

APA448Bo01 100µg

Active Insulin (INS)

Organism Species: *Bos taurus*; Bovine (Cattle)

Instruction manual

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

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Phe25~Ala54

Tags: Two N-terminal Tags, His-tag and GST-tag

Purity: >92%

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 6.4

Predicted Molecular Mass: 33.4kDa

Accurate Molecular Mass: 36kDa 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]

TPKA

FVNQHL CGSHLVEALY LVCGERGFFY

[ACTIVITY]

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 (ISR) has been identified as an interactor of INS, thus a binding ELISA assay was conducted to detect the interaction of recombinant bovine INS and recombinant human ISR. Briefly, INS were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to ISR-coated microtiter wells and incubated for 2h at 37°C. 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°C. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of of INS and ISR was shown in Figure 1, and this effect was in a dose dependent manner.

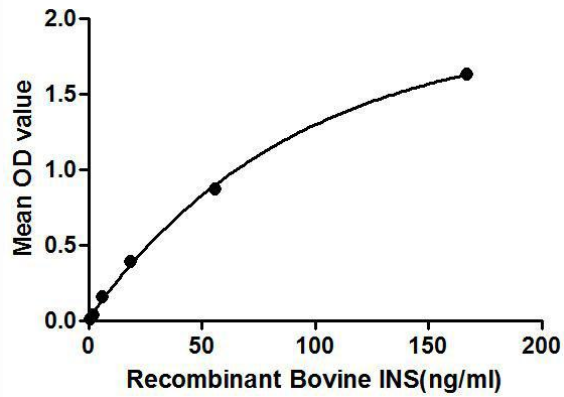


Figure 1. The binding activity of INS with ISR.

[IDENTIFICATION]

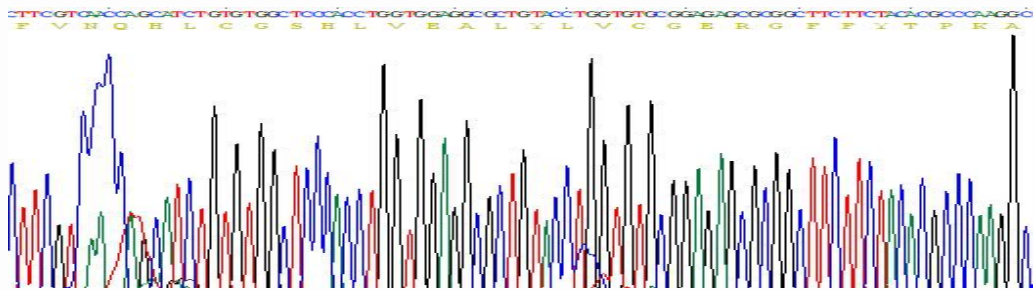


Figure 2. Gene Sequencing (extract)

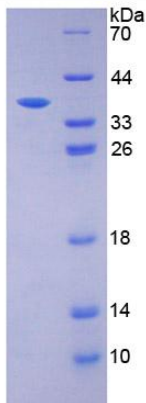


Figure 3. SDS-PAGE

Sample: Active recombinant INS, Bovine

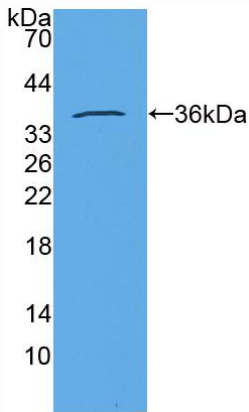


Figure 4. Western Blot

Sample: Recombinant INS, Bovine;

Antibody: Rabbit Anti-Bovine INS Ab (PAA448Bo01)