Recombinant Human MMP-8

Catalog Number: 908-MP

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Phe21-Gly467 Accession # AAZ38714
N-terminal Sequence Analysis	Phe21
Structure / Form	Pro form
Predicted Molecular Mass	51 kDa

SPECIFICATIONS

SDS-PAGE	70 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 >250 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 Tris, 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-8 (rhMMP-8) (Catalog # 908-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. Activate rhMMP-8 at 100 µg/mL with 1 mM APMA in Assay Buffer.
2. Incubate reaction at 37 °C for 1 hour.
3. Dilute activated rhMMP-8 to 1.0 ng/µL in Assay Buffer.
4. Dilute Substrate to 20 µM in Assay Buffer.
5. In a plate load 50 µL of 1.0 ng/µL rhMMP-8, and start the reaction by adding 50 µL of 20 µM Substrate to wells. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 20 µM Substrate.
6. Read at excitation and emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes.
7. 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-8: 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, -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-8 (neutrophil collagenase) is expressed in neutrophils, where it is stored in specific granules. MMP-8 release from the neutrophils is stimulated by various factors such as interleukins 1 and 8, TNF-α and GM-CSF. MMP-8 is capable of cleaving types I, II and III triple-helical collagen, gelatin peptides, fibronectin, proteoglycans, aggrecan, serpins, β-casein and peptides such as angiotensin and substance P. In addition to its function in phagocytosis, MMP-8 has a high capacity for infiltrating connective tissue, and is implicated in the breakdown of the extracellular matrix in diseases such as rheumatoid arthritis. Structurally, MMP-8 consists of several domains: a pro-domain that is cleaved upon activation, a catalytic domain containing the zinc-binding site, a short hinge region and a hemopexin-like domain. MMP-8 is heavily glycosylated.

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