Recombinant Human MMP-12

Catalog Number: 917-MP

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Leu17-Cys470 Accession # P39900
N-terminal Sequence Analysis	Leu17
Structure / Form	Pro form
Predicted Molecular Mass	52 kDa

SPECIFICATIONS

SDS-PAGE	56 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-PLGL-Dpa-AR-NH_2 (Catalog # ES001). The specific activity is >500 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in MES, NaCl and $CaCl_2$. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Recombinant Human MMP-12 (rhMMP-12) (Catalog # 917-MP)
- *p*-aminophenylmercuric acetate (APMA), (Sigma, Catalog # A-9563), 100 mM stock in DMSO
- Substrate MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH_2 (Catalog # ES001), 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

1. Dilute rhMMP-12 to 50 µg/mL in Assay Buffer.
2. Activate rhMMP-12 by adding APMA to a final concentration of 1 mM.
3. Incubate at 37 °C for 4 hours to activate.
4. Dilute activated rhMMP-12 to 0.4 ng/µL in Assay Buffer.
5. Dilute Substrate to 20 µM in Assay Buffer.
6. Load into plate 50 µL of 0.4 ng/µL rhMMP-12, and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 20 µM Substrate.
7. Read at excitation and emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes.
8. Calculate specific activity:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Well:

- rhMMP-12: 0.020 µg
- Substrate: 10 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

Matrix metalloproteinases (MMPs) are a family of zinc and calcium dependent endopeptidases with the combined ability to degrade all the components of the extracellular matrix. MMP-12 (macrophage elastase) is found in macrophages and its expression in monocytes can be induced by cytokines such as GM-CSF and CD40 signaling (1). In addition to elastin, MMP-12 can degrade a broad spectrum of substrates, including type IV collagen, fibronectin, laminin, vitronectin, proteoglycans, chondroitin sulfate, myelin basic protein, α_1-antitrypsin, and plasminogen. It can also activate MMP-2 and MMP-3. MMP-12 is required for macrophage-mediated proteolysis and matrix invasion in mice. MMP-12 is proposed to have a direct role in the pathogenesis of aortic aneurysms and in the development of pulmonary emphysema that results from chronic inhalation of cigarette smoke. Structurally, the pro MMP-12 consists of following domains: a pro domain, a catalytic domain containing the zinc-binding site, and a C-terminal hemopexin-like domain. The rhMMP-12 corresponds to the pro form that can be activated by autocatalysis under the conditions described above.

References:

1. S.D. Shapiro *et al.* (2004) in *Handbook of Proteolytic Enzymes* (eds. A.J. Barrett *et al.*) pp.540 - 544, Academic Press, San Diego.

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