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ProductInformation

Matrix Metalloproteinase-13, Proform human, recombinant, His-tagged, expressed in *E. coli*

Catalog Number **M5695**
Storage Temperature –70 °C

Synonyms: Procollagenase-3; Pro-MMP-13; EC 3.4.24.

Product Description
Matrix Metalloproteinase-13 (MMP-13), Proform is a matrix metalloproteinase that is a recombinant 452 amino acid polypeptide corresponding to human Pro-MMP-13, with an additional C-terminal His-tag with the sequence GVTHHHHHH expressed in *E. coli* and purified from periplasm. The calculated molecular mass of the recombinant protein is ~52 kDa. Upon activation with APMA activated MMP-13 is formed.

Matrix Metalloproteinase-13, Proform may be used as a control for immunoblotting and ELISA as well as for enzyme kinetics assays and substrate assays. In immunoblotting assays, pro-MMP-13 appears as a major band at ~60 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.[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 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.[6,7] 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.

ProMMP-13 consists of 452 amino acids[8] and due to N-linked glycosylation, the actual molecular mass is ~60 KDa .[9] The following domains and sequence regions can be distinguished within the protein:[8,9] N-terminal propeptide (confers latency to the proenzyme), Ca^{2+} and Zn^{2+} ion binding catalytic domain, hinge region, and C-terminal hemopexin-like domain. Latent procollagenase-3 can be activated by proteases such as stromelysin,[9] gelatinase A, MT1-MMP, and plasmin[9] or incubation with APMA.[9] The molecular mass of active collagenase-3, which begins with the N-terminal sequence YNVFPRTL is 48 kDa.

Pro-MMP-13 hydrolyzes type II collagen 5-6 times faster than type I and type III collagens. The enzyme also exhibits high activity towards gelatin and degrades SERPINS such as α_1-antichymotrypsin and plasminogen activator inhibitor-2.[9] Collagenase-3 is inhibited by TIMP-1, TIMP-2, and TIMP-3 in a 1:1 stoichiometric fashion.

Pro-MMP-13 is expressed during fetal bone development. In adult human tissues, pro-MMP-13 has been detected only in pathological conditions: malignant tumors,[8] chronic ulcers,[11] arthritic cartilage,[12] and synovium.[13] The recombinant catalytic domain of pro-MMP-13 may be used for the study of degradation of extracellular matrix proteins. The enzyme allows screening of matrix metalloproteinase inhibitors and characterization of inhibitor action.

The product is supplied in a solution containing 50 mM Tris-HCl, pH 7.5, 150 mM sodium chloride, 5 mM calcium chloride, and 0.05% Brij® 35.

Purity: >95% (SDS-PAGE)

Activation: 19.5 µL of enzyme product is mixed with 0.5 µL of 40 mM APMA (*p*-aminophenyl mercuric acetate in DMSO) solution. Incubate the mixture for 30 minutes at 37 °C. After incubation, the mixture should be stored on ice until use for activity assays.

Specific activity: 250-300 milliunits/mg (activated enzyme)

Unit definition: One unit is the activity that hydrolyzes 1 µmole of peptide (7-methoxycoumarin-4-acetyl-Pro-Leu-Gly-Leu-Dpa-Ala-Arg) within 1 minute.[14]

Inhibitors: Pro-MMP-13 is inhibited by TIMPs and by chelators of divalent cations such as EDTA or *o*-phenanthroline.

Precautions and Disclaimer
Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability
Store at –70 °C in undiluted aliquots. The enzyme may be stored at –20 °C for a few weeks without significant loss of activity. Repeated freezing and thawing is not recommended. Storage in frost-free freezers is not recommended.

References

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