APA129Ra01 100μg
Active Tissue Inhibitors Of Metalloproteinase 3 (TIMP3)
Organism Species: *Rattus norvegicus* (Rat)
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: Ser27~Thr209
Tags: N-terminal His-tag
Purity: >98%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 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: 22.4kDa
Accurate Molecular Mass: 22kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) 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 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]

SPSH PQDAFCNSDI VIRAKVVGKK
LVKEGPFGTL VYTIKQMKMY RGFSKMPHVQ YIHTEASESL CGLKLEVNKY
QYLLTGRVYE GKMYTGLCNF VERWDHLTLS QRKGLNYRYH LGCNCKIKSC
YYLPCFVTSK KECLWTDMLS NFGYPGYQSK HYACIRQKGG YCSWYRGWAP
PDKSISNAT

[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. Besides, Matrix Metalloproteinase 2 (MMP2) has been identified as an interactor of TIMP3, thus a binding ELISA assay was conducted to detect the interaction of recombinant rat TIMP3 and recombinant rat MMP2. Briefly, TIMP3 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100μL were then transferred to MMP2-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-TIMP3 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50μL stop solution to the wells and read at 450nm immediately. The binding activity of TIMP3 and MMP2 was shown in Figure 1, and this effect was in a dose dependent manner.

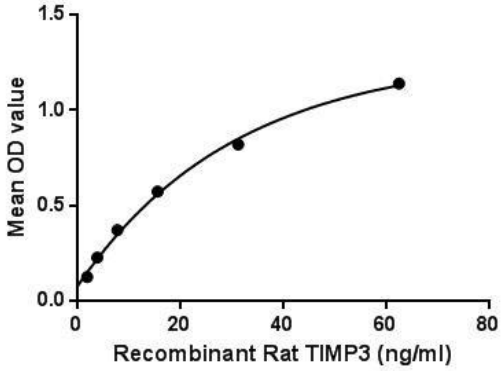

Figure 1. The binding activity of TIMP3 with MMP2.

The activity of recombinant rat TIMP3 was measured by its ability to inhibit rhMMP2 cleavage of 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. rhMMP2 was diluted to 100 ug/ml and activated with 1 mM APMA at 37 °C for 1 hour and rrTIMP3 (MW: 23.25 KD) was diluted to different concentrations with the assay buffer. Mix 8 µl of rrTIMP3 curve dilutions, 12.8 µl of activated rhMMP-2, and 59.2 µl of assay buffer, including a control containing assay buffer and the diluted rhMMP-2 and incubate the reactions for 2 hours at 37 °C. Loading 50 µl of the incubated mixtures which were diluted five-fold in assay buffer into empty wells of a plate, and start the reaction by adding 50 µl of 20 µM substrate. Include a substrate blank containing 50 µl of assay buffer and 50 µl of 20 µM substrate. Then read at excitiation and emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes. The result was shown in Figure 1 and it was obvious that recombinant rat TIMP3 significantly decreased rhMMP2 activity. The inhibition IC50 was <1 nM.

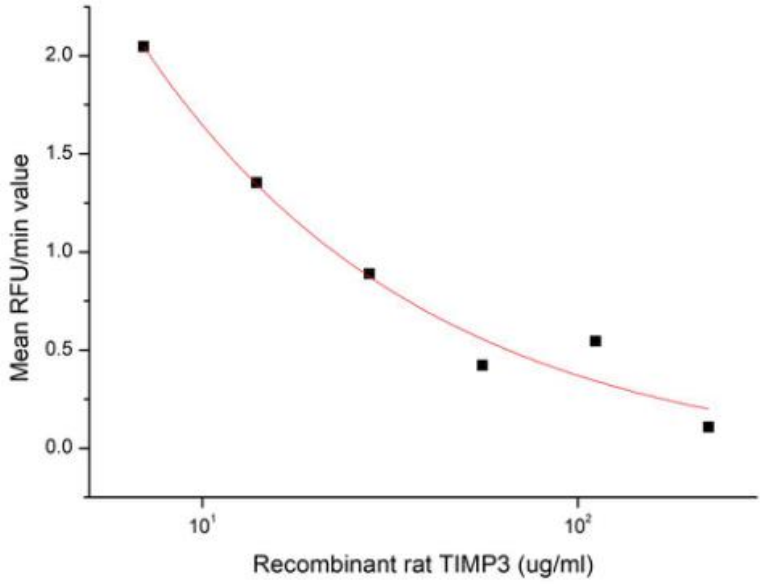

Figure 2. Inhibition of MMP2 activity by recombinant rat TIMP3

[IDENTIFICATION]

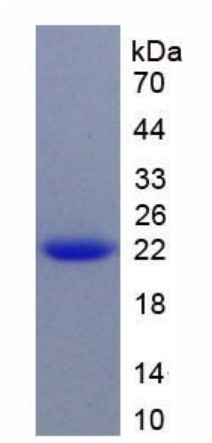

Figure 3. SDS-PAGE

Sample: Active recombinant TIMP3, Rat

[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.