Recombinant Mouse MMP-12
Catalog Number: 3467-MP

DESCRIPTION

Source: Mouse myeloma cell line, NS0-derived
Accession #: EDL24933
N-terminal Sequence: Ala18
Structure/Form: Pro form
Predicted Molecular Mass: 52 kDa

SPECIFICATIONS

SDS-PAGE: Measured by its ability to cleave a fluorogenic peptide substrate Mca-KPLGL-Dpa-AR-NH$_2$ (Catalog # ES010). The specific activity is >100 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com.

Endotoxin Level: <1.0 EU per µg of the protein by the LAL method.
Purity: >95%, by SDS-PAGE under reducing conditions and visualized by silver stain.

Activity Assay Protocol

Materials:
- Activation Buffer: 50 mM Tris, 10 mM CaCl$_2$, 150 mM NaCl, 0.05% (v/v) Brij-35, 5 µM ZnCl$_2$, pH 7.5
- Assay Buffer: 50 mM Tris, 10 mM CaCl$_2$, 150 mM NaCl, 0.05% (v/v) Brij-35, pH 7.5 (TCNB)
- Recombinant Mouse MMP-12 (rmMMP-12) (Catalog # 3467-MP)
- p-aminophenylmercuric acetate (APMA) (Sigma, Catalog # A9563), 100 mM stock in DMSO
- Substrate: MCA-Lys-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH$_2$ (R&D Systems, Catalog# ES010), 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-12 to 100 µg/mL with Activation Buffer containing 1 mM APMA.
2. To activate, incubate rmMMP-12 at 37 °C for 24 hours.
3. Dilute activated rmMMP-12 to 2 µg/mL in Assay Buffer.
4. Dilute Substrate to 20 µM in Assay Buffer.
5. In a plate load 50 µL of 2 µg/mL rmMMP-12 to wells, 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.
6. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
7. Calculate specific activity:
   \[
   \text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted V}_{\text{max}} \times \text{Conversion Factor}^{*}}{\text{amount of enzyme (µg)}}
   \]
   *Adjusted for Substrate Blank
   **Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions:
- Per Well:
  - rmMMP-12: 0.1 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-12 (macrophage elastase) is found in macrophages and its expression in monocytes can be induced by cytokines such as GM-CSF and CD40 signaling. In addition to elastin, MMP-12 can degrade a broad spectrum of substrates, including type IV collagen, fibronectin, laminin, vitronectin, proteoglycans, chondroitin sulfate, myelin basic protein, α, anti-trypsin, and plasminogen. It can also activate MMP-2 and MMP-3. MMP-12 is required for macrophage-mediated proteolysis and matrix invasion in mice. MMP-12 is proposed to have a direct role in the pathogenesis of aortic aneurysms and in the development of pulmonary emphysema that results from chronic inhalation of cigarette smoke. Structurally, the pro MMP-12 consists of following domains: a pro domain, a catalytic domain containing the zinc-binding site, and a C-terminal hemopexin-like domain. The rmMMP-12 corresponds to the pro form that can be activated under the conditions described in Activity Assay Protocol.