Recombinant Cynomolgus Monkey MMP-9

Catalog Number: 10833-MP

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived cynomolgus monkey MMP-9 protein Ala20-Asp707 Accession # XP_005569271.2
N-terminal Sequence Analysis	Ala20
Structure / Form	Proform
Predicted Molecular Mass	76 kDa

SPECIFICATIONS

SDS-PAGE	83-95 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 >750 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 Cynomolgus Monkey MMP-9 (rcynoMMP-9) (Catalog # 10833-MP)
- p-aminophenylmercuric acetate (APMA) (Sigma, Catalog # A9563), 100 mM stock in DMSO
- Substrate: Mca-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH2 (Catalog # ES001), 2 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rcynoMMP-9 to 100 µg/mL in Assay Buffer.
2. Activate rcynoMMP-9 by adding APMA to a final concentration of 1 mM.
3. Incubate at 37 °C for 24 hours.
4. Dilute activated rcynoMMP-9 to 0.2 µg/mL in Assay Buffer.
5. Dilute Substrate to 20 µM in Assay Buffer.
6. Load 50 µL of 0.2 µg/mL rcynoMMP-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.
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:

- rcynoMMP-9: 0.01 µg
- Substrate: 10 µM

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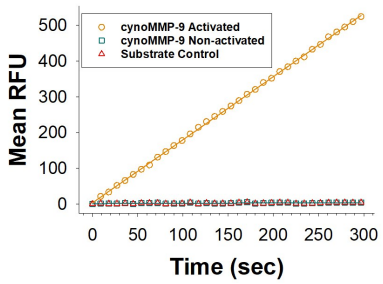
PREPARATION AND STORAGE

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.

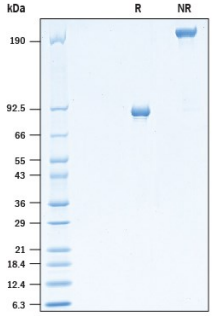
DATA

Enzyme Activity



Recombinant Cynomolgus Monkey MMP-9 Enzyme Activity Recombinant Cynomologous Monkey MMP-9 Protein (Catalog # 10833-MP) is measured by its ability to cleave the fluorogenic peptide substrate, Mca-PLGL-Dpa-AR-NH2 (Catalog # ES001).

SDS-PAGE



Recombinant Cynomolgus Monkey MMP-9 Protein SDS-PAGE. 2 µg/lane of Recombinant Cynomolgus Monkey MMP-9 (Catalog # 10833-MP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at ~90 kDa under reducing conditions.

BACKGROUND

Matrix metalloproteinase 9 (MMP-9), also known as gelatinase B, is a member of the MMP zinc-dependent family of endopeptidases. It cleaves and degrades a variety of targets including important extracellular matrix (ECM) proteins: gelatin, collagen, and elastin, as well as chemokines and extracellular domain plasma membrane proteins (1-3). MMP-9 is synthesized and secreted by several cells including neutrophils, macrophages, fibroblasts, and endothelial cells (4). The monomeric MMP-9 protein is composed of several distinct domains including a signal sequence, a pro-domain which is cleaved upon activation, and a catalytic domain at the n-terminus followed by a hinge region and the c-terminal hemopexin-like domains that contribute to substrate recognition and specificity (5,6). The catalytic domain contains fibronectin type II domains, an active site, and a zinc binding site. MMP-9 can exist as a monomer, disulfide-linked homodimer, or heterodimer in complex with lipocalin-2 (7,8). MMP-9 activity is regulated at several levels via transcription, post-transcription, translation, secretion, activation, and inhibition. As MMP-9 is involved in ECM remodeling and membrane protein cleavage, it has been widely associated to play a role in several diseases including cancers (9), autoimmune, and cardiovascular diseases (9-11). MMP-9 is consequently an important target of interest for inhibition (11-13). Additionally, it has been found to be a potential biomarker for many types of cancer including pancreatic, osteosarcoma, lung, ovarian, and breast (9).

References:

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