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Product Information

Matrix Metalloproteinase-2, human recombinant, expressed in mouse NSO cells

Catalog Number **M9070**
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, athero-sclerosis, tissue ulceration, and in cancer cell invasion and metastasis.[6] Numerous studies have shown 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, human Matrix Metalloproteinase-2 product is a highly purified recombinant enzyme from a DNA sequence encoding pro human MMP-2[11] expressed in a mouse myeloma cell line, NSO. The 631 amino acid residue recombinant protein has a predicted mass of 71 kDa and by SDS-PAGE, the apparent molecular mass is ~69 kDa.

The product is supplied lyophilized from a 0.2 μM filtered solution of 50 mM Tris, 5 mM $CaCl_2$, 150 mM NaCl, and 1 μM $ZnCl_2$, pH 7.5.

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

Molecular mass: ~69 kDa (apparent) and 71 kDa (predicted)

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

To activate pro-MMP-2, prepare an APMA (*p*-amino-phenylmercuric acetate) concentrate in DMSO. Add APMA to pro-MMP-2, to give a final APMA concentration of 1 mM. Incubate at 37 °C for 1 hour.

Specific activity: >1,000 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 (50 mM Tris, 10 mM calcium chloride, 150 mM sodium chloride, and 0.05% Brij® L23, pH 7.5) 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/µg 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
Use 0.1 mL of buffer (100 mM Tris, 10 mM calcium chloride, 150 mM sodium chloride, and 0.05% Brij L23, pH 8.0) to prepare an enzyme stock solution (100 µg/mL).

Storage/Stability
The product is shipped at ambient temperature. Upon receiving, store it immediately at –20 °C or below. Avoid repeated freeze/thaw cycles.

Upon reconstitution, the enzyme stock solution 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. Chandler, S. *et al*., Neuroscience Letter, **201**, 223-226 (1995).

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