APA097Ov01 100µg Active Matrix Metalloproteinase 1 (MMP1) Organism Species: *Ovis aries; Ovine (Sheep) 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~Asn467 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: 6.4 Predicted Molecular Mass: 55.1kDa Accurate Molecular Mass: 58kDa as determined by SDS-PAGE reducing conditions.

## [ <u>USAGE</u> ]

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.

### [<u>SEQUENCE</u>]

FPAATSEQDVETVQKYLENYYNLNSDGKKVERQRNGGLITEKLKQMQKFFGLRVTGKPDAETLNVMKQP RCGVPDVTQFVLTPGNPRWENTNLTYRIENYTPDLSRADVDQAIEKAFQLWSNVTPLTFTKVSEGQADI MISFVRGDHRDNSPFDGPGGNLAHAFQPGANIGGDAHFDDDERWTNDFQNYNLYRVAAHEFGHSLGLSH STDIGALMYPSYIFSGDVQLAQDDIDGIQAIYGPSQNPTQPVGPQTPEVCDSKLTFDAITTIRGEVMFF KDRFYMRTNPFYADVELNFISIFWPHLPNGLHAAYEVAARDEVRFFKGSNKYWAVRGQDVLRGYPRDIY RSFGFPRTVKSIDAAVSEEDTGKTYFFVANKCWRYDEYKQSMDAGYPKMIEEDFPGIGNKVDAVFQKGG FFYFFHGKRQYKFDPKTKRILTLLKANSWFNCRKN

### [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 sheep 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 rsMMP1 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 rsMMP-1 is diluted to 1 ug/mL in assay buffer. Loading into a black well plate 50 µL of 1 ug/mL rsMMP-1 and start the reaction by

adding 50  $\mu$ L of 20  $\mu$ M substrate, with a substrate blank containing 50  $\mu$ L assay buffer, 50  $\mu$ L substrate, and no rsMMP-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 sheep MMP1 is > 40 pmol/min/ $\mu$ g.



RFU (320/405)	MCA-Pro-Leu- OH (product) uM
95.78	3.52
47.46	1.76
24.20	0.88
1 <mark>1.6</mark> 3	0.44
5.71	0.22
3.05	0.11
<mark>1.5</mark> 2	0.05
0.77	0.03

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

Specific Activity (pmol/min/µg) =

Adjusted Vmax \* (RFU/min) x Conversion Factor \*\* (pmol/RFU)

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, Sheep

### [<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.