R&D SYSTEMS
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Recombinant Mouse Cathepsin D

Catalog Number: 1029-AS

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ile21-Leu410, with a C-terminal 10-His tag Accession # Q3UCD9
N-terminal Sequence Analysis	Ile21
Structure / Form	Pro form
Predicted Molecular Mass	44 kDa

SPECIFICATIONS

SDS-PAGE	52 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 >600 pmol/min/µg, as measured under 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 MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 0.1 M Sodium Acetate, 0.2 M NaCl, pH 3.5
- Recombinant Mouse Cathepsin D (rmCathepsin D) (Catalog # 1029-AS)
- Substrate: MCA-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (Catalog # ES001)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rmCathepsin D to 20 µg/mL in Assay Buffer.
2. Incubate at RT for 10 minutes.
3. Dilute activated rmCathepsin D 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 D in a 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.
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:

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

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

Final Assay Conditions

Per Well:

- rmCathepsin D: 0.020 µg
- Substrate: 10 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 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.

BACKGROUND

Cathepsin D is a lysosomal aspartic protease of the pepsin family (4). Mouse Cathepsin D is synthesized as a precursor protein, consisting of a signal peptide (residues 1-20), a propeptide (residues 21-64), and a mature chain (residues 65-410) (1-3). 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 is responsible for the generation of angiostatin, a potent endogeneous inhibitor of angiogenesis (6).

References:

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3. Hetman, *et al.* (1994) DNA Cell Biol. **13**:419.
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5. Rochefort, *et al.* (2000) Clin. Chim. Acta. **291**:157.
6. Tsukuba, *et al.* (2000) Mol. Cells **10**:601.

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