Recombinant Mouse MMP-7

Catalog Number: 2967-MP

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived mouse MMP-7 protein Met1-Leu264 Accession # AAA99983
N-terminal Sequence Analysis	Leu18
Structure / Form	Pro form
Predicted Molecular Mass	28 kDa

SPECIFICATIONS

SDS-PAGE	31 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 >2000 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	>90%, 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 Glycerol. 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 Mouse MMP-7 (rmMMP-7) (Catalog # 2967-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, (Catalog # ES010)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rmMMP-7 to 100 µg/mL in Assay Buffer.
2. Activate rmMMP-7 by adding APMA to a final concentration of 1 mM.
3. Incubate at 37 °C for 1 hour.
4. Dilute activated rmMMP-7 to 0.4 ng/µL in Assay Buffer.
5. Dilute Substrate to 120 µM in Assay Buffer.
6. In a plate, load 50 µL of 0.4 ng/µL rmMMP-7 and start the reaction by adding 50 µL of 120 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 120 µM Substrate.
7. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), 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:

- rmMMP-7: 0.020 µg
- Substrate: 60 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 (MMPs) are a family of zinc and calcium dependent endopeptidases with the combined ability to degrade all the components of the extracellular matrix. MMP-7 (matrilysin) is expressed in epithelial cells of normal and diseased tissues, and is capable of digesting a large series of proteins of the extracellular matrix including collagen IV and X, gelatin, casein, laminin, aggrecan, entactin, elastin and versican (1). MMP-7 is implicated in the activation of other proteinases such as plasminogen, MMP-1, MMP-2, and MMP-9. In addition to its roles in connective tissue remodeling and cancer, MMP-7 also regulates intestinal α-defensin activation in innate host defense, releases tumor necrosis factor-α in a model of herniated disc resorption, and cleaves FasL to generate a soluble form in a model of prostate involution. Structurally, MMP-7 is the smallest of the MMPs and consists of two domains: a pro-domain that is cleaved upon activation and a catalytic domain containing the zinc-binding site.

References:

1. Woessner, J.F. (2004) in *Handbook of Proteolytic Enzymes,* Barrett, A.J. *et al.* eds. p. 532.

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