APA097Bo61 100μg
Active Matrix Metalloproteinase 1 (MMP1)
Organism Species: *Bos taurus; Bovine (Cattle)*
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: Phe19~Asn469
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: 6.0
Predicted Molecular Mass: 53.0kDa
Accurate Molecular Mass: 68&70kDa 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.

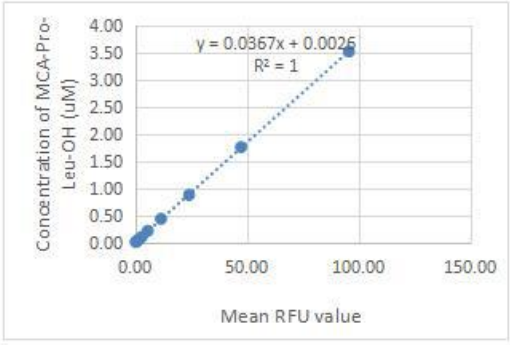
[SEQUENCE]

FPAATSETQEQDVETVKKYLENYYNLNSNGKKVERQRNGGLITEKLKQMQKFFGLRVTGKPDAETLNVM
KQPRCGVPDVAPFVLTPGKSCWENTNLTYRIENYTPDLSRADVDQAIEKAFQLWSNVTPLTFTKVSEGQ
ADIMISFVRGDHRDNSPFDGPGGNLAHAFQPGAGIGGDAHFDDDEWWTSNFQDYNLYRVAAHEFGHSLG
LAHSTDIGALMYPSYTFSGDVQLSQDDIDGIQAIYGPSQNPTQPVGPQTPEVCDSKLTFDAITTIRGEV
MFFKDRFYMRTNPLYPEVELNFISVFWPQLPNGLQAAYEVADRDEVRFFKGNKYWAVKGQDVLRGYPRD
IYRSFGFPRTVKSIDAAVSEEDTGKTYFFVANKCWRYDEYKQSMDAGYPKMIAEDFPGIGNKVDAVFQK
GGFFYFFHGRRQYKFDPQTKRILTLLKANSWFNCRKN

[ACTIVITY]

MMP1 is a zinc-dependent enzymes capable of cleaving components of the extracellular matrix, which belongs to the matrix metalloproteinase (MMP) family. MMP-1 (interstitial collagenase), can degrade a broad range of substrates including types I, II, III, VII, VIII, and X collagens as well as casein, gelatin and so on. MMP-1 is expressed by fibroblasts, keratinocytes, endothelial cells, monocytes and macrophages. Structurally, MMP-1 may be divided into several distinct domains; a pro-domain which is cleaved upon activation; a catalytic domain containing the zinc binding site; a short hinge region and a carboxyl terminal (hemopexin-like) domain. The activity of recombinant bovine MMP1 is measured

by its ability to cleave a fluorogenic peptide substrate Mca-KPLGL-Dpa-AR-NH2 in the assay buffer 50 mM Tris, 10 mM CaCl2, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5. The rbMMP1 is diluted to 50 ug/ml in assay buffer, then activated by p-aminophenylmercuric acetate (APMA) in a final concentration of 1 mM incubated at 37 ° C for 2 hours. The activated rbMMP-1 is diluted to 1 ug/mL in assay buffer. Loading into a black well plate 50 µL of 1 ug/mL rbMMP-1 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 rbMMP-1. 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 bovine MMP1 is > 600 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]

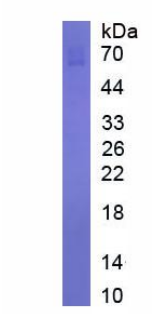


Figure 2. SDS-PAGE

Sample: Active recombinant MMP1, Cattle

[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.