

APA050Hu61 100µg
Active Insulin Like Growth Factor 1 (IGF1)
Organism Species: Homo sapiens (Human)
Instruction manual

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

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Gly49~Ala118

Tags: N-terminal His-tag

Purity: >95%

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: 7.8

Predicted Molecular Mass: 9.3kDa

Accurate Molecular Mass: 14kDa 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.)

Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[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]

GP

ETLCGAELVD ALQFVCGDRG FYFNKPTGYG SSSRRAPQTG IVDECCFRSC
DLRRLEMYCA PLKPAKSA

[ACTIVITY]

Insulin-like growth factor I (IGF1), is a hormone similar in molecular structure to insulin but have a much higher growth-promoting activity, it belongs to a family of proteins involved in mediating growth and development. It is reported that IGF1 induces the proliferation, migration, differentiation of a large types of cells including the MCF-7 breast cancer cell line. To test the effect of growth factors on 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 IGF-1. After incubated for 72h, 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

measure the absorbance at 450nm using a microplate reader after incubating the plate for 1-4 hours at 37°C.

Cell proliferation of MCF-7 cells after incubation with IGF1 for 72h observed by inverted microscope was shown in Figure 1.

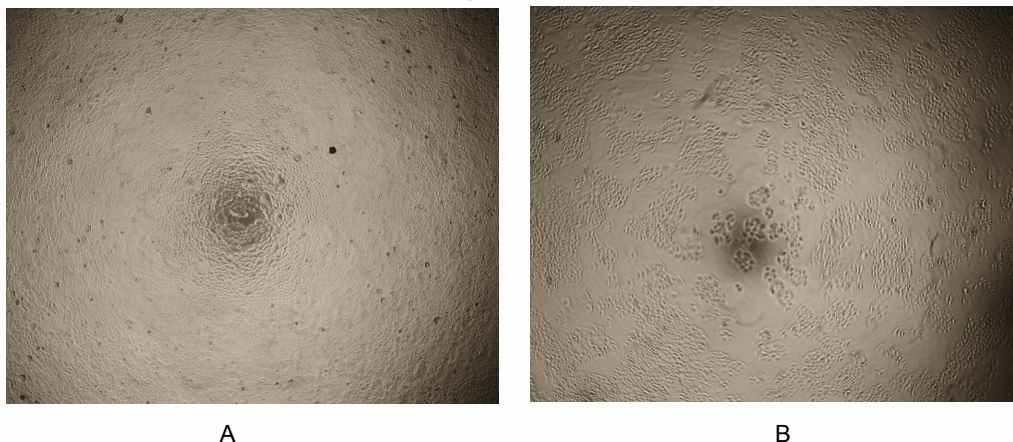


Figure 1. Cell proliferation of MCF-7 cells after stimulated with IGF1.

(A) MCF-7 cells cultured in serum-free DMEM, stimulated with 10ng/mL IGF1 for 72h;

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

The dose-effect curve of IGF1 was shown in Figure2. It was obvious that IGF1 significantly promoted cell proliferation of MCF-7 cells. The ED50 for this effect is typically 8.66~17.19 ng/mL.

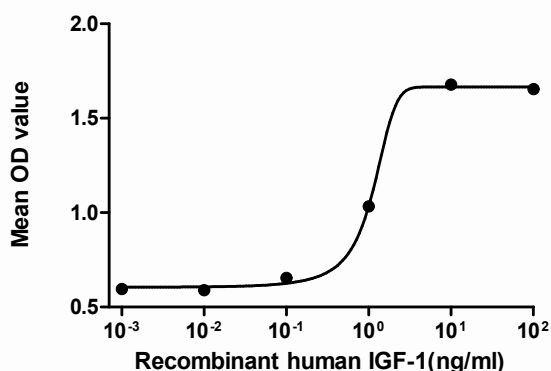


Figure 2. The dose-effect curve of IGF1 on MCF-7 cells.

[IDENTIFICATION]

IGGKGGGAGAGCCTCIGGGGGGTSAGCTGTTGGATGCTCTTAGTTGCTGCTGGGAGAGAGGGCTTTTATTTCAGCAGCCCGAGGGTATGGCTGGGGAGTGGGAGGGGGCTCGAGCAGGCATGGTGGATGAGTGGCTGGCTTGGGAGCTGTGATCTTAGGAGGCTGGAGATGATTGGCGCCCTCAGG
 G P E T L C G A E I V D A L Q F V C G D R G F Y F N K P T G Y G S S S R R A P Q T G I V D E C C F R S C D L R R L E M Y C A P L K I

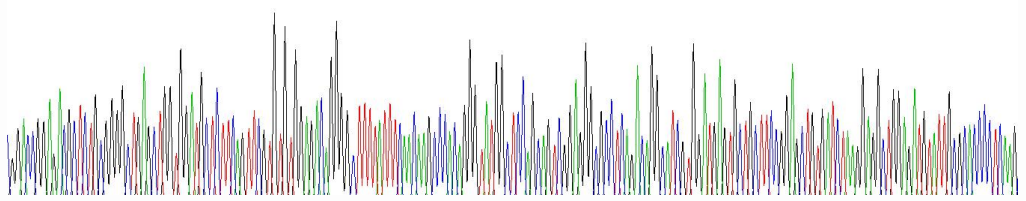


Figure 3. Gene Sequencing (extract)

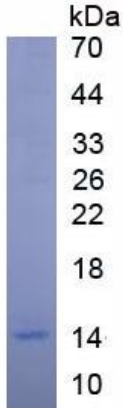


Figure 4. SDS-PAGE

Sample: Active recombinant IGF1, Human

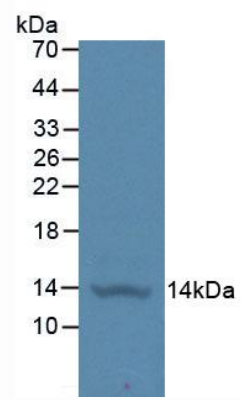


Figure 5. Western Blot

Sample: Recombinant IGF1, Human;

Antibody: Rabbit Anti-Human IGF1 Ab (PAA050Hu06)