

Product Information

MMP Control-1

Catalog Number **M2928**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

Product Description

MMP Control-1 is a concentrate of serum-free, cell culture medium from human skin fibroblasts treated with TPA (phorbol 12-myristate 13-acetate).

MMP Control-1 may be used as a qualitative positive control for immunoblotting and zymography. The concentrate contains MMP-1 (interstitial collagenase), MMP-2 (gelatinase-A), MMP-3 (stromelysin-1), MMP-9 (gelatinase-B), TIMP-1, and TIMP-2. It is not recommended as a quantitative control.

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, the structure of MMPs is characterized by a signal peptide, a propeptide, and a catalytic domain containing the highly conserved zinc-binding site. In addition, fibronectin-like repeats, a hinge region, and a C-terminal hemopexin-like domain allow categorization of MMPs into the collagenase, gelatinase, stromelysin, and membrane-type MMP subfamilies.¹⁻³ MMPs contain the motif His-Glu-Xaa-His 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 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, periodontitis, glomerulonephritis, atherosclerosis, tissue ulceration, and in cancer cell invasion and metastasis.⁶ Also, changes in the extracellular matrix gene expression may be observed in left ventricular hypertrophy.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.

Reagent

MMP Control-1 is supplied as a concentrated (20 to 30-fold) serum free, tissue culture supernatant with 0.05% sodium azide as a preservative. Product was tested and found negative for HIV and Hepatitis.

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 $-20\text{ }^{\circ}\text{C}$ is recommended. After first use, store in working aliquots at $-20\text{ }^{\circ}\text{C}$. Repeated freezing and thawing is not recommended.

Procedure

For immunoblotting, 20–40 μ L is sufficient for most applications. It is recommended that you reduce the sample with 2-mercaptoethanol.

Results

MMP-1 is present at 55 and 53 kDa, and has many breakdown products. MMP-1 does not show up well on gelatin or casein zymograms.

MMP-2 is found at 66 kDa and 62 kDa on a gelatin zymogram, and will appear weakly at the same size on a casein zymogram.

MMP-3 is found at 57–59 kDa on reduced immunoblots and is found at 45 kDa on a casein zymogram (the zymogen is less active on a zymogram).

TIMP-1 is typically seen as a “fuzzy” band at 28–30 kDa on immunoblots under reducing conditions.

TIMP-2 is found at 24 kDa on immunoblots under standard conditions.

Note: For zymography, the activity of the MMPs is affected by the substrate composition of the zymogram.

References

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4. Halbert, I., *et al.*, *Proc. Natl. Acad. Sci., USA*, **93**, 9748 (1996).
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6. Birkedal-Hansen, H., *et al.*, *Crit. Rev. Oral. Biol. Med.*, **4**, 197 (1993).
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