APA553Mu62 100μg
Active Matrix Metalloproteinase 9 (MMP9)
Organism Species: *Mus musculus (Mouse)*
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~Pro730
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: 6.0
Predicted Molecular Mass: 80.2kDa
Accurate Molecular Mass: 90kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:
1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[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 2-8°C for one month.
Aliquot and store at -80°C 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 37°C 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.

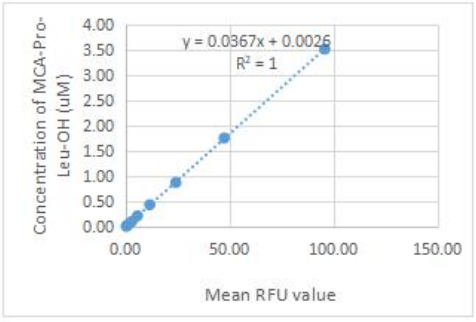
[SEQUENCE]

APYQRQPTFVVFPKDLKTSNLTDTQLAEAYLYRYGYTRAAQMMGEKQSLRPAL
LMLQKQLSLPQTGELDSQTLKAIRTPRCGVPDVGRFQTFKGLKWDHHNITYW
IQNYSEDLPRDMIDDAFARAFAVWGEVAPLTFTRVYGPEADIVIQFGVAEHG
DGYPFDGKDGLLAHAFPPGAGVQGDAHFDDDELWSLGKGVVIPTYYGNSNG
APCHFPFTFEGRSYSACTTDGRNDGTPWCSTTADYDKDGKFGFCPSERLYTEH
GNGEGKPCVFPFIFEGRSYSACTTKGRSDGYRWCATTANYDQDKLYGFCPTRV
DATVVGGNSAGELCVFPFVFLGKQYSSCTSDGRRDGRLWCATTSNFDTDKKW
GFCPDQGYSLFLVAAHEFGHALGLDHSSVPEALMYPLYSYLEGFPLNKDDIDGI
QYLYGRGSKPDPRPPATTTTEPQPTAPPTMCPTIPPTAYPTVGPTVGPTGAPSP
GPTSSPSPGPTGAPSPGPTAPPTAGSSEASTESLSPADNPCNVDVFDAIAEIQG
ALHFFKDGWYWKFLNHRGSPLQGPFLTARTWPALPATLDSAFEDPQTKRVFF
FSGRQMWVYTGKTVLGPRSLDKLGLGPEVTHVSGLLPRRLGKALLFSKGRVW
RFDLKSQKVDPQSVIRVDKEFSGVPWNSHDIFQYQDKAYFCHGKFFWRVSF
QNEVNKVDHEVNQVDDVGYVTYDLLQCP

[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 mouse 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 rmMMP9 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 rmMMP9 is diluted to 0.2 ug/mL in assay buffer. Loading into a black well plate 50 µL of 0.2 ug/mL rmMMP9 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 rmMMP9. 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 mouse MMP9 is > 5500 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]

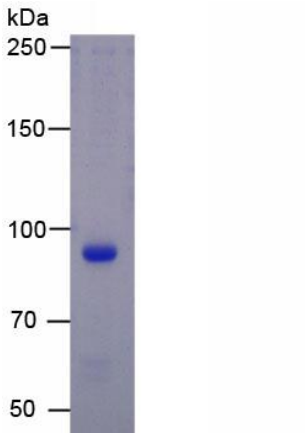


Figure 2. SDS-PAGE

Sample: Active recombinant MMP9, Mouse

[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.