

APA552Ca01 100µg

Active Tissue Inhibitors Of Metalloproteinase 1 (TIMP1)

Organism Species: *Canis familiaris*; Canine (Dog)

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: Gln32~Ala207

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: 8.7

Predicted Molecular Mass: 20.9kDa

Accurate Molecular Mass: 21kDa 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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QTALCNSQI VIRAKFVGTA EVNQTDLNRR  
YEIKMTKMPK GFSALGNASD IRFVDTPALE SVCGYLHRSQ NRSEEFVAG NLRDGHQLQIN  
TCSFVAPWSS LSTAQRRGFT KTYAAGCEGC TVFTCSSIPC KLQSDTHCLW TDHFLTGS DK  
GFQSRHLACL PREPGICTWQ SLRPRMA
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[ACTIVITY]

Tissue inhibitors of metalloproteinase 1 (TIMP1) is a member of the family of proteins that regulate the activation and proteolytic activity of the zinc enzymes known as matrix metalloproteinases (MMPs). TIMP-1 is a glycoprotein with a molecular mass of 28 kDa produced by a wide range of cell types. TIMP-1 inhibits active MMP-mediated proteolysis by forming an N-terminal, non-covalent binary complex with the MMP active site. The activity of recombinant dog TIMP1 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 TIMP1 (MW: 20.86 KD) was diluted to different concentrations with the assay buffer. Mix 8 µl of TIMP1 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 dog TIMP1 significantly decreased rhMMP2 activity. The inhibition IC₅₀ was <30 nM.

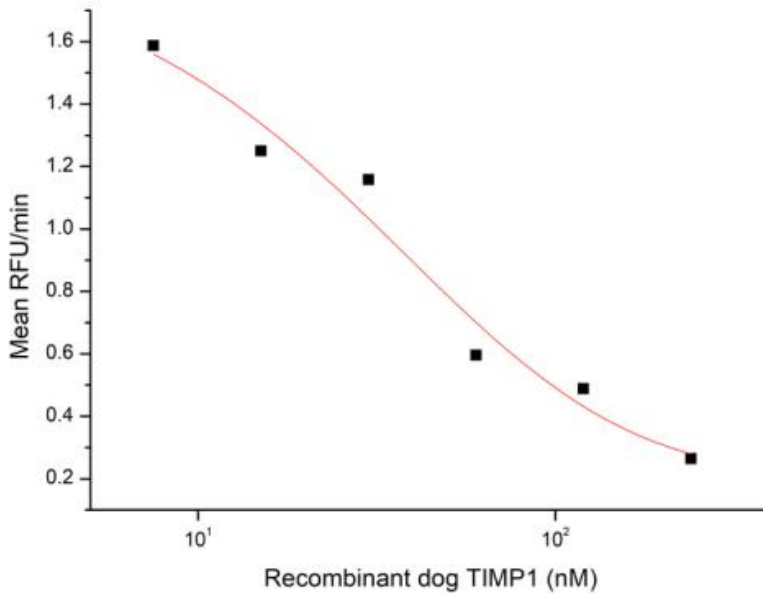


Figure 1. Inhibition of MMP2 activity by recombinant dog TIMP1

[IDENTIFICATION]

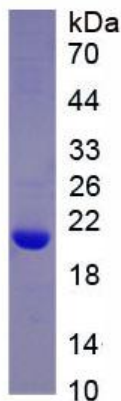


Figure 2. SDS-PAGE

Sample: Active recombinant TIMP1, Dog

[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.