Recombinant Human MMP-1

Catalog Number: 901-MP

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Phe20-Asn469 Accession # P03956
N-terminal Sequence Analysis	Phe20
Structure / Form	Pro form
Predicted Molecular Mass	52 kDa

SPECIFICATIONS

SDS-PAGE	52-55 kDa, reducing conditions
Activity	Measured by its ability to cleave a fluorogenic peptide substrate Mca-KPLGL-Dpa-AR-NH_2 (Catalog # ES010). The specific activity is > 400 pmol/min/µg, as measured under the described conditions.
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 µm filtered solution in MES, NaCl, $CaCl_2$ and Brij-35. 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-1 (rhMMP-1) (Catalog # 901-MP)
- *p*-aminophenylmercuric acetate (APMA), (Sigma, Catalog # A-9563), 100 mM stock in DMSO
- Substrate: MCA-Lys-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH_2 (R&D Systems, Catalog # ES010)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhMMP-1 to 50 µg/mL in Assay Buffer.
2. Activate 50 µg/mL rhMMP-1 by adding APMA to a final concentration of 1 mM.
3. Incubate at 37 °C for 2 hours.
4. Dilute activated rhMMP-1 to 1 ng/µL in Assay Buffer.
5. Dilute Substrate to 20 µM in Assay Buffer.
6. Load into a black well plate 50 µL of 1 ng/µL rhMMP-1 and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer, 50 µL Substrate, and no rhMMP-1.
7. Read at excitiation 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-1: 0.050 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, -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-1 (interstitial collagenase), can degrade a broad range of substrates including types I, II, III, VII, VIII, and X collagens as well as casein, gelatin, α-1 antitrypsin, myelin basic protein, L-Selectin, pro-TNF, IL-1β, IGF-BP3, IGF-BP5, pro MMP-2 and pro MMP-9. A significant role of MMP-1 is the degradation of fibrillar collagens in extracellular matrix remodeling, characterized by the cleavage of the interstitial collagen triple helix into ¾, ¼ fragments. However, as the list of substrates above illustrates, the role of MMP-1 is more diverse than originally envisaged, and may involve enzyme cascades, cytokine regulation and cell surface molecule modulation. MMP-1 is expressed by fibroblasts, keratinocytes, endothelial cells, monocytes and macrophages. Structurally, MMP-1 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.

References:

1. Cawston, T.E. (2004) in *Interstitial Collagenase*. Barrett, A.J. *et al.* (eds): Handbook of Proteolytic Enzymes, San Diego: Academic Press, p. 472.

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