

APA129Mu01 100µg

Active Tissue Inhibitors Of Metalloproteinase 3 (TIMP3)

Organism Species: Mus musculus (Mouse)

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: Gly20~Pro211 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 0.01% skl 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: 9.0

Predicted Molecular Mass: 23.3kDa

Accurate Molecular Mass: 28/22kDa as determined by SDS-PAGE reducing

conditions.

Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

- 1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
- 2. Relative charge: The composition of amino acids may affects the charge of the protein.
- 3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
- 4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
- 5. Polymerization of the target protein: Dimerization, multimerization etc.



[USAGE]

Reconstitute in ddH_2O to a concentration $\leq 0.1 mg/mL$. 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]

G AEACTCSPSH PQDAFCNSDI VIRAKVVGKK
LVKEGPFGTL VYTIKQMKMY RGFSKMPHVQ YIHTEASESL CGLKLEVNKY
QYLLTGRVYE GKMYTGLCNF VERWDHLTLS QRKGLNYRYH LGCNCKIKSC
YYLPCFVTSK NECLWTDMLS NFGYPGYQSK HYACIRQKGG YCSWYRGWAP
PDKSTSNATD P

[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. The activity of recombinant mouse 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 rmTIMP3 (MW: 23.25 KD) was diluted to different concentrations with the assay buffer. Mix 8 μl of rmTIMP3 curve dilutions, 12.8 μl of activated rhMMP-2, and 59.2 μl of assay

Cloud-Clone Corp. buffer, including a control containing assay buffer and the diluted rhMMP-2 and

incubate the reactions for 2 hours at 37 °C. Loading 50 μ l 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 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 mouse TIMP3 significantly decreased rhMMP2 activity. The inhibition IC50 was <26 nM.

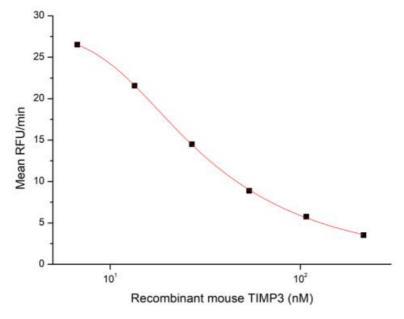


Figure 1. Inhibition of MMP2 activity by recombinant mouse TIMP3

[IDENTIFICATION]

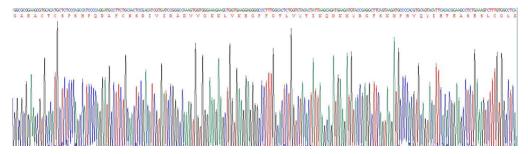


Figure 2. Gene Sequencing (extract)

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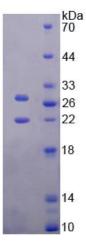


Figure 3. SDS-PAGE

Sample: Active recombinant TIMP3, Mouse

[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.