Recombinant Human Cathepsin B

Catalog Number: 953-CY

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Arg18-Ile339 (pro) & Phe74-Ile339 (mature), both with a C-terminal 10-His tag Accession # P07858
N-terminal Sequence Analysis	Arg18 & Phe74
Structure / Form	Pro and Mature forms
Predicted Molecular Mass	37 kDa (Pro) & 29 kDa (Mature)

SPECIFICATIONS

SDS-PAGE	43 kDa and 36 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate Z-LR-AMC (Catalog # ES008). The specific activity is >2,500 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 Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 25 mM MES, 5 mM DTT, pH 5.0
- Assay Buffer: 25 mM MES, pH 5.0
- Recombinant Human Cathepsin B (rhCathepsin B) (Catalog # 953-CY)
- Fluorogenic Peptide Substrate VII: Z-Leu-Arg-AMC (Catalog # ES008)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhCathepsin B to 10 µg/mL in Activation Buffer.
2. Incubate at room temperature for 15 minutes.
3. Dilute rhCathepsin B to 0.2 ng/µL in Assay Buffer.
4. Dilute substrate to 20 µM in Assay Buffer.
5. Load 50 µL of the 0.2 ng/µL rhCathepsin B in 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 B.
6. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes. 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:

- rhCathepsin B: 0.01 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.

BACKGROUND

Cathepsin B is the first described member of the family of lysosomal cysteine proteases (1). Cathepsin B possesses both endopeptidase and exopeptidase activities, in the latter case acting as a peptidyl-dipeptidase. It is known to process a number of proteins, including pro and active caspases, prorenin and secretory leucoprotease inhibitor (SLPI) (2 - 4). Therefore, Cathepsin B may play a role in activation and inactivation of caspases, activation of renin and inactivation of SLPI, the key steps in apoptosis, angiotensin production, and progression of emphysema, respectively. Because of its increased levels and redistribution of the enzyme in human and animal tumors, Cathepsin B may also have role in invasion and metastasis (5).

In addition to lysosome, Cathepsin B can be secreted or associated with plasma membrane, cytoplasm, and nucleus. It is synthesized as a preproenzyme. Following removal of the signal peptide, the inactive proenzyme undergoes further modifications including removal of the pro region to result in the active enzyme (1).

References:

1. Mort, J.S. (2004) in *Handbook of Proteolytic Enzymes*. Barrett, A.J. *et al.* (eds): Academic Press, San Diego, p. 1079.
2. Vancompernolle, K. *et al.* (1998) FEBS Lett. **438**:150.
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5. Bergquin, I.M. and B.F. Sloane (1996) Adv. Exp. Med. Biol. **389**:281.