**DESCRIPTION**

**Source**  
Mouse myeloma cell line, NS0-derived  
Glu113-Val333 & Ala114-Val333, both with a C-terminal 6-His tag  
Accession # P07711

**N-terminal Sequence Analysis**  
Glu113 & Ala114

**Structure / Form**  
Mature form

**Predicted Molecular Mass**  
26 kDa

**SPECIFICATIONS**

**SDS-PAGE**  
36 kDa, reducing conditions

**Activity**  
Measured by its ability to cleave the fluorogenic peptide substrate Z-LR-AMC (Catalog # ES008). The specific activity is >25,000 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 Sodium Acetate and NaCl. See Certificate of Analysis for details.

**Activity Assay Protocol**

**Materials**
- Assay Buffer: 50 mM MES, 5 mM DTT, 1 mM EDTA, 0.005% (w/v) Brij-35, pH 6.0
- Recombinant Human Cathepsin L (rhCathepsin L) (Catalog # 952-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 L to 40 µg/mL in Assay Buffer.
2. Incubate diluted rhCathepsin L on ice for 15 minutes.
3. Dilute incubated 40 µg/mL rhCathepsin L to 0.02 ng/µL in Assay Buffer.
4. Dilute Substrate to 80 µM in Assay Buffer.
5. Load 50 µL of 0.02 ng/µL rhCathepsin L into a black well plate, and start the reaction by adding 50 µL of 80 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 80 µM Substrate without any rhCathepsin L.
6. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
7. Calculate specific activity:
   
   \[
   \text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\text{max}}^* \times (RFU/\text{min}) \times \text{Conversion Factor}^*}{\text{amount of enzyme (µg)}}
   \]

   *Adjusted for Substrate Blank
   **Derived using calibration standard 7-Amino, 4-Methyl Coumarin (AMC) (Sigma, Catalog # A-9891).

**Final Assay Conditions**

Per Well:
- rhCathepsin L: 0.001 µg
- Substrate: 40 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 L is a lysosomal cysteine protease expressed in most eukaryotic cells. Cathepsin L is known to hydrolyze a number of proteins, including the proform of urokinase-type plasminogen activator, which is activated by Cathepsin L cleavage (1). Cathepsin L has also been shown to proteolytically inactivate α1-antitrypsin and secretory leucoprotease inhibitor, two major protease inhibitors of the respiratory tract (2). These observations, combined with the demonstration of increased Cathepsin L activity in the epithelial lining fluid of the lungs of emphysema patients, have led to the suggestion that the enzyme may be involved in the progression of this disease. Cathepsin L has also been identified as a major excreted protein of transformed fibroblasts, indicating the enzyme could be involved in malignant tumor growth (3). Human Cathepsin L activity is greatest under mildly acidic conditions, from pH 4.5 - 6.5. The stability of the enzyme decreases at higher pH values.

**References:**