

**APA552Hu61 100µg**

**Active Tissue Inhibitors Of Metalloproteinase 1 (TIMP1)**

**Organism Species: *Homo sapiens (Human)***

***Instruction manual***

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

---

---

13th Edition (Revised in Aug, 2023)

## **[ PROPERTIES ]**

**Source:** Eukaryotic expression.

**Host:** 293F cell

**Residues:** Thr25~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 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:** 8.2

**Predicted Molecular Mass:** 22.2kDa

**Accurate Molecular Mass:** 33kDa 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 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.

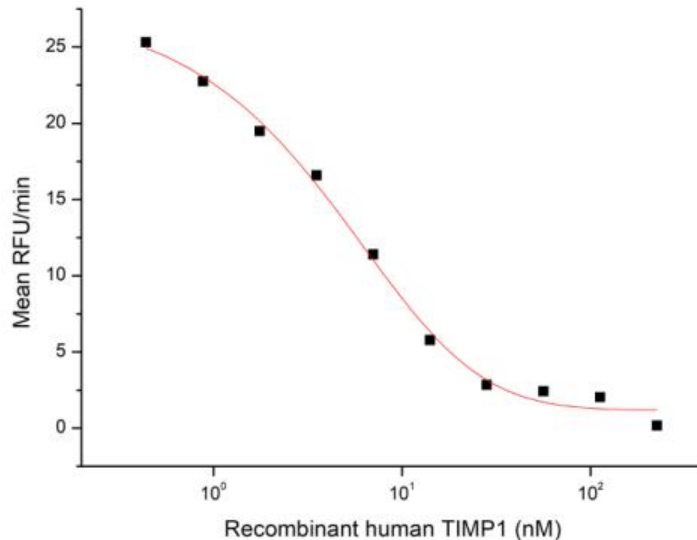
## [ SEQUENCE ]

```
TCVPPH PQTAFCNDDL VIRAKFVGTP  
EVNQTTLYQR YEIKMTKMYK GFQALGDAAD IRFVYTPAME SVCGYFHRSH  
NRSEEFLLIAG KLQDGLLHIT TCSFVAPWNS LSLAQRRTGFT KTYTVGCEEC  
TVFPCLSLIPC KLQSGTHCLW TDQLLQGSEK GFQSRHLACL PREPGLCTWQ  
SLRSQIA
```

## [ ACTIVITY ]

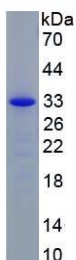
Tissue inhibitors of metalloproteinase 1 (TIMP1) is a member of the family of proteins that regulate the activation and proteolytic activity of the zinc enzymes known as matrix metalloproteinases (MMPs). TIMP-1 is a glycoprotein with a molecular mass of 28 kDa produced by a wide range of cell types. 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 human TIMP1 was measured by its ability to inhibit rhMMP2 cleavage of a fluorogenic peptide substrate MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH<sub>2</sub> in the assay buffer 50 mM Tris, 10 mM CaCl<sub>2</sub>, 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 rhTIMP1 (MW: 22.2 KD) was diluted to different concentrations with the assay buffer. Mix 8 µl of rhTIMP1 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 human TIMP1 significantly decreased rhMMP2 activity. The inhibition IC<sub>50</sub> was <4.4 nM.



**Figure 1. Inhibition of MMP2 activity by recombinant human TIMP1**

## **[ IDENTIFICATION ]**



**Figure 2. SDS-PAGE**

**Sample: Active recombinant TIMP1, Human**

## **[ 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.