



## Product Information

### Anti-Matrix Metalloproteinase-25, Hinge Region

Developed in Rabbit  
Affinity Isolated Antibody

Product Number **M 4817**

#### Product Description

Anti-Matrix Metalloproteinase-25 (MMP-25), Hinge Region is developed in rabbit using a synthetic peptide corresponding to the hinge region of human matrix metalloproteinase-25 (MMP-25), also known as membrane-type matrix metalloproteinase-6 (MT6-MMP) as immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-MMP-25 antiserum by immuno-specific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Matrix Metalloproteinase-25, Hinge Region may be used for the detection and localization of human and murine matrix metalloproteinase-25 (MMP-25, MT6-MMP). The antibody specifically binds to MMP-25 and does not cross react with the other MMP family members (MMP-1, MMP-2, MMP-3, MMP-9, etc.). By immunoblotting against the reduced protein, the antibody identifies bands at 65 kDa, as well as activation/breakdown products.

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.<sup>1-3</sup> 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 serralyisin, 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,<sup>4</sup> and tissue remodeling, and in diseases such as multiple sclerosis,<sup>2,5</sup> Alzheimer's,<sup>2</sup> malignant gliomas,<sup>2</sup> lupus, arthritis, periodontitis, glomerulonephritis, atherosclerosis, tissue ulceration, and in cancer cell invasion and metastasis.<sup>6</sup> 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- $\alpha$  and IL-1 $\beta$ . 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-25, also known as membrane-type matrix metalloproteinase-6 and leukolysin, was initially cloned from human leukocytes and found to be specifically expressed by peripheral blood leukocytes.<sup>7</sup> The expression of MMP-25 (leukolysin) at the mRNA level appears to be localized to neutrophils.<sup>8</sup> MMP-25 was also found in lung, spleen, brain tumors, and several tumor cell lines. Like the other membrane-type metalloproteinases, MMP-25 is associated with the cell membrane, but is anchored to the cell surface via a GPI (glycosyl-phosphatidyl inositol) attachment, rather than through a transmembrane domain.<sup>9</sup> MMP-25 is proteolytically active and truncated forms digest gelatin in zymograms. Like the other MT-MMPs, MMP-25 has a furin cleavage site, where prohormone convertases can activate the zymogen. MMP-25 can be shed from the cell surface, into the culture media under stimulatory conditions.

MMP-25 is expressed in brain tumors and may facilitate tumor progression through its ability to activate progelatinase A at the cell membrane in colon carcinomas or brain tumors.<sup>10</sup> MMP-25 may also play a role in the cellular migration and invasion of the extracellular matrix and basement membranes.<sup>11</sup>

The MMP-25 gene maps to human chromosome 16p13.3.

### Reagent

Anti-Matrix Metalloproteinase-25, Hinge Region is supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 50% glycerol and 0.05% 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 2-8 °C for up to six months. For extended storage, the solution may be stored -20 °C. Do not store below -22 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

### Product Profile

By immunoblotting, a minimum working dilution of 1:1,000 is recommended using a cell lysate, an alkaline phosphatase conjugated secondary antibody, and BCIP/NBT as the substrate. A starting dilution of 1:5,000 of anti-MMP-25 is recommended for chemiluminescent substrates.

Treatment of cells with concanavolin-A or the phorbol ester TPA stimulates production of MMP-25 in some cell types, and the enzyme can be recovered in cell lysates.

Note: Higher antibody dilutions may be necessary for non-human samples.

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

### References

1. Borkakoti, N., Matrix metalloproteases: variations on a theme. *Prog. Biophys. Mol. Biol.*, **70**, 73-94 (1998).
2. Yong, V.W., et al., Matrix metalloproteinases and diseases of the CNS. *Trends in Neuroscience*, **21**, 75-80 (1998).
3. Kähäri, V.M., and Saarialho-Kere, U., Matrix metalloproteinases in skin. *Exp. Dermatol.*, **6**, 199-213 (1997).
4. 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).
5. Chandler, S., et al., Matrix metalloproteinases, tumor necrosis factor and multiple sclerosis: an overview. *J. Neuroimmunol.*, **72**, 155-161 (1997).
6. Birkedal-Hansen, H., et al., Matrix metalloproteinases: a review. *Crit. Rev. Oral. Biol. Med.*, **4**, 197-250 (1993).
7. Pei, D., Leukolysin/MMP25/MT6-MMP: a novel matrix metalloproteinase specifically expressed in the leukocyte lineage. *Cell Res.*, **9**, 291-303 (1999).
8. Kang, T., et al., Subcellular distribution and cytokine- and chemokine-regulated secretion of leukolysin/MT6-MMP/MMP-25 in neutrophils. *J. Biol. Chem.*, **276**, 21960-21968 (2001).

9. Kojima, S., et al., Membrane-type 6 matrix metalloproteinase (MT6-MMP, MMP-25) is the second glycosyl-phosphatidyl inositol (GPI)-anchored MMP. FEBS Lett., **480**, 142-146 (2000).
10. Velasco, G., et al., Human MT6-matrix metalloproteinase: identification, progelatinase A activation, and expression in brain tumors. Cancer Res., **60**, 877-882 (2000).
11. English, W.R., et al., Catalytic activities of membrane-type 6 matrix metalloproteinase (MMP25). FEBS Lett., **491**, 137-142 (2001).

kaa 04/03

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.