

APA100Hu61 100µg
Active Matrix Metalloproteinase 2 (MMP2)
Organism Species: *Homo sapiens (Human)*
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: Ala30~Cys660

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.

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 5.2

Predicted Molecular Mass: 72.6kDa

Accurate Molecular Mass: 75kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.6) 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]

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                                     A PSPIIKFPGD VAPKTDKELA
VQYLNTFYGC PKESC�LFVL KDTLKKMQKF FGLPQTGDLD QNTIETMRKP
RCGNPDVANY NFFPRKPKWD KNQITYRIIG YTPDLDPETV DDAFARAFQV
WSDVTPLRFS RIHDGEADIM INFGRWEHGD GYPFDGKDGL LAHAFAFGTG
VGGDSHFDDD ELWTLGEGQV VRVKYGNADG EYCKFPFLFN GKEYNSCTDT
GRSDGFLWCS TTYNFEKDGK YGFCPHEALF TMGGNAEQQP CKFPFRFQGT
SYDSCTEGR TDGYRWCGTT EDYDRDKKYG FCPETAMSTV GGNSEGAPCV
FPFTFLGNKY ESCTSAGRSD GKMWCATTAN YDDDRKWGFC PDQGYSFLV
AAHEFGHAMG LEHSQDPGAL MAPIYTYTKN FRLSQDDIKG IQELYGASPD
IDLGTGPTPT LGPVTPEICK QDIVFDGIAQ IRGEIFFFKD RFIWRTVTPR
DKPMGPLLVA TFWPELPEKI DAVYEAPQEE KAVFFAGNEY WIYSASTLER
GYPKPLTSLG LPPDVQRVDA AFNWSKNKKT YIFAGDKFWR YNEVKKKMDP
GFPKLIADAW NAIPDNLDAV VDLQGGGHSY FFKGAYYLKL ENQSLKSVKF
GSIKSDWLGC
    
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[ACTIVITY]

MMP2 is a zinc-dependent enzymes capable of cleaving components of the extracellular matrix, which belongs to the matrix metalloproteinase (MMP) family .It is a gelatinase A, 72 kDa type IV collagenase which can hydrolyze gelatin under certain conditions. Gelatin zymography is mainly used for the detection of the gelatinases, MMP-2 and MMP-9 and It is extremely sensitive because levels of 10 pg of MMP-2 can already be detected .Briefly , various concentrations of MMP2 (50ng, 25ng, 13ng, 6.5ng, 3.3ng, 1.7ng) were denatured by SDS loading buffer, electrophoresed through sodium dodecylsulphate–polyacrylamide gel (SDS–PAGE; 10% gels) containing gelatin (1 mg/ml) with nonreducing conditions. After renaturation, incubation and CCB-stained, active MMP2 would hydrolyze gelatin nearby, which was indicated by the white binds on the gel. In this experiment we use trypsin as positive control. The result was shown in figure 1.

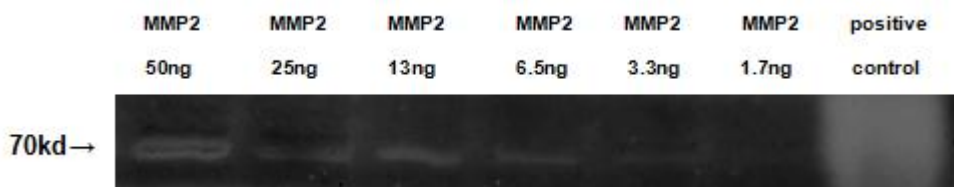
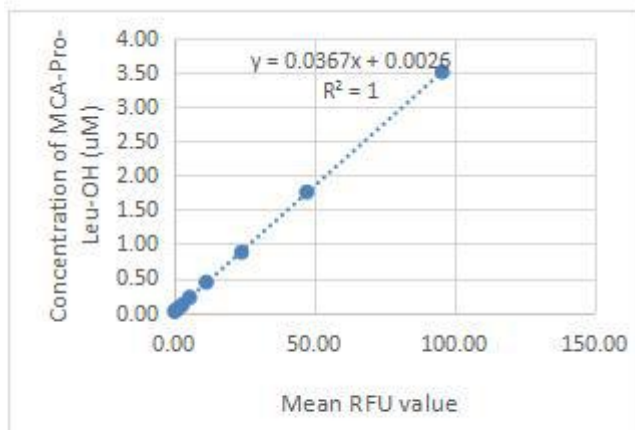


Figure 1. Hydrolysis of gelatin by recombinant human MMP2

The activity of recombinant human MMP2 is also 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 rhMMP2 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 rhMMP2 is diluted to 2.5 ug/mL in assay buffer. Loading into a black well plate 50 μL of 2.5 ug/mL rhMMP2 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 rhMMP2. Then read at excitation and emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes. The specific activity of recombinant human MMP2 is > 170 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 2. The standard curve of MCA-Pro-Leu-OH

Specific Activity (pmol/min/μg) =

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

*Adjusted for Substrate Blank

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

[IDENTIFICATION]

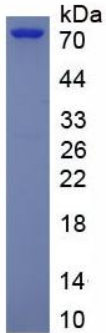


Figure 3. SDS-PAGE

Sample: Active recombinant MMP2, Human

[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.