

APB897Hu01 100µg
Active Insulin Degrading Enzyme (IDE)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Ala753~Pro973

Tags: N-terminal His-tag

Purity: >90%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% SKL, 5% Trehalose and Proclin300.

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 7.2

Predicted Molecular Mass: 29.6kDa

Accurate Molecular Mass: 30kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

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AHTKPLLP  SQLVRYREVQ  LPDRGWFVYQ  QRNEVHNNCG  IEIYYQTMQ  
STSENMFLF  FCQIISEPCF  NTLRTKEQLG  YIVFSGPRRA  NGIQGLRFII  
QSEKPPHYL  SRVEAFLITM  EKSIEDMTEE  AFQKHIQALA  IRRLDKPKKL  
SAECAKYWGE  IISQQYNFDR  DNTEVAYLKT  LTKEDIKIFY  KEMLAVDAPR  
RHKVSVHVL  REMDSCPVVG  EFP
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[ACTIVITY]

Insulin Degrading Enzyme (IDE) is an evolutionarily conserved 110-kDa zinc metalloprotease. It has been described principally as a cytosolic enzyme but is also found in multiple cellular compartments including endosomes, peroxisomes, mitochondria, the cell surface and in secreted form. IDE is a major enzyme responsible for insulin degradation. In addition to insulin, IDE degrades many targets including glucagon, atrial natriuretic peptide, and beta-amyloid peptide, regulates proteasomal degradation and other cell functions. In addition, IDE can degrade IGF2, thus affecting the concentration and activity of IGF2 in the cell. Thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human IDE and recombinant rabbit IGF2. Briefly, IDE was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ l were then transferred to IGF2-coated microtiter wells and incubated for 1h at 37°C.

Wells were washed with PBST and incubated for 1h with anti-IDE pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 °C , wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37°C . Finally, add 50 µL stop solution to the wells and read at 450/630 nm immediately. The binding activity of recombinant human IDE and recombinant rabbit IGF2 was shown in Figure 1, the EC50 for this effect is 0.08 ug/mL.

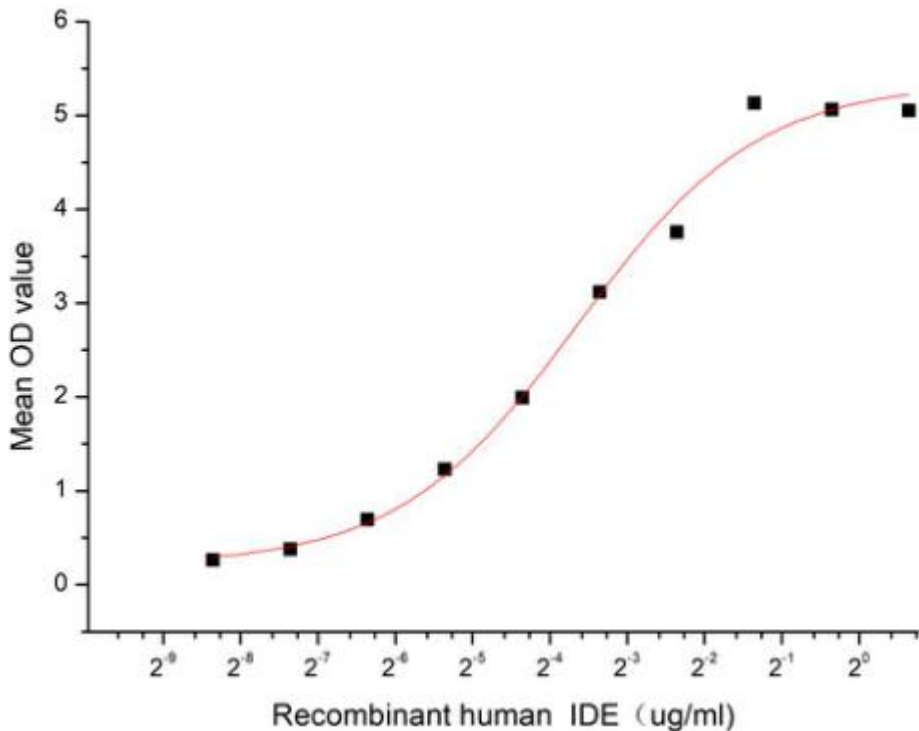


Figure 1. The binding activity of recombinant human IDE and recombinant rabbit IGF2

[IDENTIFICATION]

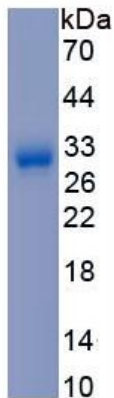


Figure 2. SDS-PAGE

Sample: Active recombinant IDE, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.