

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ala23-Val306 & Leu27-Val306, both with a C-terminal 10-His tag Accession # Q9WUU7
N-terminal Sequence Analysis	Ala23 & Leu27
Structure / Form	Pro form
Predicted Molecular Mass	33 kDa

SPECIFICATIONS

SDS-PAGE	40 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPPGFSAFK(Dnp)-OH (Catalog # ES005). The specific activity is >1,200 pmol/min/µg, 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 Sodium Acetate and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials	<ul style="list-style-type: none"> ● Assay Buffer: 25 mM NaOAc, 5 mM DTT, pH 3.0 ● Recombinant Mouse Cathepsin X/Z/P (rmCathepsin X/Z/P) (Catalog # 1033-CY) ● Fluorogenic Peptide Substrate V: MCA-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys-(DNP)-OH (Catalog # ES005) ● F16 Black Maxisorp Plate (Nunc, Catalog # 475515) ● Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent
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Assay	<ol style="list-style-type: none"> 1. Dilute rmCathepsin X/Z/P to 10 µg/mL in Assay Buffer. 2. Incubate at room temperature for 5 minutes to activate. 3. Dilute activated rmCathepsin X/Z/P to 0.4 ng/µL in Assay Buffer. 4. Dilute Substrate to 20 µM in Assay Buffer. 5. Load 50 µL of the 0.4 ng/µL rmCathepsin X/Z/P into a black well plate, and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 20 µM Substrate without any rmCathepsin X/Z/P. 6. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes. 7. Calculate specific activity: $\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* \text{ (RFU/min)} \times \text{Conversion Factor}^{**} \text{ (pmol/RFU)}}{\text{amount of enzyme (}\mu\text{g)}}$ <p>*Adjusted for Substrate Blank **Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975)</p>
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Final Assay Conditions	Per Well: <ul style="list-style-type: none"> ● rmCathepsin X/Z/P: 0.02 µg ● Substrate: 10 µM
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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. <ul style="list-style-type: none"> ● 6 months from date of receipt, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

Cathepsin X (also known as Cathepsin Z and P) is a relatively new cysteine protease of the papain family (1-5). Compared to other members of the papain family, Cathepsin X has a short proregion and unique insertions. The cysteine residue in the proregion forms a covalent and reversible bond with the active site cysteine residue (6). Acting as a carboxypeptidase, Cathepsin X displays a unique specificity (7-10). It is ubiquitously expressed in human tissues and conserved in other species such as mouse, nematode and echiuran. The nematode enzyme is apparently involved in molting of third stage larvae (11).

References:

1. Deussing, *et al.* (2000) *Biochim. Biophys. Acta* **1491**:93.
2. Santamaria, *et al.* (1998) *J. Biol. Chem.* **273**:16816.
3. Nagler and Menard (1998) *FEBS Lett.* **434**:135.
4. Pungercar and Ivanovski (2000) *Pflugers Arch.* **439**:R116.
5. Pungercar, *et al.* (2000) *Pflugers Arch.* **439**:R119.
6. Sivaraman, *et al.* (2000) *J. Mol. Biol.* **295**:935.
7. Menard, *et al.* (2001) *Biol. Chem.* **382**:839.
8. Therrien, *et al.* (2001) *Biochemistry* **40**:2702.
9. Klemencic, *et al.* (2000) *Eur. J. Biochem.* **267**:5404.
10. Guncar, *et al.* (2000) *Structure Fold Des.* **8**:305.
11. Lustigman, *et al.* (1996) *J. Biol. Chem.* **271**:30181.