

APA128Bo01 100µg

Active Tissue Inhibitors Of Metalloproteinase 2 (TIMP2)

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: Cys27~Pro220

Tags: N-terminal His-tag

Purity: >90%

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: 7.2

Predicted Molecular Mass: 25.4kDa

Accurate Molecular Mass: 28kDa 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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CSCS PVHPQQAFCN ADIVIRAKAV NKKEVDSGND
IYGNPIKRIQ YEIKQIKMFK GPDQDIEFIY TAPAAAVCGV SLDIGGKKEY LIAGKAEBNG
NMHITLCDFI VPWDTLSATQ KKSLNHRYQM GCECKITRCP MIPCYISSPD ECLWMDWVTE
KNINGHQAKF FACIKRSDGS CAWYRGAAPP KQEFLDIEDP
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[ACTIVITY]

Tissue inhibitors of metalloproteinases or TIMPs are a family of proteins that regulate the activation and proteolytic activity of the zinc enzymes known as matrix metalloproteinases (MMPs). There are four members of the family, TIMP-1, TIMP-2, TIMP-3, and TIMP-4. TIMP-2 is a non N-glycosylated protein with a molecular mass of 22 kDa produced by a wide range of cell types, which inhibits MMPs non-covalently by the formation of binary complexes. TIMP-2 also has erythroid-potentiating and cell growth promoting activities. The activity of recombinant bovine TIMP2 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 rbTIMP2 (MW: 51.75 KD) was diluted to different concentrations with the assay buffer. Mix 8 µl of rhTIMP2 curve dilutions, 12.8 µl of activated rbMMP-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 μ l of assay buffer and 50 μ l of 20 μ M 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 TIMP2 significantly decreased rhMMP2 activity. The inhibition IC₅₀ was <30 nM.

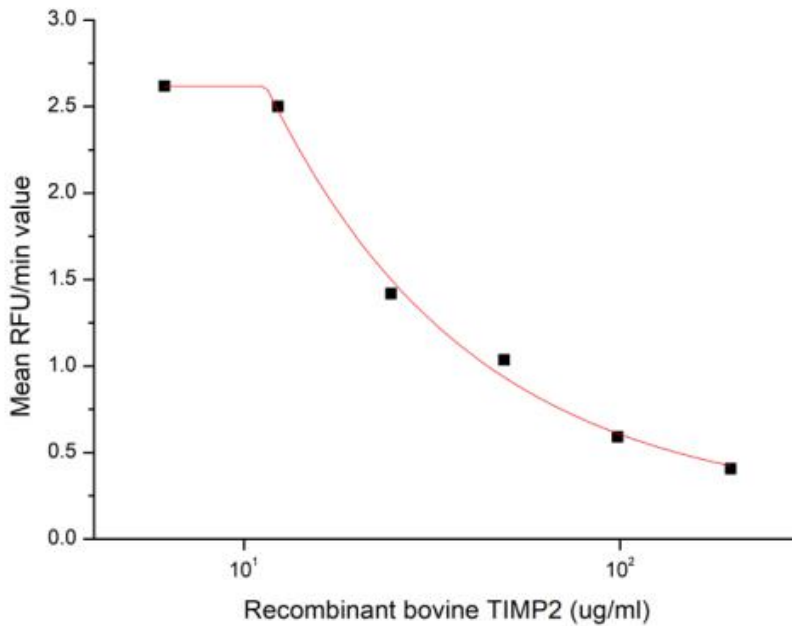


Figure 1. Inhibition of MMP2 activity by recombinant bovine TIMP2

[IDENTIFICATION]

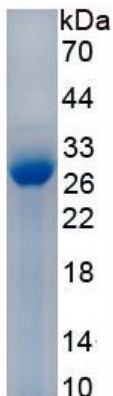


Figure 2. SDS-PAGE**Sample: Active recombinant TIMP2, Cattle****[IMPORTANT NOTE]**

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.