Active Insulin Like Growth Factor 1 (IGF1) Instruction Manual

SBPA031Mu02

Mus musculus (Mouse)

Buffer Formulation	PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.
Traits	Freeze-dried powder
Purity	> 97%
Isoelectric Point	9.7
Applications	Cell culture; Activity Assays.

ACTIVITY TEST

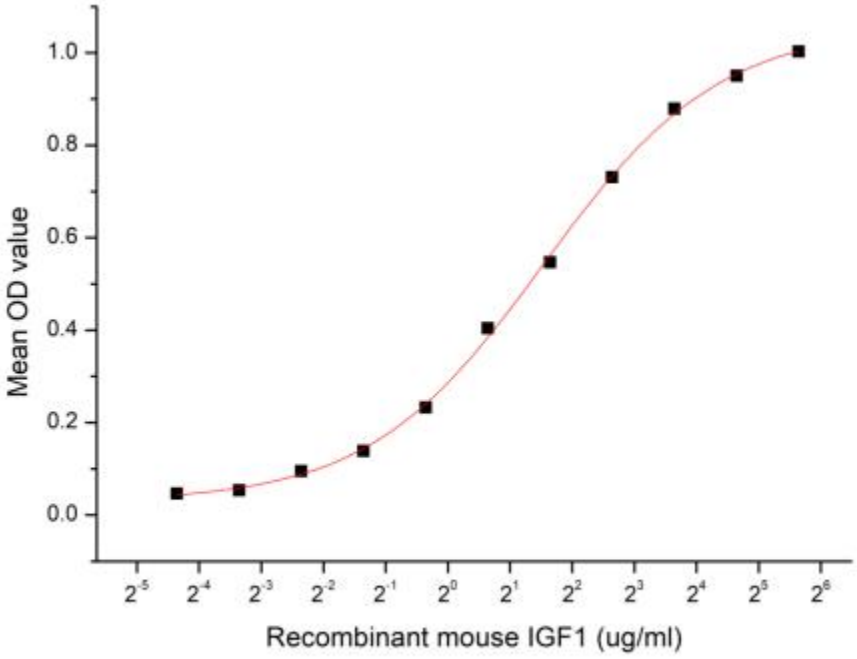
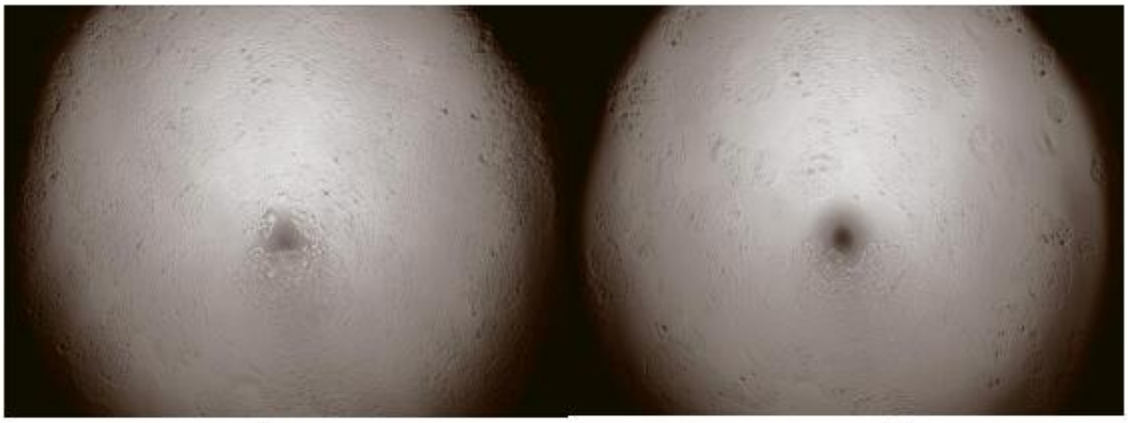


