Recombinant Human Legumain/Asparaginyl Endopeptidase

Catalog Number: 2199-CY

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human Legumain/Asparaginyl Endopeptidase protein Ile18-Tyr433, with an N-terminal 7-His tag Accession # AAH03061.1
N-terminal Sequence Analysis	His
Structure / Form	Pro form
Predicted Molecular Mass	49 kDa

SPECIFICATIONS

SDS-PAGE	60 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, N-carbobenzyloxy-Ala-Ala-Asn-7-amido-4-methylcoumarin (Z-AAN-AMC). The specific activity is >250 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 Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 50 mM Sodium Acetate, 100 mM NaCl, pH 4.0
- Assay Buffer: 50 mM MES, 250 mM NaCl, pH 5.0
- Recombinant Human Legumain/Asparaginyl Endopeptidase (rhLegumain) (Catalog # 2199-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 rhLegumain to 100 µg/mL in Activation Buffer.
2. Incubate for 2 hours at 37 °C.
3. Dilute rhLegumain to 1 ng/µL in Assay Buffer.
4. Dilute Substrate to 200 µM in Assay Buffer.
5. Load into a black well plate 50 µL of 1 ng/µL rhLegumain and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 200 µM 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:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Well:

- rhLegumain: 0.050 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, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

Legumain is a lysosomal cysteine protease whose activity is found in several tissues tested (1, 2). 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 (3). Over-expression of Legumain in tumors is significant for invasion/metastasis (4). 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 the cleavage, which result in the removal of the pro peptides in both C- and N-termini (5). 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 (6-8). Human Legumain is synthesized as a 433 amino acid precursor with a signal peptide (residues 1-17). The pro enzyme (residues 18-433) was expressed with an N-terminal His tag. This activity of Legumain can be inhibited by recombinant human Cystatins C and E/M and recombinant mouse Cystatin C (Catalog # 1196-PI, 1286-PI and 1238-PI, respectively).

References:

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