

DESCRIPTION

Source Mouse myeloma cell line, NS0-derived
Val18-Tyr435, with an N-terminal 7-His tag
Accession # O89017

N-terminal Sequence Analysis His

Structure / Form Pro form

Predicted Molecular Mass 49 kDa

SPECIFICATIONS

SDS-PAGE 61 kDa, reducing conditions

Activity Measured by its ability to cleave the fluorogenic peptide substrate, N-carbobenzoyloxy-Ala-Ala-Asn-7-amido-4-methylcoumarin (Z-AAN-AMC). The specific activity is >350 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, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 0.1 M NaOAc, 0.1 M NaCl, pH 4.5
- Assay Buffer: 50 mM MES, 250 mM NaCl, pH 5.5
- Recombinant Mouse Legumain/Asparaginyl Endopeptidase (rmLegumain) (Catalog # 2058-CY)
- Substrate: Z-Ala-Ala-Asn-AMC (Bachem, Catalog # I-1865), 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 rmLegumain to 50 μg/mL in Activation Buffer.
2. Incubate for 4 hours at 37 °C.
3. Dilute rmLegumain to 2 ng/μL in Assay Buffer.
4. Dilute Substrate to 200 μM in Assay Buffer.
5. Load 50 μL of 2 ng/μL rmLegumain in the plate, and start the reaction by adding 50 μL of 200 μM Substrate. Include a Substrate Blank containing Assay Buffer and Substrate.
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:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Well:

- rmLegumain: 0.100 μg
- Substrate: 100 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

Legumain is a lysosomal cysteine protease found in all mouse tissues examined, but was particularly abundant in kidney and placenta (1). Legumain plays a pivotal role in the endosomal/lysosomal degradation system because the Legumain deficiency causes the accumulation of pro cathepsins B, H and L, another group of lysosomal cysteine proteases (2). Over-expression of Legumain in tumors is significant for invasion/metastasis (3). Also known as asparaginyl endopeptidase, it specifically cleaves peptide bonds with Asn at the P1 position. Nevertheless, it also cleaves peptide bonds with Asp at the P1 position. Auto-activation of pro Legumain involves both types of cleavage, which results in the removal of the pro peptides in both C- and N-termini (4). In addition, Legumain activates pro MMP-2 and processes bacterial antigens for MHC class II presentation and pro thymosin α to thymosin α_1 and thymosin α_{11} , two acidic peptides with immunoregulatory properties (5-7). Mouse Legumain is synthesized as a 435 amino acid precursor with a signal peptide (residues 1 to 17). The pro enzyme (residues 18 to 435) was expressed with an N-terminal His tag. The purified pro enzyme can be activated under the conditions as described above. Legumain activity can be inhibited by rmCystatin C and recombinant human cystatins C and E/M (Catalog # 1238-PI, 1196-PI, and 1286-PI).

References:

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3. Liu, C. *et al.* (2003) *Cancer Res.* **63**: 2957.
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