

APA129Hu01 50µg
Active Tissue Inhibitors Of Metalloproteinase 3 (TIMP3)
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: Prokaryotic expression.

Host: *E. coli*

Residues: Cys26~Pro211

Tags: N-terminal His-tag

Purity: >92%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.01% skl, 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: 9.3

Predicted Molecular Mass: 25.2kDa

Accurate Molecular Mass: 26kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in ddH₂O 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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                CSPSH PQDAFCNSDI VIRAKVVGKK  
LVKEGPFRTL VYTIKQMKMY RGFTKMPHVQ YIHTEASESL CGLKLEVNKY  
QYLLTGRVYD GKMYTGLCNF VERWDQLTSL QRKGLNYRYH LGCNCKIKSC  
YYLPCFVTSK NECLWTDMLS NFGYPGYQSK HYACIRQKGG YCSWYRGWAP  
PDKSIINATD P
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[ACTIVITY]

Tissue Inhibitors Of Metalloproteinase 3 (TIMP3) is a protein belongs to the tissue inhibitor of metalloproteinases family. They are inhibitors of the matrix metalloproteinases. TIMP-3 is the only member of the TIMP family which is found exclusively in the extracellular matrix (ECM). It is regulated in a cell cycle-dependent fashion in certain cell types and may serve as a marker for terminal differentiation. The activity of recombinant human TIMP3 was measured by its ability to inhibit recombinant human MMP2 cleavage of a fluorogenic peptide substrate Mca-PLGL-Dpa-AR-NH₂ in the assay buffer 50 mM Tris, 10 mM CaCl₂, 150 mM NaCl, 0.05% Brij-35 (v/v), pH 7.5. The rhMMP-2 (APA100Hu61) was activated with 1 mM APMA at 37 °C for 1h. Then 16 µl 126 ug/mL rhTIMP-3, 25.6 µL of 100 ug/ml rhMMP-2 and 118.4 µL of assay buffer was incubated for 2 hours at 37 °C, including a control (in duplicate) containing assay buffer and the diluted rhMMP-2. The mixtures was diluted 5 fold in assay buffer followed by adding 50 ul 20 uM substrate, including a control containing assay buffer and substrate. Then read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively in kinetic mode for 5 minutes. Under these conditions, the enzyme amount of 50% inhibition of rhMMP-2 activity per minute is defined as a unit. The specific activity of TIMP3 is >6000 U/mg.

Calculation

$$\text{TIMP3 activity (U/mg)} = \frac{\frac{A405_1 / \text{min} - A405_2 / \text{min}}{A405_1 / \text{min}} \times 100\%}{50\%} / M$$

Where:

$A405_1 / \text{min}$ = rhMMP-2 activity of $\Delta A405\text{nm/min}$

$A405_2 / \text{min}$ = inhibition of rhMMP-2 activity of TIMP3

M = mass of enzyme

[IDENTIFICATION]

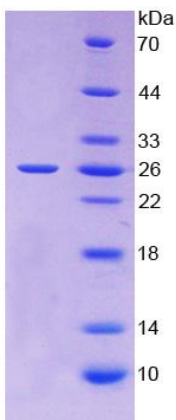


Figure 1. SDS-PAGE

Sample: Active recombinant TIMP3, Human

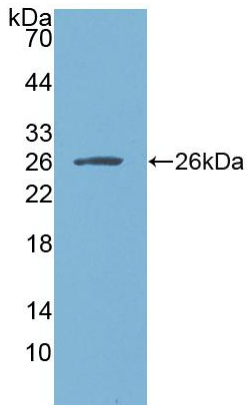


Figure 2. Western Blot

Sample: Recombinant TIMP3, Human;

Antibody: Rabbit Anti-Human TIMP3 Ab (PAA129Hu01)

[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.