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Recombinant Human MMP-2

Catalog Number: 902-MPN

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human MMP-2 protein Ala30-Cys660 Accession # P08253
N-terminal Sequence Analysis	Ala30
Predicted Molecular Mass	71 kDa (proform)

SPECIFICATIONS

SDS-PAGE	60-70 kDa, 30 kDa, 21 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 >1,000 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	Lyophilized from a 0.2 µm filtered solution in Tris, $CaCl_2$, NaCl and $ZnCl_2$. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 100 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 8.0
- 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-2 (rhMMP-2) (Catalog # 902-MPN)
- *p*-aminophenylmercuric acetate (APMA), (Sigma, Catalog # A-9563), prepare a 100 mM stock in DMSO
- Fluorogenic Peptide Substrate I: MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH_2 (Catalog # ES001), 1 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 rhMMP-2 to 100 µg/mL in Activation Buffer.
2. Activate rhMMP-2 by adding APMA to a final concentration of 1 mM.
3. Incubate at 37 °C for 1 hour.
4. Dilute activated rhMMP-2 to 0.2 ng/µL in assay buffer.
5. Dilute substrate to 20 µM in assay buffer.
6. Load in plate 50 µL of the 0.2 ng/µL rhMMP-2 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.
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-2: 0.010 µg
- Substrate: 10 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile 100 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, and 0.05% Brij-35, pH 8.0.
Shipping	The product is shipped with polar packs. 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, -70 °C under sterile conditions after reconstitution.

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-2 (gelatinase A), a type IV collagenase, can degrade a broad range of substrates including type IV, V, VII and X collagens as well as elastin and fibronectin. 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-2 has been shown to be associated with many connective tissue cells as well as neutrophils, macrophages and monocytes. Structurally, MMP-2 may be divided into several distinct domains: a pro-domain which is cleaved upon activation; a catalytic domain containing the zinc binding site; a fibronectin-like domain thought to play a role in substrate targeting; and a carboxyl terminal (hemopexin-like) domain containing 2 N-linked glycosylation sites.

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