

## 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- $\alpha$  *in vitro* (1). In addition, IDE has been shown to degrade the amyloid  $\beta$  (A $\beta$ ) 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 A $\beta$  degradation in the brain, resulting in increased cerebral accumulation of A $\beta$  peptide. 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 A $\beta$  peptide in the brain.

## 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. (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. USA* **100**:4162.

## Description

<b>Source</b>	<i>Spodoptera frugiperda</i> , Sf21 (baculovirus)-derived Met42 - Leu1019, with an N-terminal Met and 7-His tag Accession # P14735
<b>N-terminal Sequence Analysis</b>	Met
<b>Predicted Molecular Mass</b>	114 kDa

## Specifications

<b>SDS-PAGE</b>	105 kDa, reducing conditions
<b>Activity</b>	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPPGFSAFK(Dnp)-OH, R&D Systems, Catalog # ES005. The specific activity is > 1,000 pmoles/min/ $\mu$ g, as measured under the described conditions. See Activity Assay Protocol.
<b>Endotoxin Level</b>	<1.0 EU per 1 $\mu$ g of the protein by the LAL method.
<b>Purity</b>	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
<b>Formulation</b>	Supplied as a 0.2 $\mu$ m filtered solution in Tris and NaCl. See Certificate of Analysis for details.

## Preparation and Storage

<b>Shipping</b>	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<b>Do not freeze.</b> <ul style="list-style-type: none"> <li>● 3 months from date of receipt, 2 to 8 °C as supplied.</li> <li>● 1 month, 2 to 8 °C under sterile conditions after opening.</li> </ul>

## Activity Assay Protocol

### Materials

- Assay Buffer: 50 mM Tris, 1 M NaCl pH 7.5
- Recombinant human Insulysin (R&D Systems, Catalog # 2496-ZN)
- Substrate: MCA-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(DNP)-OH (R&D Systems, Catalog # ES005), 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 rhInsulysin to 0.2  $\mu$ g/mL in Assay Buffer.
2. Dilute Substrate to 20  $\mu$ M in Assay Buffer.
3. Load 50  $\mu$ L of the 0.2  $\mu$ g/mL rhInsulysin into a plate, and start the reaction by adding 50  $\mu$ L of 20  $\mu$ M Substrate. Include a Substrate Blank containing 50  $\mu$ L Assay Buffer and 50  $\mu$ L of 20  $\mu$ M 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:

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

\*Adjusted for Substrate Blank

\*\*Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

### Final Assay Conditions Per Well

- rhInsulysin: 0.01  $\mu$ g
- Substrate: 10  $\mu$ M

8/3/2009

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NOT FOR USE IN HUMANS.