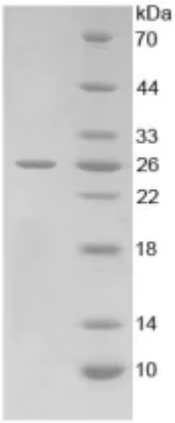
Active Tissue Inhibitors Of Metalloproteinase 3 (TIMP3)

Catalog#:PEH12221

概述（Summary）

别称（Alternative Names）	SFD; TIMP Metallopeptidase Inhibitor 3; Metallopeptidase Inhibitor 3; Sorsby Fundus Dystrophy; Pseudoinflammatory
纯度（Purity）	Greater than 90% as determined by reducing SDS-PAGE.
内毒素（Endotoxin level）	Please contact with the lab for this information
蛋白构建（Construction）	【Cys26-Pro211】
Accession #	P35625
表达宿主（Host）	E.coli
种属（Species）	Homo sapiens (Human)
预测分子量（Predicted MW）	25.2 kDa
制剂（Form）	Lyophilized from a 0.2 μm filtered solution of 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% SKL, 5% Trehalose and Proclin300.
运输方式（Shipping）	The product is shipped with ice bag. Upon receipt, store it immediately at the temperature listed below.
稳定性&储存（Stability &Storage）	12 months at -20°C to -80°C in lyophilized state.6 months at -20°C to -80°C under sterile conditions after reconstitution.7-10 days at 2°C to 8°C under sterile conditions after reconstitution.Please minimize freeze-thaw cycles.
复溶（Reconstitution）	Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.
背景介绍（References）	Complexes with metalloproteinases (such as collagenases) and irreversibly inactivates them by binding to their catalytic zinc cofactor. May form part of a tissue-specific acute response to remodeling stimuli. Known to act on MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-13, MMP-14 and MMP-15.

电泳图（SDS-PAGE image）



Greater than 90% as determined by reducing SDS-PAGE.

活性图（Bioactivity image）

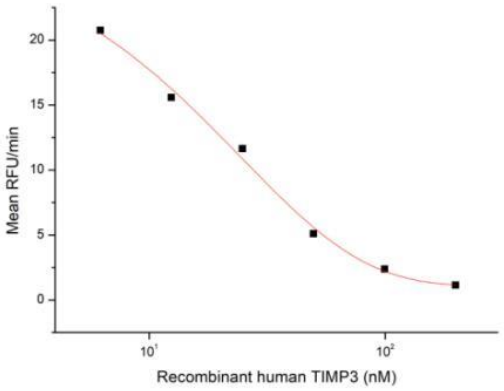


Figure 1. Inhibition of MMP2 activity by recombinant human TIMP3

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 human TIMP3 was measured by its ability to inhibit recombinant human MMP2 cleavage of a fluorogenic peptide substrate Mca-PLGL-Dpa-AR-NH2 in the assay buffer 50 mM Tris, 10 mM CaCl2, 150 mM NaCl, 0.05% Brij-35 (v/v), pH 7.5. The rhMMP-2 (APA100Hu61) was activated with 1 mM APMA at 37 °C for 1h. Then 16 µl 126 ug/mL rhTIMP-3, 25.6 µL of 100 ug/ml rhMMP-2 and 118.4 µL of assay buffer was incubated for 2 hours at 37 °C, including a control (in duplicate) containing assay buffer and the diluted rhMMP-2. The mixtures was diluted 5 fold in assay buffer followed by adding 50 ul 20 uM substrate, including a control containing assay buffer and substrate. Then read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively in kinetic mode for 5 minutes. Under these conditions, the enzyme amount of 50% inhibition of rhMMP-2 activity per minute is defined as a unit. The specific activity of TIMP3 is >6000 U/mg. 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 human 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 rhTIMP3 (MW: 25.28 KD) was diluted to different concentrations with the assay buffer. Mix 8 µl of rhTIMP3 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 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 human TIMP3 significantly decreased rhMMP2 activity. The inhibition IC50 was <15 nM.

Calculation

$$\text{TIMP3 activity（U/mg）} = \frac{\frac{A405_1/\text{min} - A405_2/\text{min}}{A405_1/\text{min}} \times 100\%}{50\%} / M$$

Where:

$A405_1/\text{min}$ = rhMMP-2 activity of ΔA405nm/min

$A405_2/\text{min}$ = inhibition of rhMMP-2 activity of TIMP3

M = mass of enzyme

注意事项（Note）

For Research Use Only , Not for Diagnostic Use.