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-2
mouse, recombinant
expressed in mouse NSO cells

Catalog Number **M9445**
Storage Temperature –20 °C

EC 3.4.24.24
Synonyms: MMP-2; Gelatinase-A, 72 kDa Type IV Collagenase

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.[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 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 (ECM) 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, periodontis, 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-2 (MMP-2) degrades gelatin, type IV, V, VII, X, and XI collagens, fibronectin, elastin, laminin, vitronectin, tenascin, proteoglycans, and a range of extracellular matrix conponents *in vivo*. MMP-2 and MMP-9 play an important role in the final degradation of fibrillar collagens after initial cleavage by collagenases. Interestingly, reports provide evidence that both gelatinases 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 cleaves 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 play an important role in the remodeling of collagenous ECM.

In general, inducers such as PMA, EGF, IL-1β, or TNF-α enhance MMP-9 production without altering MMP-2 levels, and TGF-β, which down regulates most MMPs, enhances both MMP-2 and MMP-9 expression.[10] MMP-2 is constitutively expressed in several types of cells in culture (i.e., epidermal keratinocytes, dermal fibroblasts).

This recombinant, mouse Matrix Metalloproteinase-2 product is a highly purified recombinant enzyme from a DNA sequence encoding pro mouse MMP-2 (amino acid residues 1-662)[11] expressed in a mouse myeloma cell line, NSO. The amino acid sequences of the proenzymes are identical between mouse and rat. The predicted N-terminus of the proenzyme starts at Ala^{30}. The N-terminal sequence of the purified recombinant mouse MMP-2 corresponds to I_{34}IKFPGDVAP. By SDS-PAGE under reducing conditions, the apparent molecular mass is ~72 kDa. The product is supplied as a 0.2 μm filtered solution of 25 mM Tris, pH 7.5, with 5 mM calcium chloride, 75 mM sodium chloride, 0.025% BRIJ® 35, and 50% glycerol.

MMP-2 may be used to study enzyme kinetics, cleave target substrates, and screen for inhibitors.

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

To activate rmMMP-2, prepare an APMA (*p*-amino-phenylmercuric acetate) concentrate in DMSO. Incubate rmMMP-2 (40 μg/mL) with APMA (1 mM) in TCNB buffer (50 mM Tris, pH 7.5, with 10 mM calcium chloride, 150 mM sodium chloride, and 0.05% BRIJ 35) at 37 °C for 2 hours.

Specific activity: >1,500 pmoles/min/μg

The specific activity is measured with 10 μM of the fluorogenic substrate and 10 ng activated enzyme in 100 μL of TCNB buffer 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-NH_2. Cleavage of the substrate can be measured at excitation and emission wavelengths of 320 nm and 405 nm, respectively.

Endotoxin: <1.0 EU per 1 μg of the protein (LAL method)

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.

Preparation Instructions
It is recommended that TCNB buffer (50 mM Tris, pH 7.5, with 10 mM calcium chloride, 150 mM sodium chloride, and 0.05% BRIJ 35) be used to dilute the enzyme before and during assays.

Storage/Stability
The product ships with wet ice and storage at −20 °C is recommended. Avoid repeated freeze/thaw cycles.

Upon reconstitution, the enzyme can be aliquoted and stored under sterile conditions at −20 °C or below.

References

1. Borkakoti, N., Prog. Biophy. Mol. Biol., **70**, 73 (1998).
2. Yong, V.W., et al., Trends in Neuroscience, **21**, 75 (1998).
3. Kähäri, V.M., and Saarialho-Kere, U., Exp. Dermatol., **6**, 199 (1997).
4. Halbert, I., *et al*., Proc. Natl. Acad. Sci., USA, **93**, 9748 (1996).
5. Chandler, S., *et al*., J. Neuroimmunol., **72**, 155 (1997).
6. Birkedal-Hansen, H., *et al*., Crit. Rev. Oral. Biol. Med., **4**, 197 (1993).
7. Hasty, K.A., *et al*., J. Exp. Med., **159**, 1455 (1984).
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. Eukar. Gene Exp., **7**, 159 (1997).
11. Reponen, *et al*., J. Biol. Chem., **267**, 7856-7862 (1992).

KAA,MAM 07/07-1