APA129Hu01 100μg
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 ddH_2O 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^oC for one month.
Aliquot and store at -80^oC 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]

CSPSH PQDAFCNSDI VIRAKVVGKK LVKEGPFGTL VYTIKQMKMY RGFTKMPHVQ YIHTEASESL CGLKLEVNKY QYLLTGRVYD GKMYTGLCNF VERWDQLTLS QRKGLNYRYH LGCNCKIKSC YYLPCFVTSK NECLWTDMLS NFGYPGYQSK HYACIRQKGG YCSWYRGWAP PDKSIINATD P

[ACTIVITY]

Tissue Inhibitors Of Metalloproteinase 3 (TIMP3) is an 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

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

Where:

$A405_1/\min$ = rhMMP-2 activity of ΔA405nm/min

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

M = mass of enzyme

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<15 nM.

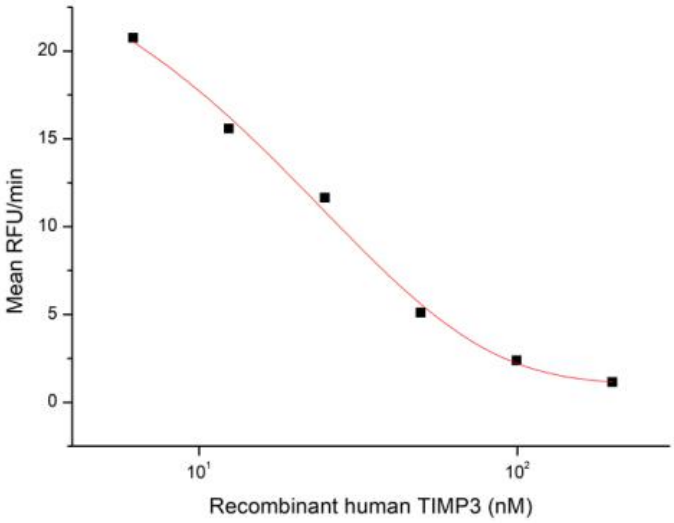

Figure 1. Inhibition of MMP2 activity by recombinant human TIMP3

[IDENTIFICATION]

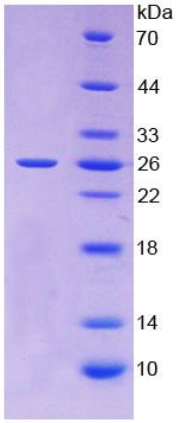

Figure 2. SDS-PAGE

Sample: Active recombinant TIMP3, 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.