APA101Hu62 100μg
Active Matrix Metalloproteinase 3 (MMP3)
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: Tyr18~Cys477
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 .
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: 5.9
Predicted Molecular Mass: 53.8kDa
Accurate Molecular Mass: 56&58kDa 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.

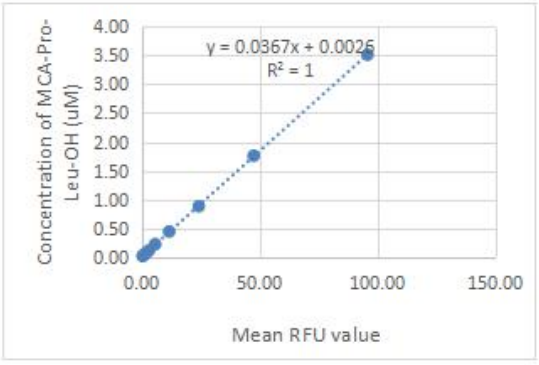
[SEQUENCE]

YPL DGAARGEDTS MNLVQKYLEN YYDLKKDVKQ
FVRRKDSGPV VKKIREMQKF LGLEVTGKLD SDTLEVMRKP RCGVPDVGHF
RTFPGIPKWR KTHLTYRIVN YTPDLPKDAV DSAVEKALKV WEEVTPLTFS
RLYEGEADIM ISFAVREHGD FYPFDGPGNV LAHAYAPGPG INGDAHFDDD
EQWTKDTTGT NLFLVAAHEI GHSLGLFHSA NTEALMYPLY HSLTDLTRFR
LSQDDINGIQ SLYGPPPDSP ETPLVPTEPV PPEPGTPANC DPALSFDAVS
TLRGEILIFK DRHFWRKSLR KLEPELHLIS SFWPSLPSGV DAAYEVTSKD
LVFIFKGNQF WAIRGNEVRA GYPRGIHTLG FPPTVRKIDA AISDKEKNKT
YFFVEDKYWR FDEKRNSMEP GFPKQIAEDF PGIDSKIDAV FEEFGFFYFF
TGSSQLEFDP NAKKVTHTLK SNSWLNC

[ACTIVITY]

Matrix metalloproteinases are a family of zinc and calcium dependent endopeptidases with the combined ability to degrade all the components of the extracellular matrix. MMP-3 (stromelysin-1), can degrade a broad range of substrates including collagen alpha chains, aggrecan, laminin, fibronectin, and elastin. MMP-3 does not cleave the triple helical region of interstitial collagens, a characteristic which distinguishes the stromelysins from the collagenases. MMP-3 is expressed by fibroblasts, chrondrocytes, osteoblasts, endothelial cells, smooth muscle cells and macrophages. Structurally, MMP-3 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 human MMP-3 is measured by its ability to cleave a fluorogenic peptide substrate Mca-Arg-Pro-Lys-Pro-Val-Glu-Nval-Trp-Arg-Lys(Dnp)-NH2 in the assay buffer 50 mM Tris, 10 mM CaCl2, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5. The rhMMP3 is diluted to 200 ug/ml in assay buffer, then activated with 5 ug/ml chymotrypsin at 37 ℃ for 30min followed by adding 2 mM PMSF to stop activation. The activated rhMMP3 is diluted to 6.25 ug/mL in assay buffer. Loading into a black well plate 50 µL of 6.25 ug/mL rhMMP3 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 rhMMP3. 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 human MMP3 is > 90 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) =

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

*Adjusted for Substrate Blank

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

[IDENTIFICATION]

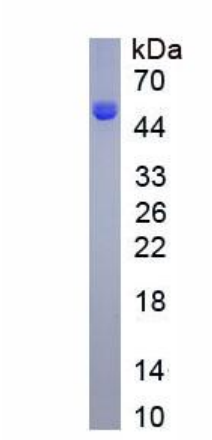


Figure 2. SDS-PAGE

Sample: Active recombinant MMP3, 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.