Recombinant Human Cathepsin D

Catalog Number: 1014-AS

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human Cathepsin D protein Leu21-Leu412, with a C-terminal 10-His tag Accession # P07339.1
N-terminal Sequence Analysis	Leu21
Structure / Form	Pro form
Predicted Molecular Mass	44 kDa

SPECIFICATIONS

SDS-PAGE	50 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-PLGL-Dpa-AR-NH_2 (Catalog # ES001). The specific activity is >350 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 MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 0.1 M NaOAc, 0.2 M NaCl, pH 3.5
- Recombinant Human Cathepsin D (rhCathepsin D) (Catalog # 1014-AS)
- 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 rhCathepsin D to 20 µg/mL in Assay Buffer.
2. Aliquot 50 µL of 20 µg/mL rhCathepsin D.
3. Incubate at 37 °C for 30 minutes.
4. Dilute incubated rhCathepsin D to 1 ng/µL in Assay Buffer.
5. Dilute Substrate to 60 µM in Assay Buffer.
6. Load 50 µL of 1 ng/µL rhCathepsin D in a plate, and start the reaction by adding 50 µL of 60 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 60 µM Substrate.
7. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively in kinetic mode for 5 minutes.
8. 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 D: 0.050 µg
- Substrate: 30 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

Cathepsin D is a lysosomal aspartic protease of the pepsin family (1). Human cathepsin D is synthesized as a precursor protein, consisting of a signal peptide (residues 1-18), a propeptide (residues 19-64), and a mature chain (residues 65-412) (2-4). The mature chain can be processed further to the light (residues 65-161) and heavy (residues 169-412) chains. It is expressed in most cells and overexpressed in breast cancer cells (5). It is a major enzyme in protein degradation in lysosomes, and also involved in the presentation of antigenic peptides. Mice deficient in this enzyme showed a progressive atrophy of the intestinal mucosa, a massive destruction of lymphoid organs, and a profound neuronal ceroid lipofucinosis, indicating that cathepsin D is essential for proteolysis of proteins regulating cell growth and tissue homeostasis (6). Cathepsin D secreted from human prostate carcinoma cells are responsible for the generation of angiostatin, a potent endogeneous inhibitor of angiogenesis (6).

References:

1. Conner *et al.* in *Handbook of Proteolytic Enzymes* Barrett (1998) Academic Press, San Diego, p. 828.
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4. Redecker, *et al.* (1991) DNA Cell Biol. **10:**423.
5. Rochefort, *et al.* (2000) Clin. Chim. Acta. **291**:157.
6. Tsukuba, *et al.* (2000) Mol. Cells **10**:601.

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