R&D SYSTEMS
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Recombinant Human TIMP-4

Catalog Number: 974-TSF

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Cys30-Pro224 Accession # Q99727
N-terminal Sequence Analysis	Cys30
Predicted Molecular Mass	22 kDa

SPECIFICATIONS

SDS-PAGE	23 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 2.5 nM, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
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% (w/v) Brij-35, pH 7.5 (TCNB)
- Recombinant human TIMP-4 (rhTIMP-4) (Catalog # 974-TSF)
- Recombinant Human MMP-2 (rhMMP-2) (Catalog # 902-MP)
- 4-Aminophenylmercuric acetate (APMA) (Sigma, Catalog # A-9563), 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 with 1 mM APMA in Assay Buffer.
2. Incubate at 37 °C for 1 hour (to activate).
3. Prepare a curve of rhTIMP-4 (MW: 22,400 Da) in Assay Buffer. Make serial dilutions of: 5000, 2000, 1000, 500, 300, 200, 150, 100, 20, and 2 nM.
4. After activation, dilute 100 µg/mL rhMMP-2 to 12.5 µg/mL in Assay Buffer.
5. Mix 16 µL of rhTIMP-4 curve dilutions, 25.6 µL of diluted rhMMP-2, and 118.4 µL of Assay Buffer. Include a control (in duplicate) containing 134.4 µL of Assay Buffer and 25.6 µL of diluted rhMMP-2.
6. Incubate reactions for 2 hours at 37 °C.
7. After incubation, dilute the mixtures five fold in Assay Buffer.
8. Dilute Substrate to 10 µM in Assay Buffer.
9. Load 50 µL of the diluted incubated mixtures in a plate, and start the reaction by adding 50 µL of 10 µM Substrate.
10. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively in kinetic mode for 5 minutes.
11. Derive the 50% inhibiting concentration (IC_{50}) for rhTIMP-4 by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
12. The specific activity for rhMMP-2 at each point may be determined 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
- rhTIMP-4: 50, 20, 10, 5, 3, 2, 1.5, 1, 0.2, and 0.02 nM
- Substrate: 5 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 200 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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BACKGROUND

Tissue inhibitors of metalloproteinases (TIMPs) are a family of secreted proteins that regulate the activation and proteolytic activity of the zinc enzymes known as matrix metalloproteinases (MMPs). There are four known members of the family, TIMP-1, -2, -3 and -4. TIMP-4 is produced by a wide range of tissues, particularly brain, heart, ovary and skeletal muscle (1, 2). Limited studies have shown that TIMP-4 has a tumor suppressive effect against Wilm's tumor, exhibits negative correlation with glioma maligancy and is found in breast carcinoma cells (3-5). TIMP-4 inhibits MMP-mediated proteolysis by forming a non-covalent binary complex with the MMP active site through its N-terminal domain. In addition, it binds to the hemopexin-like domain of pro-MMP-2 through its C-terminal domain in a manner similar to TIMP-2 (6). However, unlike TIMP-2, TIMP-4 does not promote pro-MMP-2 activation by MT1-MMP (MMP-14) (7). Although TIMP-4 is a potent inhibitor of most MMPs, it is not an effective inhibitor of ADAMs, such as TACE (8, 9).

References:

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