APA553Po61 100μg
Active Matrix Metalloproteinase 9 (MMP9)
Organism Species: ***Sus scrofa; Porcine (Pig)***
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: Ala19~Asp708
Tags: N-terminal His-tag
Purity: >90%
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: 5.7
Predicted Molecular Mass: 78.8kDa
Accurate Molecular Mass: 78kDa 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 2-8°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 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]

APKPRQPTFVVFPGDLRTNLIDRQLAEEYLYRYGYTQVAEMTDRKQSLEPALKLLQQRLSLPQTG
ELDTTTLNAMRAPRCGVPDLGKFQTFEGDLKWHHHDMTYWIQNYSEDLSRDVIDDAFVRAFS
LWSEATPLNCTRVYGPNADIVIQFGVKEHGDGYPFDGKDGLLAHAFPPGPGIQGDAHFDDEEL
WSLGKGVVVPTYFGNAKGAACHFPFTFEGRSYSACTTDGRSDDMLWCSTTADYDTDRQFGFCP
SERLFTRDGNADGKPCVFPFTFEGRSYSSCTTDGRSDGYRWCGTTANYDQDKLYGFCPTRADST
VTGGNSAGDLCVFPFTFLGKEYSACTREGRSDGHLWCATTSNFDKDKKWGFCPDQGYSLFLVA
AHEFGHALGLDHSTVPEALMYPMYSFTEEHPLHEDDVRGIQYLYGSRPKPEPRPPTTTTAEPQST
VPPTVCVTGPPTTRPSERSTAGPTGPPSAGPTGPPTAGPSAAPTVSLGPGEDICNVDIFDAIAEIR
NRLHFFKDGKYWRLSEGKEHRVQGPFLIKDRWPALPHKLDSAFEEPLSKKIFFFSGRQVWVYTG
ASVLGPRRLDKLGLGPEVARVTGALPREAGKVLLFSQQNFWRFDVKTQKVDSRSATRVDQMFP
GVPLNTHDIFQYQEKAYFCQARFFWRVSSRNEVNQVDYVGYVTFDLLKCPED

[ACTIVITY]

Matrix Metalloproteinase 9 (MMP9), or gelatinase B, is a zinc-dependent endopeptidase in the MMP family. It mediates extracellular matrix remodeling by degrading gelatin, collagen, and other components, participating in tissue repair and inflammation. Synthesized as latent pro-MMP9 and activated via proteolysis, its dysregulation links to cancer metastasis, arthritis, and neurodegenerative diseases. Controlled by TIMPs, it’s a key biomarker and therapeutic target for multiple pathologies.Serum samples are first concentrated (5-fold) via Sephadex G-25. MMP-9 is separated by electrophoresis, then transferred to a nitrocellulose membrane (via capillary blotting) pre-coated with fluorescent substrate (MCA-Pro-Arg-Ser-Leu-DPA-Ser-Gly-NH_2). Active MMP-9 cleaves the substrate, separating fluorophore (MCA) from quencher (DPA). Fluorescence (excitation: ~328nm, emission: ~393nm) indicates MMP-9 activity, enabling quantification.Dilute rhMMP-9 to 100 μg/mL in Assay Buffer.Add APMA to a final concentration of 1 mM to activate rhMMP-9; incubate at 37 ° C for 24 hours.Dilute activated rhMMP-9 to 0.4 ng/μL in Assay Buffer.Dilute the substrate to 20 μM in

Assay Buffer.Add 50 μL of diluted rhMMP-9 to each well, then add 50 μL of diluted substrate to initiate the reaction (include a substrate blank with 50 μL Assay Buffer + 50 μL substrate).Read with a fluorescent plate reader in kinetic mode at 320 nm (excitation)/405 nm (emission) for 5 minutes.For the standard curve: Dilute MCA-Pro-Leu-OH to 25 μg/mL in Assay Buffer, then perform 2-fold serial dilutions (15 gradients). Add 100 μL per well (include a blank with 100 μL Assay Buffer); sonicate the standard before use and read at 320/405 nm.The specific activity of recombinant pig MMP9 is > 40 pmol/min/ug.

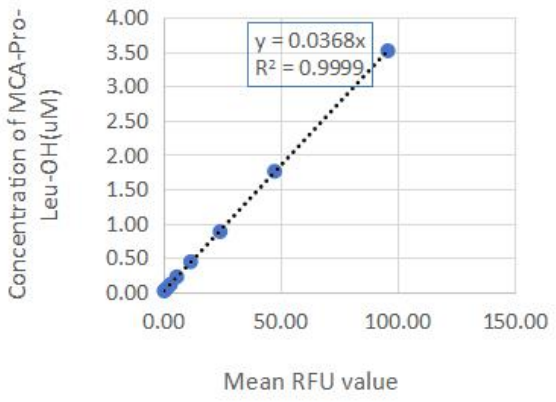


Figure 1. The standard curve of MCA-Pro-Leu-OH

RFU(320/405)	MCA-Pro-Leu-OH 浓度 umol/L
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
0.32	0.01

Specific Activity (pmol/min/μg) =

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

*Adjusted for Substrate Blank

**Derived using calibration standard ADP

[IDENTIFICATION]

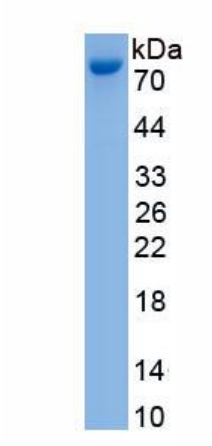


Figure 2. SDS-PAGE

Sample: Active recombinant MMP9, Pig

[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.