ANTI-MATRIX METALLOPROTEINASE-24 (MMP-24, MT5-MMP), CYTOPLASMIC DOMAIN
Developed in Rabbit, Affinity Isolated Antibody

Product Number M 6559

Product Description
Anti-Matrix Metalloproteinase-24 (MMP-24, MT5-MMP), is developed in rabbit using a synthetic peptide corresponding to the cytoplasmic domain of mouse MMP-24 as immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-MMP-24 antisera by immuno-specific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to the peptide. MMP-24 is also known as membrane-type matrix metalloproteinase-5 (MT5-MMP).

Rabbit Anti-MMP-24 (MT5-MMP), Cytoplasmic Domain may be used for the detection and localization of human and mouse MMP-24, which has greater than 95% homology, by various immunochemical techniques including immunoblotting, immunoprecipitation, immunohistochemistry, cell sorting, and ELISA.

Rabbit Anti-MMP-24, Cytoplasmic Domain specifically binds to MMP-24 and does not cross-react with the other MMP family members (MMP-1, MMP-2, MMP-3, MMP-9, etc). The cytoplasmic domain of MMP-24 is detached when MT-MMPs shed from the membrane, thus this antibody should not detect these forms of MMP-24. By immunoblotting against the reduced protein, the antibody identifies a band at 65 kDa (zymogen) and also the activation and breakdown products. Anti-MMP-24, cytoplasmic domain also recognizes non-reduced (native) MMP-24.

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. 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, and tissue remodeling, and in diseases such as multiple sclerosis, Alzheimer’s, malignant gliomas, lupus, arthritis, periodontitis, glomerulonephritis, atherosclerosis, tissue ulceration, and in cancer cell invasion and metastasis. 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-24 (MMP-24, MT5-MMP) has been described as a proteoglycanase, which is critical...
in the turnover of ECM (extracellular matrix) components in the brain.\(^8\) MMP-24 contains a candidate signal sequence, a propeptide region, a potential furin recognition motif, a zinc-binding catalytic domain, a hemopexin-like domain, a 24-residue hydrophobic region, which acts as a potential transmembrane domain, and a short cytosolic domain.\(^1\) Unlike other MT-MMPs, MMP-24 (MT5-MMP) sheds from the cell surface as soluble proteinases, allowing its versatility as both a cell bound and soluble proteinase in ECM remodeling processes.\(^1\)

MMP-24 is elevated in several tumor cell lines, and is also produced by some normal cell lines. It is predominantly expressed in the brain, indicating a role in nervous system development.\(^6\) MMP-24 is also highly expressed during embryonic development. The mouse MMP-24 gene maps to chromosome 2.\(^10\)

Reagent
Rabbit Anti-MMP-24 (MT5-MMP), Cytoplasmic Domain is supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 50 % glycerol and 0.1 % sodium azide. The protein concentration is approximately 1 mg/ml.

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
For continuous use, store at 2 °C to 8 °C for up to six months. For extended storage, the solution may be stored 0 °C to −20 °C. The antibody is supplied with 50 % glycerol to prevent freezing. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile
A working dilution of 1:2,000 is determined by immunoblotting using a concentrated cell culture media from a stimulated cell line, an alkaline phosphatase conjugated secondary antibody and BCIP/NBT as the substrate. Higher antibody concentrations may be necessary for non-human samples.

Note: Since cell types differ greatly in the quantity of MMP-24 produced, the conditioned media may require mitogen stimulation to visualize the bands by immunoblotting. Treatment of cells with concanavalin-A or the phorbol ester TPA stimulates production of MMP-24 in some cell types, and the enzyme can be recovered in cell lysates.

In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimum working dilutions by titration assay.

References
5. Halpert, I., et al., Matrilysin is expressed by lipid-laden macrophages at sites of potential rupture in atherosclerotic lesions and localizes to areas of versican deposition, a proteoglycan substrate for the enzyme. Proc. Natl. Acad. Sci., USA, 93, 9748-9753 (1996).

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