Recombinant Human Cathepsin X/Z/P

Catalog Number: 934-CY

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ala21-Val303 (Gly23Val & Ser48Thr), with a C-terminal 10-His tag Accession # Q9UBR2
N-terminal Sequence Analysis	Ala21
Structure / Form	Pro form
Predicted Molecular Mass	33 kDa

SPECIFICATIONS

SDS-PAGE	41 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPPGFSAFK(Dnp)-OH (Catalog # ES005). The specific activity is >800 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

- Activation Buffer: 25 mM Sodium Acetate, pH 3.5
- Assay Buffer: 25 mM Sodium Acetate, 5 mM Dithiothreitol (DTT), pH 3.5
- Dithiothreitol (DTT), 1 M stock in deionized water
- Recombinant Human Cathepsin X/Z/P (rhCathepsin X/Z/P) (Catalog # 934-CY)
- Substrate: 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

Assay

1. Activate rhCathepsin X/Z/P.
   a. Dilute rhCathepsin X/Z/P to 20 µg/mL in Activation Buffer.
   b. Dilute DTT stock to 10 mM in Activation Buffer.
   c. Mix equal volumes of 20 µg/mL rhCathepsin X/Z/P and 10 mM DTT.
   d. Incubate at room temperature for 5 minutes.
2. Dilute activated rhCathepsin X/Z/P to 0.4 ng/µL in Assay Buffer.
3. Dilute Substrate to 20 µM in Assay Buffer.
4. Load 50 µL of the 0.4 ng/µL rhCathepsin X/Z/P in a black well 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 without any rhCathepsin X/Z/P.
5. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
6. Calculate specific activity:

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

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

Final Assay Conditions

Per Well:

- rhCathepsin X/Z/P: 0.02 µg
- Substrate: 10 µM

PREPARATION AND STORAGE

Shipping

The product is shipped with dry ice or equivalent. 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

Cathepsin X (also known as Cathepsin Z and P) is a 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).

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