**Recombinant Human MMP-3**

**Catalog Number:** 513-MP

### DESCRIPTION

**Source**
Mouse myeloma cell line, NS0-derived human MMP-3 protein

Tyr18-Cys477 (Lys45Glu) Accession # P08254

**N-terminal Sequence Analysis**
Tyr18

**Structure / Form**
Pro form

**Predicted Molecular Mass**
52 kDa

### SPECIFICATIONS

**SDS-PAGE**
54-56 kDa doublet, reducing conditions

**Activity**
Measured by its ability to cleave the fluorogenic peptide substrate, Mca-µF16 Black Maxisorp Plate (Nunc, Catalog # 475515)

**Endotoxin Level**
<1.0 EU per 1 µg of the protein by the LAL method.

**Purity**
>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.

**Formulation**
Supplied as a 0.2 µL µL µL µL 2 µL µL 3 months, 10 rhMMP 3 months, Dilute activated rhMMP Measured by its ability to cleave the fluorogenic peptide substrate, Mca-µF16 Black Maxisorp Plate (Nunc, Catalog # 475515)

**Stability & Storage**
The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

**Materials**
- Assay Buffer: 50 mM Tris, 10 mM CaCl₂, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Recombinant Human MMP-3 (rhMMP-3) (Catalog # 513-MP)
- Chymotrypsin (Sigma, Catalog # C-3142), 1 mg/mL stock in 1 mM HCl
- Phenylmethyl Sulfonyl Fluoride (PMSF) (Sigma, Catalog # P-7626), 0.2 M stock in 2-Propanol
- Substrate: MCA-Arg-Pro-Lys-Pro-Val-Glu-NVAL-Trp-Arg-Lys(DNP)-NH₂ (Catalog # ES002), 2 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

**Activity Assay Protocol**

1. Activate rhMMP-3 at 20 µg/mL in Assay Buffer containing 5 µg/mL Chymotrypsin.
2. Incubate reaction at 37 °C for 30 minutes.
3. Stop activation with 2 mM PMSF. Pre-activate rhMMP 1 week before use. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 20 µM Substrate.
4. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity

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\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}^* \text{ (RFU/min) } \times \text{ Conversion Factor}^{**}}{\text{amount of enzyme (µg)}}
\]

*Adjusted for Substrate Blank

**Final Assay Conditions**

- **Per Well:**
  - rhMMP-3: 0.125 µg
  - Substrate: 10 µM

**PREPARATION AND STORAGE**

**Shipping**
The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage**

Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

**BACKGROUND**

Matrix metalloproteinases are a family of zinc and calcium dependent endopeptidases with the combined ability to degrade all the components of the extracellular matrix. MMP-3 (stromelysin-1), can degrade a broad range of substrates including collagen α chains, aggregan, laminin, fibronectin, elastin, casein, α-1 antitrypsin, myelin basic protein, IL-1α, IGFBP-3, pro MMP-1, pro MMP-7, pro MMP-8, pro MMP-9 and pro MMP-13. MMP-3 does not cleave the triple helical region of interstitial collagens, a characteristic which distinguishes the stromelysins from the collagenases. The MMP-3 substrate repertoire extends beyond extracellular matrix proteins and implicates MMP-3 in roles other than direct tissue remodelling, for instance, enzyme cascades and cytokine regulation. MMP-3 is expressed by fibroblasts, chondrocytes, osteoblasts, endothelial cells, smooth muscle cells and macrophages. Structurally, MMP-3 may be divided into several distinct domains; a pro-domain which is cleaved upon activation; a catalytic domain containing the zinc binding site; a short hinge region and a carboxyl terminal (hemopexin-like) domain.