Figure 1. The binding activity of IGF1 with IGFBP5

Insulin-like growth factor 1 (IGF1), also called somatomedin C is a hormone similar in molecular structure to insulin. It plays an important role in childhood growth and continues to have anabolic effects in adults. A synthetic analog of IGF1, mecasermin, is used for the treatment of growth failure. Besides, Insulin Like Growth Factor Binding Protein 5 (IGFBP5) has been identified as an interactor of IGF1, thus a binding ELISA assay was conducted to detect the interaction of recombinant mouse IGF1 and recombinant mouse IGFBP5. Briefly, IGF1 were diluted serially in PBS, with 0.01%

BSA (pH7.4). Duplicate samples of 100 µl were then transferred to IGFBP5-coated microtiter wells and incubated for 1h at 37℃. Wells were washed with PBST and incubated for 1h with anti-IGF1 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50µL stop solution to the wells and read at 450 nm immediately. The binding activity of IGF1 and IGFBP5 was shown in Figure 1, and this effect was in a dose dependent manner, the EC50 was 2.92 ug/ml.



A B

Figure 1. Cell proliferation of MCF-7 cells after stimulated with IGF1.

(A) MCF-7 cells cultured in DMEM, stimulated with 1ng/ml IGF1 for 96h;
(B) Unstimulated MCF7 cells cultured in DMEM for 96h.

To measure the effect of IGF1 on cell proliferation, breast cancer MCF-7 cells were seeded into triplicate wells of 96-well plates at a density of 5,000 cells/well and allowed to attach, replaced with serum-free overnight, then the medium was replaced with 1% serum standard DMEM prior to the addition of various concentrations of recombinant mouse IGF1. After incubated for 96h, 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℃. Proliferation of MCF-7 cells after incubation with IGF1 for 96h observed by inverted microscope was shown in Figure1. Cell viability was assessed by CCK-8 assay after incubation with recombinant IGF1 for 96h. The result was shown in Figure2. It was obvious that IGF1 significantly increased cell viability of MCF-7 cells.

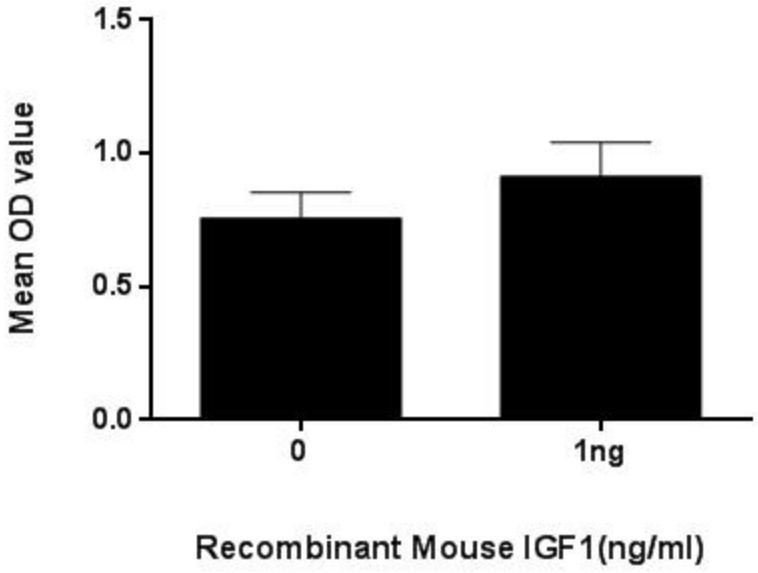


Figure 2. Cell proliferation of MCF-7 cells after stimulated with IGF1.

USAGE

Reconstitute in 10mM PBS (pH7.4) 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

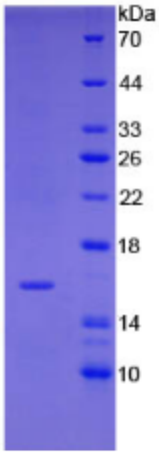


Figure. SDS-PAGE

[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.