Recombinant Human MMP-15/MT2-MMP

Catalog Number: 916-MP

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human MMP-15/MT2-MMP protein Glu47-Pro565 (Arg128Pro) (Arg129Gly), with a C-terminal 5-His tag Accession # P51511
N-terminal Sequence Analysis	Glu47
Predicted Molecular Mass	61 kDa

SPECIFICATIONS

SDS-PAGE	60-65 kDa, reducing conditions
Activity	Measured by its ability to cleave a fluorogenic peptide substrate Mca-KPLGL-Dpa-AR-NH_2 (Catalog # ES010). The specific activity is >200 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	>70%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in MES, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 5 µM $ZnCl_2$, 0.05% (w/v) Brij-35, pH 7.5
- Assay Buffer: 50 mM Tris, 500 mM NaCl, 5 mM $CaCl_2$, 1 µM $ZnCl_2$, 0.02% (w/v) Brij-35, pH 8.0
- Recombinant Human MMP-15/MT2-MMP (rhMMP-15) (Catalog # 916-MP)
- Recombinant Human Active Trypsin 3/PRSS3 (rhTrypsin 3) (Catalog # 3714-SE))
- 4-(2-Aminoethyl-benzensulfonyl fluoride hydrochloride) (AEBSF) (Tocris, Catalog # 5175), 100 mM stock in deionized water
- Substrate: MCA-Lys-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH_2 (Catalog # ES010)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Activate rhMMP-15 at 100 µg/mL with 0.1 µg/mL rhTrypsin 3 in Activation Buffer.
2. Incubate at 37 °C for 2 hours.
3. Add AEBSF for a final concentration of 1 mM and incubate at room temperature for 15 minutes to stop the reaction.
4. Dilute activated rhMMP-15 to 1 ng/µL in Assay Buffer.
5. Dilute Substrate to 20 µM in Assay Buffer.
6. Load 50 µL of the 1 ng/µL rhMMP-15 in 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 without any rhMMP-15.
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-15: 0.05 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.

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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. They play critical roles in tissue remodeling, angiogenesis, tumor invasion, and rheumatoid arthritis (1). MMP-15, also known as MT2-MMP, is a membrane-type MMP that is expressed in many tumor tissues including urothelial carcinoma, oral cancer, ovarian carcinoma, melanoma, and astrocytoma (2). Structurally, MMP-15 consists of the following domains:a pro domain containing a furin cleavage site, a catalytic domain containing the zinc-binding site, a hinge region, a hemopexin-like domain, a transmembrane domain, and a cytoplasmic tail (1). Recombinant Human (rh) MMP-15, consists of the pro domain, catalytic domain, hinge region and hemopexin-like domain. The pro domain contains the mutations R128P and R129G, which prevent activation by furin cleavage. Activation of rhMMP-15 is possible by treatment with rhTrypsin 3 as described in the Activity Assay Protocol.

References:

1. Takino, T. *et al.* (1995) J. Biol. Chem. **270**:23013.
2. Yana I. and M. Seiki (2004) Handbook of Proteolytic Enzymes (ed. Barrett, *et al.*) p. 549-551, Academic Press, San Diego.

PRODUCT SPECIFIC NOTICES

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