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ProductInformation

Matrix Metalloproteinase-2 (MMP-2)
from human fibroblasts

Product Number **M 1927**

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

Product Description

Matrix Metalloproteinase-2 (MMP-2) is a matrix metalloproteinase that has been substrate-affinity purified from cell culture media of stimulated human fibroblasts. MMP-2 is free of its endogenous inhibitor, TIMP-2, and other matrix metalloproteinases.

Matrix Metalloproteinase-2 (MMP-2) may be used in various immunochemical techniques such as immunoblotting, ELISA, enzyme kinetics assays, and substrate assays. This enzyme consists as a mixture of the zymogen and active enzyme. By immunoblotting, bands are detected at approximately 72 kDa (pro-form) and 68 kDa (active form).

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 characterizes 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.[1-3] 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,[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.

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).

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 have an important role in the final degradation of fibrillar collagens after initial cleavage by collagenases. Interestingly, recent 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 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 play a more important role in the remodeling of collagenous ECM (extracellular matrix) than had been previously thought.

In general, inducers such as PMA, EGF, IL-1β, or TNFα enhance MMP-9 production without altering MMP-2 levels, and TGFβ, 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). The human MMP-2 gene has the chromosomal location of 16q13.

Reagent
The product is supplied as a solution in 10 mM sodium phosphate, pH 7.4, 250 mM sodium chloride, 2% DMSO, and 50% (v/v) glycerol.

Storage/Stability
The product ships on dry ice and storage at –70 °C in aliquots is recommended. Repeated freezing and thawing, and storage in "frost-free" freezers are not recommended. TIMP-2, an endogenous inhibitor to MMP-2, is often complexed with this enzyme *in vivo*, but it has been removed from this preparation, leaving MMP-2 unstable and it must be stored carefully.

Product Profile
Purity: minimum 95% (SDS-PAGE, visualized by silver stain)

Note: Low protein levels produced (pg/ml) often require concentration of cell culture media to visualize the bands by immunoblotting. MMP-2 and MMP-9 may be enriched from conditioned cell culture media by binding to gelatin-agarose, and eluting with 10 % DMSO.

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).

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