Recombinant Mouse MMP-9

Catalog Number: 909-MM

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived mouse MMP-9 protein Ala20-Pro730 Accession # P41245
N-terminal Sequence Analysis	Ala20
Structure / Form	Pro form
Predicted Molecular Mass	78 kDa

SPECIFICATIONS

SDS-PAGE	80-105 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 >1,500 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, $CaCl_2$, NaCl 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% Brij-35 (w/v), pH 7.5 (TCNB)
- Recombinant Mouse MMP-9 (rmMMP-9) (Catalog # 909-MM)
- *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 rmMMP-9 to 100 µg/mL in Assay Buffer.
2. Activate rmMMP-9 by adding APMA to a final concentration of 1 mM.
3. Incubate at 37 °C for 2 hours.
4. Dilute activated rmMMP-9 to 0.4 ng/µL in Assay Buffer.
5. Dilute Substrate to 20 µM in Assay Buffer.
6. Load into a black well plate 50 µL of the 0.4 ng/µL rmMMP-9 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 without any rmMMP-9.
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:

- rmMMP-9: 0.02 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.

DATA

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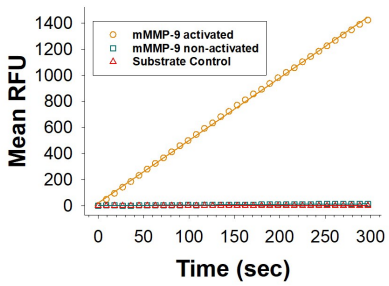
Enzyme Activity



Recombinant Mouse MMP-9 (Catalog # 909-MM) is measured by its ability to cleave the fluorogenic peptide substrate, Mca-PLGL-Dpa-AR-NH2 (Catalog # ES001).

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-9 (gelatinase B) can degrade a broad range of substrates including gelatin, collagen types IV and V, elastin and proteoglycan core protein. It is believed to act synergistically with interstitial collagenase (MMP-1) in the degradation of fibrillar collagens as it degrades their denatured gelatin forms. MMP-9 is produced by keratinocytes, monocytes, macrophages and PMN leukocytes. MMP-9 is present in most cases of inflammatory responses. Structurally, MMP-9 may be divided into five distinct domains: a pro-domain which is cleaved upon activation, a gelatin-binding domain consisting of three contiguous fibronectin type II units, a catalytic domain containing the zinc binding site, a proline-rich linker region, and a carboxyl terminal hemopexin-like domain. Compared to the Recombinant Human MMP-9 (Catalog # 911-MP), the mouse enzyme contains extra sequences in the linker region and in the hemopexin-like domain, respectively.

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