Recombinant Human MMP-9 Activated

Catalog Number: 11602-MP

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human MMP-9 protein Ala20-Asp707 (Gln279Arg) Accession # P14780.2 The proform was activated.
N-terminal Sequence Analysis	Phe107 & Gly547
Structure / Form	Activated

SPECIFICATIONS

SDS-PAGE	60-85, 38-42, 16-21 kDa, under 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 >1000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, $CaCl_2$, NaCl 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-9 Activated (rhMMP-9) (Catalog # 11602-MP)
- Substrate: MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH2 (Catalog # ES001)
- Black 96-well plate
- Plate Reader with Fluorescence Read Capability

Assay

1. Dilute rhMMP-9 to 0.4 µg/mL in Assay Buffer.
2. Dilute Substrate to 20 µM in Assay Buffer.
3. Load 50 µL of 0.4 µg/mL rhMMP-9 into a plate and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 20 µM Substrate.
4. Read at excitation and emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes.
5. 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.

Final Assay Conditions

Per Well:

- rhMMP-9: 0.020 µg
- Substrate: 10 µM

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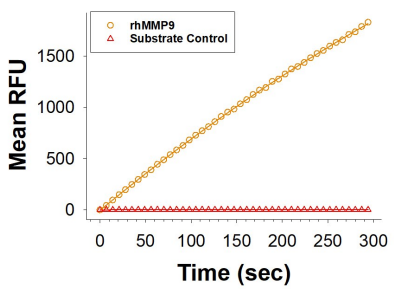
PREPARATION AND STORAGE

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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

Enzyme Activity



Recombinant Human MMP-9 Activated Protein Enzyme Activity. Recombinant Human MMP-9 Activated (Catalog # 11602-MP) is measured by its ability to cleave the fluorogenic peptide substrate, Mca-PLGL-Dpa-AR-NH2 (Catalog # ES001).

BACKGROUND

Recombinant human Active Matrix Metalloproteinase 9 (MMP-9), also known as gelatinase B, is a member of the MMP family of zinc and calcium-dependent endopeptidases. MMP-9 protein is synthesized as a pre-proenzyme in specific cells such as neutrophils, macrophages, fibroblasts and endothelial cells (1). Expressed MMP-9 contains a signal peptide to transport it to the extracellular matrix (ECM), a hinge region, a propeptide region, a catalytic domain, and a hemopexin-like domain that is important for substrate recognition (2, 3). In addition to having three fibronectin type II domains that contribute to substrate binding and an active site, the catalytic domain of MMP-9 also contains a zinc-binding region that interacts with a cysteine in the propeptide to maintain latency. Consequently, removal of the propeptide through cleavage by proteases in the ECM, such as MMP-3, activates the protein (2, 3). MMP-9 has specificity for targets containing an established preferred consensus sequence (3-5) and has a broad range of substrates within the ECM including gelatin, collagen, elastin that contributes to its role in ECM remodeling and extracellular domain cell surface protein release from the plasma membrane (3). Due to its activity in the ECM, MMP-9 plays a role in many biological processes and can serve as a biomarker in tumor invasion and mestastasis (3, 6) of many cancers including colon, ovarian, breast, osteosarcoma, and lung cancers (7-11) making it a therapeutic target (6, 8, 12). MMP-9 modeling of the ECM also leads to a pivotal role in other inflammation- and autoimmune-related diseases (3, 13, 14).

References:

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