

## 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  $\alpha$  chains, aggrecan, laminin, fibronectin, elastin, casein,  $\alpha$ -1 antitrypsin, myelin basic protein, IL-1 $\beta$ , 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.

## Description

<b>Source</b>	Murine myeloma cell line, NS0-derived Tyr18 - Cys477 (Lys45Glu) Accession # P08254
<b>N-terminal Sequence Analysis</b>	Tyr18
<b>Structure / Form</b>	Pro form
<b>Predicted Molecular Mass</b>	52 kDa

## Specifications

<b>SDS-PAGE</b>	54 - 56 kDa doublet, reducing conditions
<b>Activity</b>	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH <sub>2</sub> , R&D Systems, Catalog # ES002. The specific activity is > 150 pmoles/min/ $\mu$ g, as measured under the described conditions. See Activity Assay Protocol.
<b>Endotoxin Level</b>	<1.0 EU per 1 $\mu$ g of the protein by the LAL method.
<b>Purity</b>	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
<b>Formulation</b>	Supplied as a 0.2 $\mu$ m filtered solution in Tris, CaCl <sub>2</sub> , NaCl, Brij-35 and Glycerol. See Certificate of Analysis for details.

## Preparation and Storage

<b>Shipping</b>	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>6 months from date of receipt, -20 to -70 °C as supplied.</li> <li>3 months, -20 to -70 °C under sterile conditions after opening.</li> </ul>

## Activity Assay Protocol

### Materials

- Assay Buffer: 50 mM Tris, 10 mM CaCl<sub>2</sub>, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Recombinant human MMP-3 (R&D Systems, Catalog # 513-MP)
- Chymotrypsin (Sigma, Catalog # C-3142)
- 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)-NH<sub>2</sub> (R&D Systems, 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

### Assay

- Activate rhMMP-3 at 20  $\mu$ g/mL in Assay Buffer containing 5  $\mu$ g/mL Chymotrypsin.
- Incubate reaction at 37 °C for 30 minutes.
- Stop activation with 2 mM PMSF. Pre-warm the PMSF to 37 °C prior to adding to sample.
- Dilute activated rhMMP-3 to 2.5 ng/ $\mu$ L in Assay Buffer.
- Dilute Substrate to 20  $\mu$ M in Assay Buffer.
- In a plate load 50  $\mu$ L of 2.5 ng/ $\mu$ L rhMMP-3, and start the reaction by adding 50  $\mu$ L of 20  $\mu$ M Substrate to wells. Include a Substrate Blank containing 50  $\mu$ L Assay Buffer and 50  $\mu$ L of 20  $\mu$ M Substrate.
- Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
- Calculate specific activity

$$\text{Specific Activity (pmoles/min}/\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* \text{ (RFU/min)} \times \text{Conversion Factor}^{**} \text{ (pmole/RFU)}}{\text{amount of enzyme } (\mu\text{g})}$$

\*Adjusted for Substrate Blank

\*\*Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975)

### Final Assay Conditions Per Well

- rhMMP-3: 0.125  $\mu$ g
- Substrate: 10  $\mu$ M

12/28/2009

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS