

APA448Mu01 100µg

Active Insulin (INS)

Organism Species: *Mus musculus* (Mouse)

Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Phe25~Ser54 and Gly90~Asn110 linked by GGGGS

Tags: N-terminal His-tag

Purity: >98%

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% sarcosyl, 5% trehalose, and Proclin300.

Predicted isoelectric point: 7.1

Predicted Molecular Mass: 10.3kDa

Accurate Molecular Mass: 10kDa as determined by SDS-PAGE reducing conditions.

Applications: Cell culture; Activity Assays; In vivo assays.

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

[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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FVKQHL CGSHLVEALY LVCGERGFFY  
TPMSGGGGSG IVDQCCTSIC SLYQLENYCN
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[ACTIVITY]

INS (Insulin) is a peptide hormone produced by beta cells of the pancreatic islets, which decreases blood glucose concentration and increases cell permeability to monosaccharides, amino acids and fatty acids. It has been reported that insulin triggers phosphorylation of a number of substrates by binding to its receptors, which was important for cell proliferation, cell cycle progression, cell division and differentiation. To detect the effect of Insulin on cell proliferation, MCF-7 cells were seeded into triplicate wells of 96-well plates at a density of 2,000 cells/well and allowed to attach overnight, then the medium was replaced with serum-free standard DMEM prior to the addition of various concentrations of INS. After incubated for 48h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 μ L of CCK-8 solution was added to each well of the plate, then the absorbance at 450nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37°C.



Figure 1. Cell proliferation of MCF-7 cells after stimulated with INS.

- (A) MCF-7 cells cultured in DMEM, stimulated with 100ng/mL INS for 72h;
- (B) Unstimulated MCF-7 cells cultured in DMEM for 72h.

The dose-effect curve of INS was shown in Figure 2. It was obvious that INS significantly promoted cell proliferation of MCF-7 cells. The ED50 for this effect is typically 11.71 to 57.11 ng/mL.

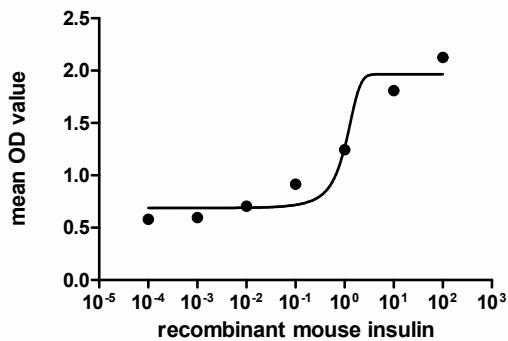


Figure 2. The dose-effect curve of INS on MCF-7 cells.

[IDENTIFICATION]

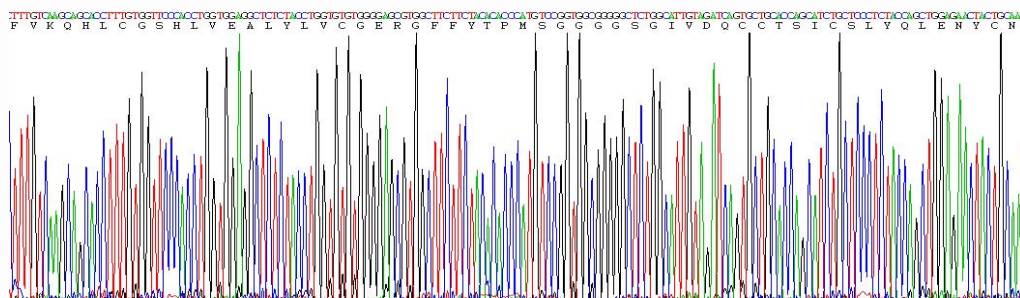


Figure 3. Gene Sequencing (extract)

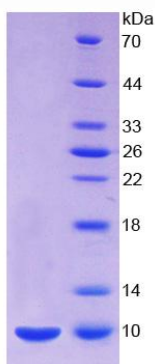


Figure 4. SDS-PAGE

Sample: Active recombinant INS, Mouse

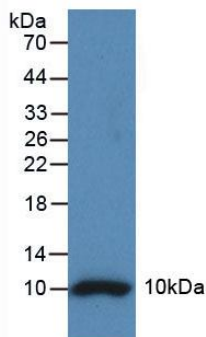


Figure 5. Western Blot

Sample: Recombinant INS, Mouse;

Antibody: Rabbit Anti-Mouse INS Ab (PAA448Mu01)