crit. Rev. Eukaryot. Gene Exp., **7**, 159 (1997).

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