

APA129Bo02 100µg

Active Tissue Inhibitors Of Metalloproteinase 3 (TIMP3)

Organism Species: *Bos taurus*; Bovine (Cattle)

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: Cys24~Pro211

Tags: N-terminal His-tag

Purity: >95%

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

Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 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: 9.1

Predicted Molecular Mass: 25.4kDa

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

[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**]

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CTCSPSHPQDAFCNSDIVIRAKVVGKLLKEGPFMTMVTIKQMKMYRGFTKMPHVQYIHTAEASESLCGLKLEVNKYQYLLTGRVYDGMKMTGLCNFVERWDQLTSLQQRKGLNRYHYLGCNCKIKSCYYLPCFVTTKNECLWTDMLSNFGYPGYQSKHYACIRQKGGYCSWYRGWAPPDKSIINATDP
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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 bovine 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 rbTIMP3 (MW: 23.25 KD) was diluted to different concentrations with the assay buffer. Mix 8 µl of rbTIMP3 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 excitation 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

bovine TIMP3 significantly decreased rhMMP2 activity. The inhibition IC₅₀ was <15 nM.

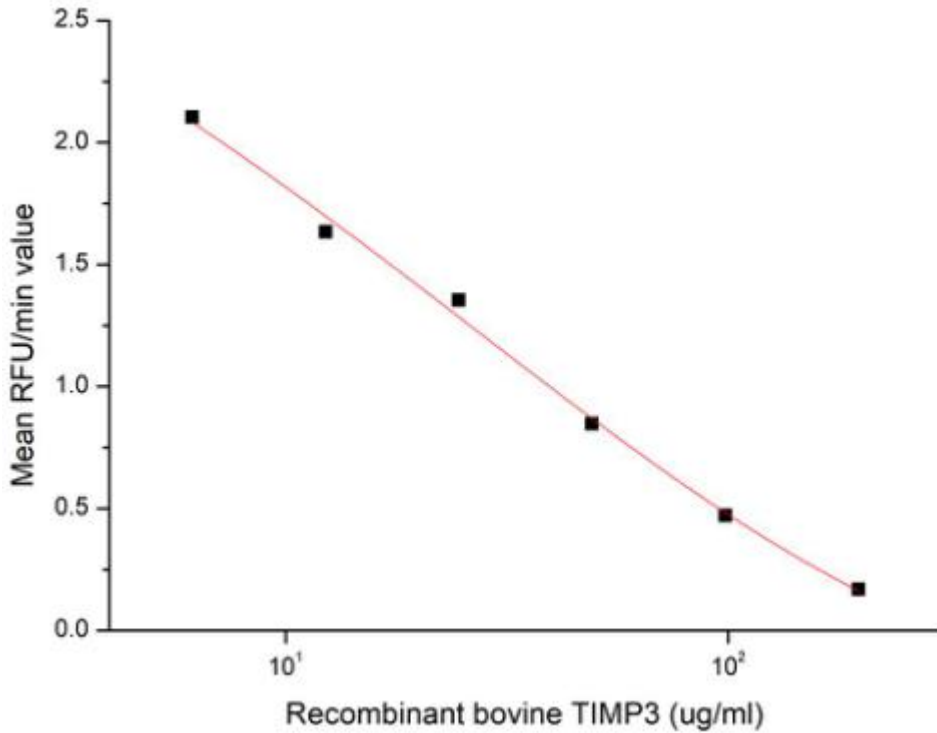


Figure 1. Inhibition of MMP2 activity by recombinant bovine TIMP3

[IDENTIFICATION]

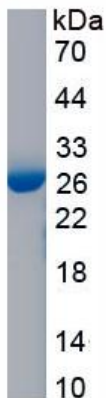


Figure 2. SDS-PAGE

Sample: Active recombinant TIMP3, Cattle

[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.