R&D SYSTEMS
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Recombinant Mouse Cathepsin H

Catalog Number: 1013-CY

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Glu22-Val333, with a C-terminal 10-His tag Accession # Q3UCD6
N-terminal Sequence Analysis	Glu22
Structure / Form	Pro form
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	40 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Arg-7-amido-4-methylcoumarin (R-AMC). The specific activity is >400 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com
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

- Activation Buffer: 50 mM MES, 10 mM $CaCl_2$, 150 mM NaCl, pH 6.5
- Assay Buffer: 50 mM MES, pH 6.5
- Dithiothreitol (DTT) (Sigma, Catalog # D-0632), 1 M stock in deionized water
- Recombinant Mouse Cathepsin H (rmCathepsin H) (Catalog # 1013-CY)
- Bacterial Thermolysin (Catalog # 3097-ZN)
- Phosphoramidon (Catalog # EI006), 20 mM in methanol
- Substrate: Arg-7-amido-4-methylcoumarin (R-AMC) (Chem-Impex, Catalog # 5859), 10 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 rmCathepsin H to 200 µg/mL in Activation Buffer.
2. Dilute Thermolysin to 100 µg/mL in Activation Buffer.
3. Mix equal volumes of 100 µg/mL Thermolysin and 200 µg/mL rmCathepsin H.
4. Incubate at 37 °C for 1 hour.
5. Stop reaction by adding an equal volume of 2 mM Phosphoramidon in Assay Buffer to reaction mixture.
6. Incubate at room temperature for 10 minutes.
7. Then add an equal volume of Assay Buffer containing 20 mM DTT to reaction mixture. The concentration of rmCathepsin H is now 25 ng/µL.
8. Incubate reaction at room temperature for 30 minutes.
9. Dilute activated rmCathepsin H to 0.5 ng/µL in Assay Buffer.
10. Dilute Substrate to 200 µM in Assay Buffer.
11. In a plate load 50 µL of 0.5 ng/µL rmCathepsin H to wells, and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 200 µM Substrate.
12. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
13. 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 7-Amino, 4-Methyl Coumarin (AMC) (Sigma, Catalog # A-9891)

Final Assay Conditions

Per Well:

- rmCathepsin H: 0.025 µg
- Substrate: 100 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 H is a lysosomal cysteine protease of the papain family (1). It is synthesized as a precursor protein, consisting of a signal peptide (residues 1-20), a propeptide (residues 21-95), a mini chain (residues 96-103), a heavy chain (residues 114-290) and a light chain (residues 291-333) (2, 3). A truncated form with a 12 amino acid deletion in the signal peptide region is secreted (4). Cathepsin H is the only known mono-aminopeptidase in the papain family (5). Cathepsin H expression is significantly increased in disease states such as in prostate tumors, sera of asthmatic patients, and mucosa of colorectal cancer patients (4, 6, 7).

References:

1. Kirschke, H. (2004) in *Handbook of Proteolytic Enzymes* (ed. Barrett, *et al.*) p. 1089, Academic Press, San Diego.
2. Lafuse, W.P. *et al.* (1995) J. Leukoc. Biol. **57**:663.
3. Soderstrom, M. *et al.* (1999) Biochim. Biophys. Acta **1446**:35.
4. Waghray, A. *et al.* (2002) J. Biol. Chem. **277**:11533.
5. Guncar, G. *et al.* (1998) Structure **6**:51.
6. Cimerman, N. *et al.* (2001) Clin. Chim. Acta **310**:113.
7. del Re, E.C. *et al.* (2000) Br. J. Cancer. **82**:1317.

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