Recombinant Human MMP-13

Catalog Number: 511-MM

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Leu20-Cys471 Accession # P45452
N-terminal Sequence Analysis	Leu20
Structure / Form	Pro form
Predicted Molecular Mass	52 kDa

SPECIFICATIONS

SDS-PAGE	58 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 >2,000 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com
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 MES, 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-13 (rhMMP-13) (Catalog # 511-MM)
- p-Aminophenylmercuric acetate (APMA), (Sigma, Catalog # A-9563) 100 mM stock in DMSO
- Fluorogenic Peptide Substrate I: 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-13 to 100 µg/mL in Assay Buffer.
2. Add APMA to a final concentration of 1 mM.
3. Incubate at 37 °C for 2 hours to activate.
4. Dilute activated rhMMP-13 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-13 into a black well plate 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 (top read), 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-13: 0.010 µg
- Substrate: 10 µM

PREPARATION AND STORAGE

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, -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-13 (Collagenase-3) has been demonstrated to degrade a range of extracellular matrix proteins, including collagen types I, II, III, IV, IX, X and XIV, gelatin, aggrecan, perlecan and fibronectin. MMP-13 is distinguished from the other human collagenases by its effecient degradation of type II collagen. MMP-13 is expressed by fibroblasts, chrondrocytes and squamous epithelial cells. Structurally, MMP-13 may be divided into several distinct domains; a pro-domain which is cleaved upon activation; a catalytic domain containing the zinc binding site; a short hinge region and a carboxyl terminal (hemopexin-like) domain.

References:

1. Jeffery, J.J. (1998) in *Collagenase 3.* A.J. Barrett, *et al.* (eds): Handbook of Proteolytic Enzymes, San Diego: Academic Press, p. 1167.