APA448Mu01 10μg
Active Insulin (INS)
Organism Species: *Mus musculus* (Mouse)
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: 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 0.01% skl, 5%Trehalose.
Original Concentration: 400μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 7.1
Predicted Molecular Mass: 10.3kDa
Accurate Molecular Mass: 10kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in ddH_2O 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 37ºC 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]

FVKQHL CGSHLVEALY LVCGERGFFY TPMSGGGGSG IVDQCCTSIC SLYQLENYCN

[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, 10μL 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.

A B

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.

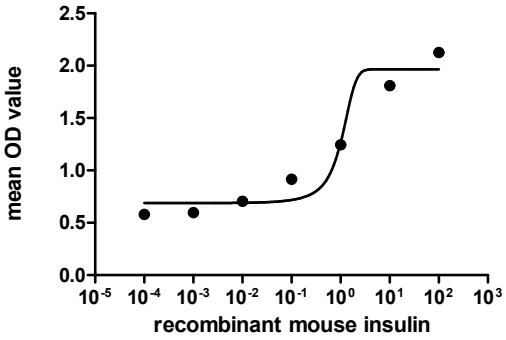

Figure 2. The dose-effect curve of INS on MCF-7 cells.

Insulin (INS) is a polypeptide hormone originating in the beta cells of the pancreas and serving as a principal regulator for the storage and production of carbohydrates. Insulin decreases blood glucose concentration. It increases cell permeability to monosaccharides, amino acids and fatty acids. It accelerates glycolysis, the pentose phosphate cycle, and glycogen synthesis in liver. Besides, Insulin Receptor (INSR) has been identified as an interactor of INS, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant mouse INS and recombinant rat INSR. Briefly, INS were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 ul were then transferred to INSR-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-INS pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution , wells were incubated 15-25 minutes at 37℃. Finally, add 50 μL stop solution to the wells and read at 450 nm immediately. The binding activity of of recombinant mouse INS and recombinant rat INSR was shown in Figure 1, the EC50 was 8.68 ug/ml.

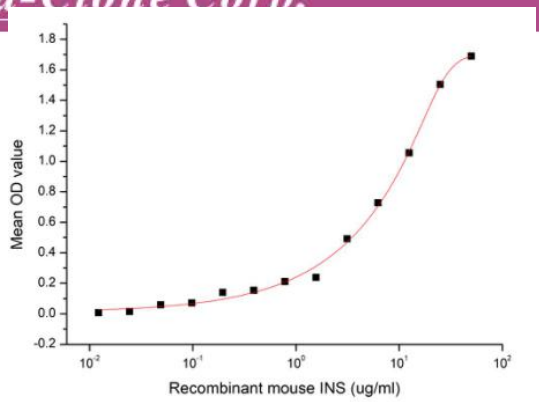

Figure 3. The binding activity of recombinant mouse INS and recombinant rat INSR

[IDENTIFICATION]

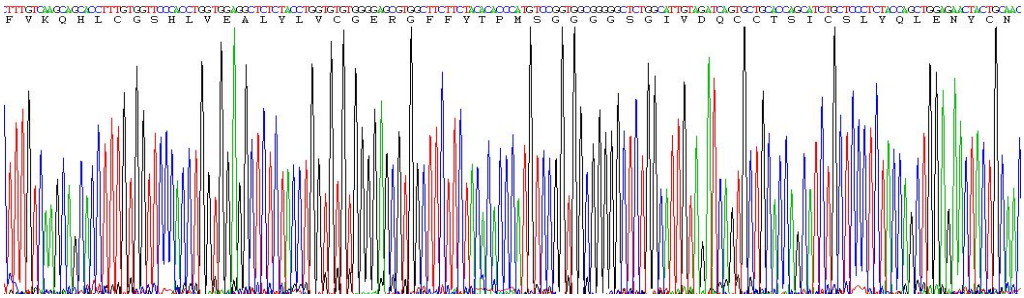

Figure 4. Gene Sequencing (extract)

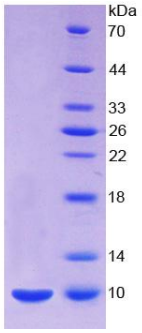

Figure 5. SDS-PAGE

Sample: Active recombinant INS, Mouse

[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.