APA553Hu61 100μg
Active Matrix Metalloproteinase 9 (MMP9)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.
Host: 293F cell
Residues: Ala20~Asp707
Tags: N-terminal His-tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .
Original Concentration: 200μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 5.9
Predicted Molecular Mass: 78.0kDa
Accurate Molecular Mass: 100kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.
Store at 2-8ºC for one month.
Aliquot and store at -80ºC for 12 months.
Stability Test: The thermal stability is described by the loss rate. The loss rate

was determined by accelerated thermal degradation test, that is, incubate the protein at 37ºC for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

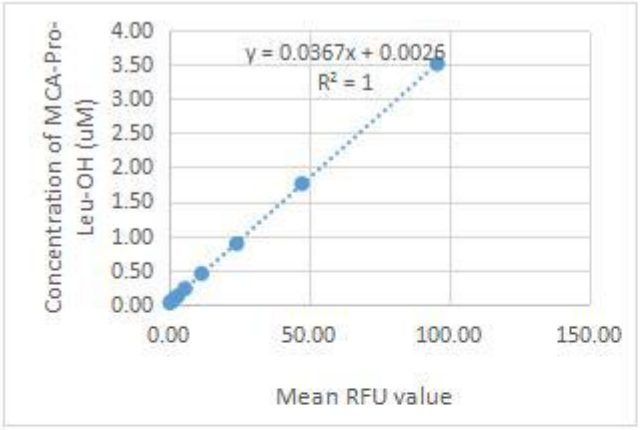
[SEQUENCE]

APRQRQSTLVLFPGDLRTNLTDRQLAEEYLYRYGYTRVAEMRGESKSLGPALLLLQKQLSLPETGELDSATLKAMRTPRCGV
PDLGRFQTFEGDLKWHHHNITYWIQNYSEDLPRAVIDDAFARAFALWSAVTPLTFTRVYSRDADIVIQFGVAEHGDGYPFDG
KDGLLAHAFPPGPGIQGDAHFDDDELWSLGKGVVVPTRFGNADGAACHFPFIFEGRSYSACTTDGRSDGLPWCSTTANYDTD
DRFGFCPSERLYTQDGNADGKPCQFPFIFQGQSYSACTTDGRSDGYRWCATTANYDRDKLFGFCPTRADSTVMGGNSAGELC
VFPFTFLGKEYSTCTSEGRGDGRLWCATTSNFDSDKKWGFCPDQGYSLFLVAAHEFGHALGLDHSSVPEALMYPMYRFTEGP
PLHKDDVNGIRHLYGPRPEPEPRPPTTTTPQPTAPPTVCPTGPPTVHPSERPTAGPTGPPSAGPTGPPTAGPSTATTVPLSP
VDDACNVNIFDAIAEIGNQLYLFKDGKYWRFSEGRGSRPQGPFLIADKWPALPRKLDSVFEERLSKKLFFFSGRQVWVYTGA
SVLGPRRLDKLGLGADVAQVTGALRSGRGKMLLFSGRRLWRFDVKAQMVDPRSASEVDRMFPGVPLDTHDVFQYREKAYFCQ
DRFYWRVSSRSELNQVDQVGYVTYDILQCPED

[ACTIVITY]

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-9 (gelatinase B) can degrade a broad range of substrates including gelatin, collagen types IV and V, elastin and proteoglycan core protein. 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-9 is produced by keratinocytes, monocytes, macrophages and PMN leukocytes. MMP-9 is present in most cases of inflammatory responses. The activity of recombinant human MMP9 is measured by its ability to cleave a fluorogenic peptide substrate MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH2 in the assay buffer 50 mM Tris, 10 mM CaCl2, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5. The rhMMP9 is diluted to 100 ug/ml in assay buffer, then activated by p-aminophenylmercuric acetate (APMA) in a final concentration of 1 mM incubated at 37 ° C for 1 hours. The activated rhMMP9 is diluted to 0.2 ug/mL in assay buffer. Loading into a black well plate 50 µL of 0.2 ug/mL rhMMP9 and start the reaction by adding 50 µL of 20 µM substrate, with a substrate blank containing 50 µL assay buffer, 50 µL substrate, and no rhMMP9. Then read at excitiation and

emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes. The specific activity of recombinant human MMP9 is > 3000 pmol/min/μg.



RFU (320/405)	MCA-Pro-Leu-OH (product) uM
95.78	3.52
47.46	1.76
24.20	0.88
11.63	0.44
5.71	0.22
3.05	0.11
1.52	0.05
0.77	0.03

Figure 1. The standard curve of MCA-Pro-Leu-OH

Specific Activity (pmol/min/μg) =

$$\frac{\text{Adjusted Vmax * (RFU/min) x Conversion Factor ** (pmol/RFU)}}{\text{amount of enzyme (ug)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH

[IDENTIFICATION]

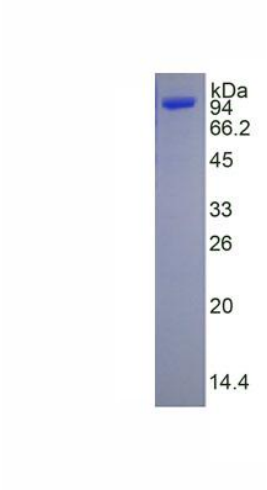


Figure 2. SDS-PAGE

Sample: Active recombinant MMP9, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.