Recombinant Rat Insulysin/IDE

Catalog Number: 6958-ZN

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived rat Insulysin/IDE protein Met42-Leu1019, with an N-terminal Met and 5-His tag Accession # P35559
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	114 kDa

SPECIFICATIONS

SDS-PAGE	95-115 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPPGFSAFK(Dnp)-OH (Catalog # ES005). The specific activity is >1,600 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 Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, Glycerol, and Brij-35. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 1 M NaCl pH 7.5
- Recombinant Rat Insulysin/IDE (rrInsulysin) (Catalog # 6958-ZN)
- Substrate: MCA-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(DNP)-OH (Catalog # ES005), 2 mM in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rrInsulysin to 0.2 µg/mL in Assay Buffer.
2. Dilute Substrate to 20 µM in Assay Buffer.
3. Load 50 µL of the 0.2 µg/mL rrInsulysin into a 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.
4. Read at excitation and emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes.
5. 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 MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rrInsulysin: 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, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

Insulysin, or insulin-degrading enzyme (IDE), is a zinc metallopeptidase of the inverzincin family. IDE is primarily located in the cytosol, but has been detected as a secreted enzyme and associated with the plasma membrane as well (1). The enzyme is expressed in many tissues, with the highest levels in liver, kidney, brain, and testis (2). IDE hydrolyzes a variety of regulatory peptides, including insulin, glucagon, atrial natriuretic factor, and transforming growth factor-a *in vitro* (1). In addition, IDE has been shown to degrade the amyloid b (Ab) peptide, which polymerizes into the plaques associated with Alzheimer's disease (3). Deficiencies in IDE activity may contribute to the pathogenesis of type 2 diabetes mellitus (DM2) and Alzheimer's disease. The IDE region of human chromosome 10q has been genetically linked to DM2 (4). When the IDE gene was specifically disrupted in mice, IDE -/- animals developed hyperinsulinemia and glucose intolerance, characteristics of DM2 (5). The IDE -/- mice were also shown to have a significant decrease in Ab degradation in the brain, resulting in increased cerebral accumulation of Ab peptide (6). This *in vivo* evidence is consistent with the hypotheses that IDE is important for the degradation of insulin in cells and for the clearance of Ab peptide in the brain. Rat Insulysin displays 95.5% and 98.5 % sequence homology with human and mouse insulysin, respectively.

References:

1. Affholter, J. A. *et al.* (1988) Science **242**:1415.
2. Duckworth, W. C. *et al.* (1998) Endocr. Rev. **19**:608.
3. Akiyama, H. *et al.* (1990) Biochem. Biophys. Res. Commun. **170**:1325.
4. Selkoe, D. J. *et al.* (2001) Neuron **32**:177.
5. Ghosh, S. *et al.* (2000) Am. J. Hum. Genet. **67**:1174.
6. Farris, W. *et al.* (2003) Proc. Natl. Acad. Sci. **100**:4162.

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