

APA130Bo01 100µg
Active Tissue Inhibitors Of Metalloproteinase 4 (TIMP4)
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: Ala50~Pro224

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: 8.1

Predicted Molecular Mass: 24.1kDa

Accurate Molecular Mass: 27kDa 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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STDPADPQKM IRYEIKQIKM FKGFEKVNDI QYIYTPFDSS LCGVKLEANS QKRYLLTGQI  
LSDGKVFVHL CNYIEPWENL SFLQRESLNH HYHLNCGCQI TTCYAVPCTI SAPNECLWTD  
WLLERKLYGY QAQHYVCMKH VDGSCSWYQG RLPLRKEFVD IIQP
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[ACTIVITY]

Tissue Inhibitors of Metalloproteinase 4 (TIMP4) is an enzyme that in humans is encoded by the TIMP4 gene. This gene belongs to the tissue inhibitor of metalloproteinases gene family. The proteins encoded by this gene family are inhibitors of the matrix metalloproteinases, a group of peptidases involved in degradation of the extracellular matrix. The secreted, netrin domain-containing protein encoded by this gene is involved in regulation of platelet aggregation and recruitment and may play role in hormonal regulation and endometrial tissue remodeling. The activity of recombinant bovine TIMP4 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 rbTIMP4 (MW: 24.05 KD) was diluted to different concentrations with the assay buffer. Mix 8 µl of rbTIMP4 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 μ l of 20 μ M 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 TIMP4 significantly decreased rhMMP2 activity. The inhibition IC₅₀ was <27 nM.

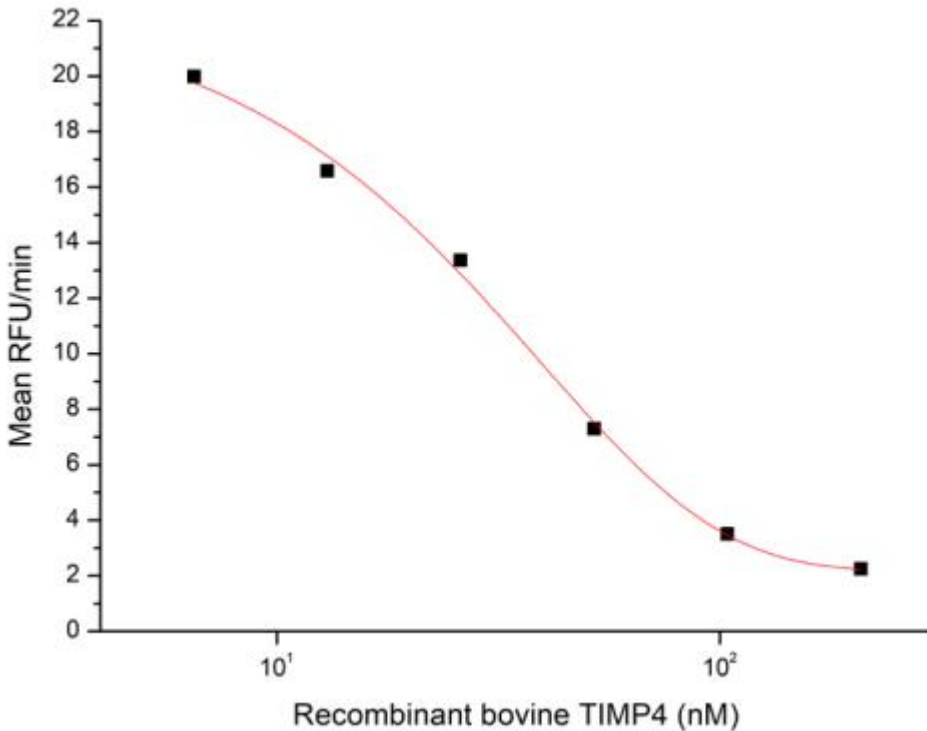


Figure 1. Inhibition of MMP2 activity by recombinant bovine TIMP4

[IDENTIFICATION]

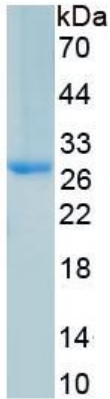


Figure 2. SDS-PAGE

Sample: Active recombinant TIMP4, 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.