

# Active Insulin (INS) Instruction Manual

## SBPA128Mu01

### Mus musculus (Mouse)

#### Buffer Formulation

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

#### Traits

Freeze-dried powder

#### Purity

> 97%

#### Isoelectric Point

7.1

#### Applications

Cell culture; Activity Assays.

### ACTIVITY TEST

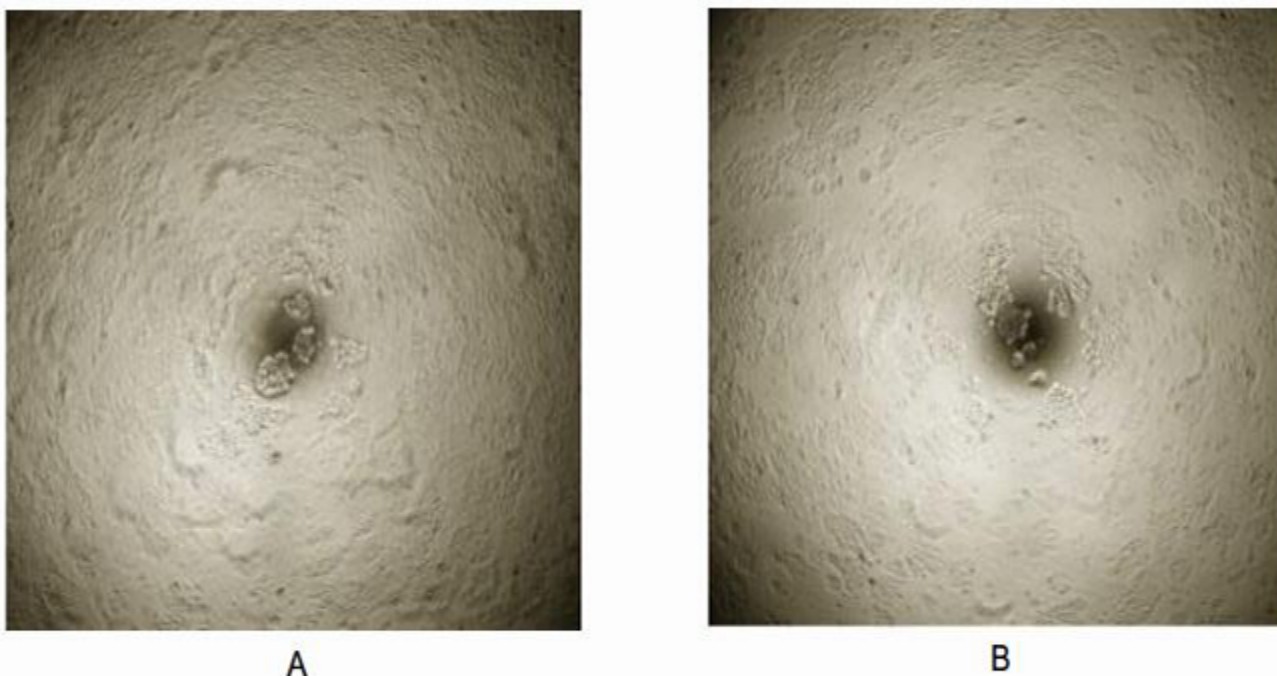
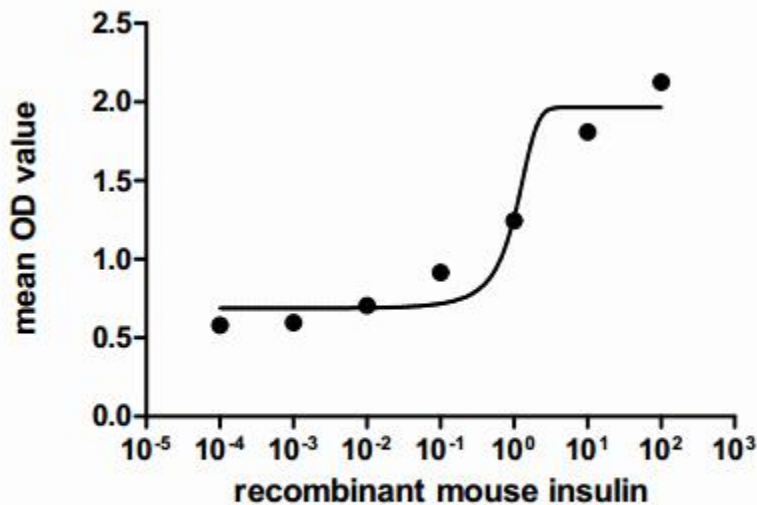


Figure 1. Cell proliferation of MCF-7 cells after stimulated with INS.

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

(B) Unstimulated MCF-7 cells cultured in DMEM for 72h.

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 $\mu$ 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 $^{\circ}$ C.



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

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.

## USAGE

Reconstitute in 20mM Tris, 150mM NaCl (PH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

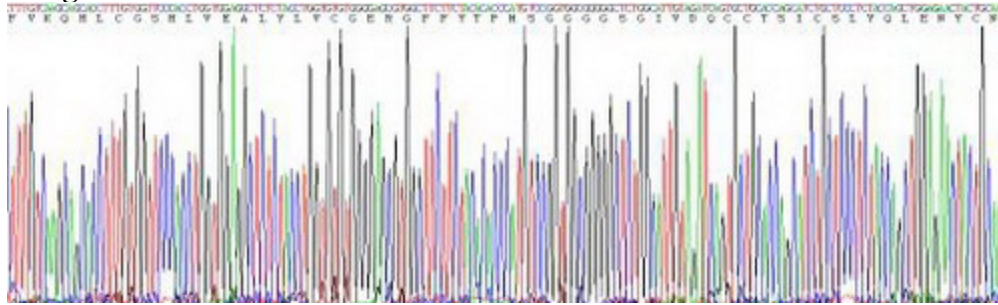
## STORAGE

Avoid repeated freeze/thaw cycles. Store at 2-8°C for one month. Aliquot and store at -80°C for 12 months.

## STABILITY

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.

## Image



SDS-PAGE Image

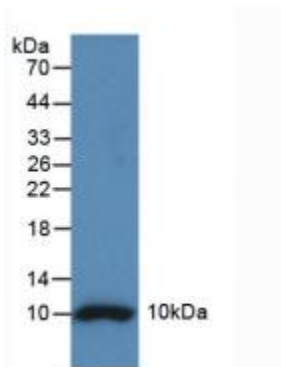


Figure. Western Blot; Sample: Recombinant INS, Mouse.

## **[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.

