

DESCRIPTION

Source Mouse myeloma cell line, NS0-derived
Val18-Val334, with a C-terminal 10-His tag
Accession # O60911

N-terminal Sequence Analysis Val18

Structure / Form Pro form

Predicted Molecular Mass 37 kDa

SPECIFICATIONS

SDS-PAGE 40 kDa, reducing conditions

Activity Measured by its ability to cleave the fluorogenic peptide substrate Z-LR-AMC (Catalog # ES008).
The specific activity is >400 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: 25 mM Sodium Acetate, 0.1 M NaCl, 5 mM DTT, pH 5.5
 - Recombinant Human Cathepsin V (rhCathepsin V) (Catalog # 1080-CY)
 - Substrate: Z-Leu-Arg-AMC ((Catalog # ES008), 2 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 rhCathepsin V to 1 μg/mL in Assay Buffer.
 2. Dilute Substrate to 20 μM in Assay Buffer.
 3. Load into a black well plate 50 μL of 1 μg/mL rhCathepsin V and start the reaction by adding 50 μL of 20 μM Substrate.
 4. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
 5. Calculate specific activity:

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

*Adjusted for Substrate Blank

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

- Final Assay Conditions**
- Per Well:
- rhCathepsin V: 0.050 μg
 - Substrate: 10 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.

BACKGROUND

Cathepsin V (also known as Cathepsin L2 and U) is a cysteine protease of the papain family (1). It is expressed as a proenzyme in lysosomes, which can be autocatalytically converted to the mature form at pH 4.0 (2). Cathepsin V shows high amino acid identity with Cathepsin L (77% for the proenzymes and 80% for the mature enzymes) (3). However, Cathepsin V is specifically expressed in the thymus, testis, and corneal epithelium whereas Cathepsin L is expressed throughout the body.

References:

1. Turk, V. *et al.* (2001) EMBO J. **20**:4629.
2. Bromme, D. *et al.* (1999) Biochemistry **38**:2377.
3. Somoza, J.R. *et al.* (2000) Biochemistry **39**:12543.