Recombinant Mouse Cystatin F

Catalog Number: 4557-PI

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ala19-Gln144, with a C-terminal 6-His tag Accession # O89098
N-terminal Sequence Analysis	Ala19
Structure / Form	Disulfide-linked homodimer
Predicted Molecular Mass	15 kDa

SPECIFICATIONS

SDS-PAGE	20 kDa, reducing conditions
Activity	Measured by its ability to inhibit active Cathepsin L cleavage of a fluorogenic peptide substrate Z-LR-AMC (Catalog # ES008). The IC_{50} value is <60 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	Supplied as a 0.2 µm filtered solution in Tris, NaCl and Brij-35. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, pH 6.0
- Dithiothreitol (DTT) (Sigma, Catalog # D0632), 1 M stock
- Recombinant Mouse Cystatin F (rmCystatin F) (Catalog # 4557-PI)
- Recombinant Human Cathepsin L (rhCathepsin L) (Catalog # 952-CY)
- Substrate: Z-Leu-Arg-AMC (Catalog # ES008) , 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 rhCathepsin L to 40 µg/mL in Assay Buffer with 5 mM DTT.
2. Incubate on ice for 15 minutes.
3. After incubation, dilute activated rhCathepsin L to 0.2 µg/mL in Assay Buffer.
4. Prepare a curve of rmCystatin F (MW: 15249 Da) in Assay Buffer. Make the following serial dilutions: 6000, 1000, 500, 250, 125, 62.5, 31.25, 15.625, and 5.208 nM.
5. Gently mix equal volumes of the rmCystatin L curve dilutions and the diluted active rhCathepsin L. Include a control (in duplicate) containing Assay Buffer and the diluted active rhCathepsin L.
6. Incubate mixtures at room temperature for 15 minutes.
7. Make a 5 fold dilution of the incubated curve dilutions in Assay Buffer.
8. Dilute Substrate to 20 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 20 µM Substrate.
10. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively in kinetic mode for 5 minutes.
11. Derive the 50% inhibition concentration (IC_{50}) for rmCystatin F by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
12. The specific activity for rhCathepsin L 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 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

- rhCathepsin L: 0.001 µg
- rmCystatin F curve: 300, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.781 and 0.260 nM
- Substrate: 10 µM

PREPARATION AND STORAGE

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 opening.

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BACKGROUND

Cystatin F, also known as leukocystatin and CMAP (Cystatin-like Metastasis-Associated Protein), is a new member of the Cystatin superfamily (1-3). Cystatin F is selectively expressed by hematopoietic cells and may be a biomarker for both liver metastasis and inflammatory lung disorders (3, 4). As a cysteine protease inhibitor, it shows selectivity towards Cathepsin L and legumain (1, 2, 5). Compared to other secreted Cystatins including C, D, E/M, S, SA and SN, which contain two intrachain disulfide bonds, Cystatin F has two extra Cys residues that may be involved in interchain disulfide bonds. rmCystatin F and rhCystatin F (Catalog # 1889-PI) exhibit disulfide bond-linked dimer formation, which was also the case for an *E. coli* expressed fusion protein containing mature human Cystatin F and glutathione S-transferase (2).

References:

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4. Werle, B. *et al. (*2003) Biol. Chem. **384**:281.
5. Gruninger-Leitch, F. *et al. (*2000) Nat. Biotechnol. **18**:66.

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