Recombinant Human Cathepsin S

Catalog Number: 1183-CY

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human Cathepsin S protein Gln17-Ile331 (pro) & Ser109-Ile331 (mature), both with a C-terminal 10-His tag Accession # P25774
N-terminal Sequence Analysis	Gln17 predicted & Ser109
Structure / Form	Pro and mature forms
Predicted Molecular Mass	39 kDa (Pro) and 28 kDa (mature)

SPECIFICATIONS

SDS-PAGE	37-41 kDa and 26-27 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH_2 (Catalog # ES002). The specific activity is >300 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, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM NaOAc, 5 mM DTT, 250 mM NaCl, pH 4.5
- Recombinant Human Cathepsin S (rhCathepsin S) (Catalog # 1183-CY)
- Substrate: MCA-Arg-Pro-Lys-Pro-Val-Glu-NVAL-Trp-Arg-Lys(DNP)-NH_2 (Catalog # ES002)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhCathepsin S to 10 µg/mL in Assay Buffer.
2. Incubate at room temperature for 2 hours.
3. Dilute rhCathepsin S to 0.5 ng/µL in Assay Buffer.
4. Dilute Substrate to 20 µM in Assay Buffer.
5. Load 50 µL of the 0.5 ng/µL rhCathepsin S 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 rhCathepsin S.
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:

- rhCathepsin S: 0.025 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 S is a lysosomal cysteine protease of the papain family (1). It plays a major role in the processing of the MHC class II-associated invariant chain (2). It has been implicated in the pathogenesis of several diseases such as Alzheimer's disease and degenerative disorders associated with the cells of the mononuclear phagocytic system (1). Human Cathepsin S is synthesized as a preproenzyme of 331 amino acid residues consisting a signal peptide (residues 1-16), a pro region (residues 17-114), and the mature enzyme (residues 115-331) (3-5). Cathepsin S is less abundant in tissues than Cathepsins B, L and H. The highest levels have been found in lymph nodes, spleen, macrophages and other phagocytic cells.

References:

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