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

1st 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.

Applications: Cell culture; Activity Assays; In vivo assays.

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

Predicted isoelectric point: 7.8

Predicted Molecular Mass: 9.3kDa

Accurate Molecular Mass: 14kDa as determined by SDS-PAGE reducing conditions.

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 2-8ºC for one month.

Aliquot and store at -80ºC 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]

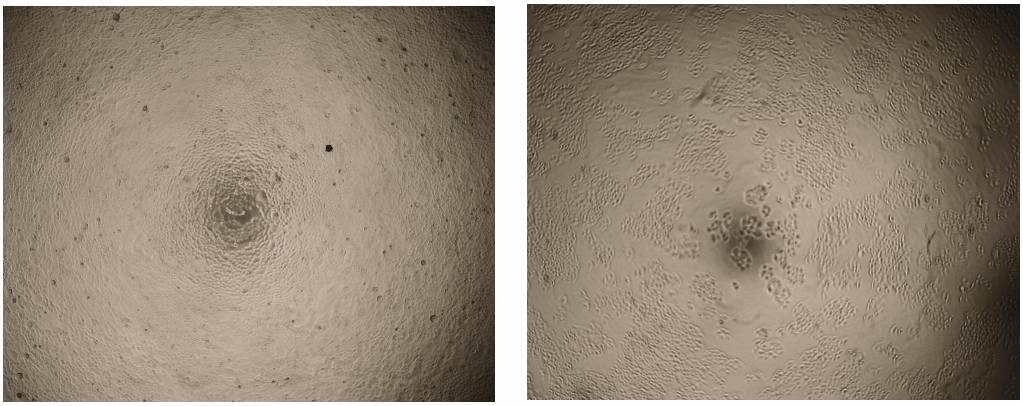
GP
ETLCGAELVD ALQFVCGDRG FYFNKPTGYG SSSRRAPQTG IVDECCFRSC
DLRRLEMYCA PLKPAKSA

[ACTIVITY]

Mechanism: 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 µL 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.



A B

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.

Cell proliferation was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with various concentrations of IGF1 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.

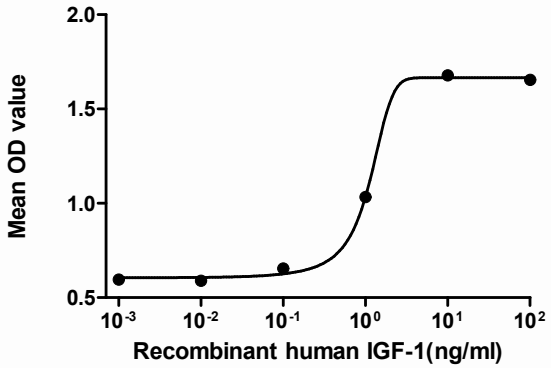


Figure 2. The dose-effect curve of IGF1 on MCF-7 cells.

[IDENTIFICATION]

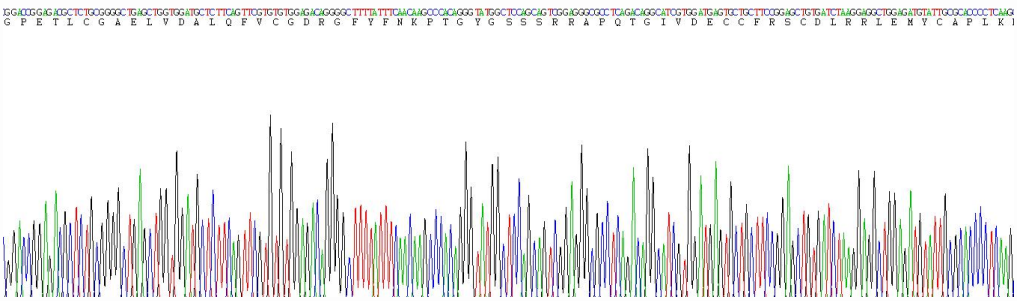


Figure 3. Gene Sequencing (extract)

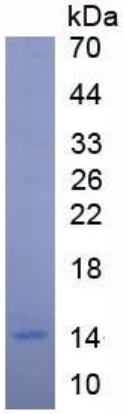


Figure 4. SDS-PAGE

Sample: Active recombinant IGF1, Human

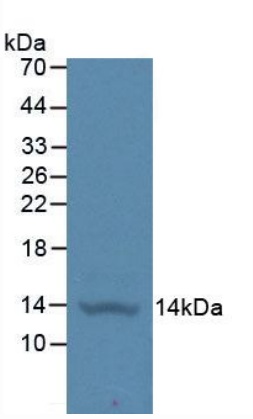


Figure 5. Western Blot

Sample: Recombinant IGF1, Human;

Antibody: Rabbit Anti-Human IGF1 Ab (PAA050Hu06)