Recombinant Human TIMP-3

Catalog Number: 973-TM

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human TIMP-3 protein Cys24-Pro211 Accession # P35625
N-terminal Sequence Analysis	Cys24
Predicted Molecular Mass	22 kDa

SPECIFICATIONS

SDS-PAGE	26 kDa, reducing conditions
Activity	Measured by its ability to inhibit human MMP-2 cleavage of a fluorogenic peptide substrate Mca-PLGL-Dpa-AR-NH_2 (Catalog # ES001). The IC_{50} value is approximately 3 nM, under conditions the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Lyophilized from a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% Brij-35 (v/v), pH 7.5 (TCNB)
- Recombinant Human TIMP-3 (rhTIMP-3) (Catalog # 973-TM)
- Recombinant Human MMP-2 (rhMMP-2) (Catalog # 902-MP)
- 4-Aminophenylmercuric acetate (APMA), 100 mM stock in DMSO
- Substrate: MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH_2 ((Catalog # ES001)), 2 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhMMP-2 to 100 µg/mL in Assay Buffer.
2. Activate 100 µg/mL rhMMP-2 with 1 mM APMA.
3. Incubate at 37 °C for 1 hour.
4. Prepare a curve of rhTIMP-3 (MW: 21,700 Da) in Assay Buffer. Make serial dilutions of: 5,000, 2,000, 1,000, 500, 300, 200, 150, 100, 20, and 2 nM.
5. After activation, dilute 100 µg/mL rhMMP-2 to 12.5 µg/mL in Assay Buffer.
6. Mix 16 µL of rhTIMP-3 curve dilutions, 25.6 µL of diluted rhMMP-2, and 118.4 µL of Assay Buffer.
7. Include a control (in duplicate) containing Assay Buffer and the diluted rhMMP-2.
8. Incubate reactions for 2 hours at 37 °C.
9. After incubation, dilute the mixtures 5 fold in Assay Buffer.
10. Dilute Substrate to 10 µM in Assay Buffer.
11. Load 50 µL of the diluted incubated mixtures in a plate, and start the reaction by adding 50 µL of 10 µM Substrate.
12. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively in kinetic mode for 5 minutes.
13. Derive the IC_{50} value for rhTIMP-3 from the curve.
14. Calculate specific activity for each point using the following formula (if needed):

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rhMMP-2: 0.020 µg
- Substrate: 5 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile, deionized water.
Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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BACKGROUND

Tissue inhibitors of metalloproteinases (TIMPs) are a family of proteins that regulate the activation and proteolytic activity of the zinc enzymes known as matrix metalloproteinases (MMPs). There are four members of the family, TIMP-1, TIMP-2, TIMP-3 and TIMP-4. TIMP-3 is a glycoprotein with a molecular mass of 30 kDa produced by a wide range of cell types. TIMP-3 inhibits active MMP-mediated proteolysis by forming a non-covalent binary complex with the MMP active site through its N-terminal domain. In addition, TIMP-3 is the only known member of the TIMP family that is an effective inhibitor of ADAMs such as TACE (1).

TIMP-3 is unique among the TIMPs because of its high affinity for binding to the extracellular matrix (2). Point mutations in the TIMP-3 C-terminal domain have been reported to result in Sorsby's fundus dystrophy, a disease leading to macular degeneration and loss of vision.

References:

1. Amour, A. *et al.* (1998) FEBS Lett. **435**:39.
2. Leco, K.J. *et al.* (1994) J. Biol. Chem. **269**:9352.

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