APA097Bo01 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: Prokaryotic expression.

Host: *E. coli*

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 0.01% SKL, 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: 5.8

Predicted Molecular Mass: 55.1kDa

Accurate Molecular Mass: 55kDa 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]

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                 FP  AATSETQEQD  VETVKKYLEN  YYNLNSNGKK
VERQRNGGLI  TEKLKQMQKF  FGLRVTGKPD  AETLNVMKQP  RCGVPDVAPF
VLTPGKSCWE  NTNLTYRIEN  YTPDLSRADV  DQAIEKAFQL  WSNVTPLTFT
KVSEGQADIM  ISFVRGDHRD  NSPFDGPGGN  LAHAFQPGAG  IGGDAHFDDD
EWWTSNFQDY  NLYRVAAHEF  GHSLGLAHST  DIGALMYPSY  TFSGDVQLSQ
DDIDGIQAIY  GPSQNPTQPV  GPQTPEVCDS  KLTFDAITTI  RGEVMFFKDR
FYMRTNPLYP  EVELNFISVF  WPQLPNGLQA  AYEVADRDEV  RFFKGNKYWA
VKGQDVLRGY  PRDIYRSFGF  PRTVKSIDAA  VSEEDTGKTY  FFVANKCWRY
DEYKQSMDAG  YPKMIAEDFP  GIGNKVDAVF  QKGGFFYFFH  GRRQYKFDPQ
TKRILTLLKA  NSWFNCRKN
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[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 > 150 pmol/min/μg.
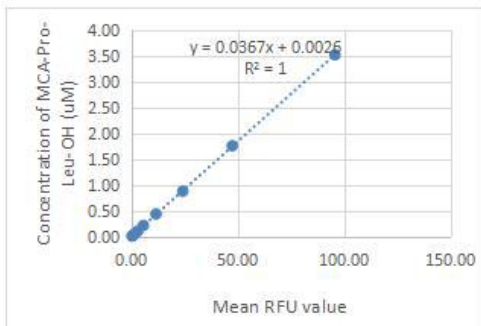
RFU (320/405)	MCA-Pro-Leu-OH (product) uM
95.78	3.52
47.46	1.76
24.20	0.88
11.63	0.44
5.71	0.22
3.05	0.11
1.52	0.05
0.77	0.03

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

Specific Activity (pmol/min/μg) =

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

*Adjusted for Substrate Blank

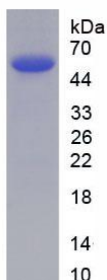**Derived using calibration standard MCA-Pro-Leu-OH

[IDENTIFICATION]

Figure 2. SDS-PAGE

Sample: Active recombinant MMP1, Cattle

[<u>IMPORTANT NOTE</u>]

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.