Recombinant Human MMP-9

Catalog Number: 911-MPN

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ala20-Asp707(Gln279Arg) Accession # P14780
N-terminal Sequence Analysis	Ala20
Structure / Form	Pro form
Predicted Molecular Mass	76 kDa

SPECIFICATIONS

SDS-PAGE	97 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 >1,300 pmol/min/µg 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 Tris-HCl, 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% Brij-35 (w/v), pH 7.5 (TCNB)
- Recombinant Human MMP-9 (rhMMP-9) (Catalog # 911-MPN)
- *p*-aminophenylmercuric acetate (APMA), (Sigma, Catalog # A-9563), prepare a 100 mM stock in DMSO
- Substrate: MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH_2 (Catalog # ES001)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhMMP-9 to 100 µg/mL in Assay Buffer.
2. Activate rhMMP-9 by adding APMA to a final concentration of 1 mM.
3. Incubate at 37 °C for 24 hours.
4. Dilute activated rhMMP-9 to 0.2 ng/µL in Assay Buffer.
5. Dilute Substrate to 20 µM in Assay Buffer.
6. Load 50 µL of the 0.2 ng/µL rhMMP-9 into a plate and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing 50 µL of 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-9: 0.010 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-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 maybe 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. In addition to the human enzyme, the recombinant mouse MMP-9 is also available (Catalog # 909-MM).

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449