



## Product Information

### **Anti-Matrix Metalloproteinase-28, Prohormone Region**

Developed in Rabbit  
Affinity Isolated Antibody

Product Number **M 4691**

#### **Product Description**

Anti-Matrix Metalloproteinase-28 (MMP-28), Prohormone Region is developed in rabbit using a synthetic peptide corresponding to the prohormone convertase region (furin family cleavage site) of human matrix metalloproteinase epilysin (MMP-28) as immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-MMP-28 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-28, Prohormone Region may be used for the detection and localization of human matrix metalloproteinase epilysin (MMP-28). By immunoblotting against the reduced protein, the antibody identifies bands at 62 kDa and 58 kDa, as well as breakdown products at 50 kDa, 48 kDa, and 46 kDa. The antibody specifically binds to MMP-28 and does not cross react with the other MMP family members (MMP-1, MMP-2, MMP-3, MMP-9, etc.).

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 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,<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-28, also known as epilysin, was first cloned from human skin keratinocytes and described to reflect its role in the remodeling of the epidermis.<sup>7</sup> It was found in testis, as well as heart, brain, placenta, lung, prostate, intestine, and colon. MMP-28 was later cloned from human lung and found in lung, kidney, brain, skeletal muscle, and several tumor cell lines.

At least three MMP-28 transcripts of 2.6, 2.0, and 1.2 kb have been reported possibly representing alternatively spliced forms of MMP-28.<sup>7</sup> There are two human sequences (isoform 1 and isoform 2) which encode proteins of 520 and 393 amino acids with predicted masses of 58.9 and 44.5 kDa respectively. The mouse sequence contains two inserts of 41 and 39 amino acids respectively, relative to the human sequence. Mouse epilysin has a predicted molecular mass of approx. 70 kDa. Mouse and human epilysin are highly conserved and share 97% identical residues.

Epilysin (MMP-28) contains the key domains of the other MMPs: a signal peptide, conserved cysteine-containing prodomain (with a furin cleavage site), conserved histidine-containing catalytic domain, hinge domain, and hemopexin domain.<sup>7,8</sup> MMP-28 has a furin cleavage site, similar to MMP-11, and is cleaved by the prohormone convertase family of enzymes. Sequence identity between MMP-28 and the other MMPs is low overall. MMP-28 is most closely related to MMP-19 (39% sequence identity). Recombinant MMP-28 degrades casein. EDTA and batimastat (selective MMP inhibitor) inhibits the proteolytic activity of epilysin.

MMP-28 is expressed in tumors as well as normal adult and fetal tissues. It is involved in homeostasis, wound repair, and cell proliferation (during epithelial repair).<sup>9</sup>

The MMP-28 gene maps to chromosome 17q11.2 and includes 8 exons and 7 introns.<sup>8</sup>

### Reagent

Anti-Matrix Metalloproteinase-28, Prohormone 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

A minimum working dilution of 1:1,000 is determined by immunoblotting using stimulated human keratinocytes, an alkaline phosphatase conjugated secondary antibody and BCIP/NBT as the substrate. A starting dilution of 1:5,000 is recommended for chemiluminescent substrates.

Higher antibody concentrations may be necessary for non-human samples.

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

### References

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