APA051Hu01 100μg
Active Insulin Like Growth Factor 2 (IGF2)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Ala25~Glu91
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 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: 7.7
Predicted Molecular Mass: 8.5kDa
Accurate Molecular Mass: 9kDa 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 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]

AYRPSE TLCGGELVDT LQFVCGDRGF YFSRPASRVS RRSRGIVEEC CFRSCDLALL ETYCATPAKS E

[ACTIVITY]

Insulin-like growth factor 2 (IGF2) is one of three protein hormones that share structural similarity to insulin. It has growth-regulating, insulin-like and mitogenic activities. IGF2 exerts its effects by binding to the IGF-1 receptor and to the short isoform of the insulin receptor. IGF2 may also bind to the IGF2 receptor (also called the cation-independent mannose 6-phosphate receptor), which acts as a signalling antagonist; that is, to prevent IGF2 responses. Besides, Extracellular Matrix Metalloproteinase Inducer (EMMPRIN) has been identified as an interactor of IGF2, thus a binding ELISA assay was conducted to detect the interaction of recombinant human IGF2 and recombinant human EMMPRIN. Briefly, IGF2 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to EMMPRIN-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-IGF2 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℃. Finally, add 50μL stop solution to the wells and read at 450nm immediately. The binding activity of IGF2 and EMMPRIN was shown in Figure 1, and this effect was in a dose dependent manner.

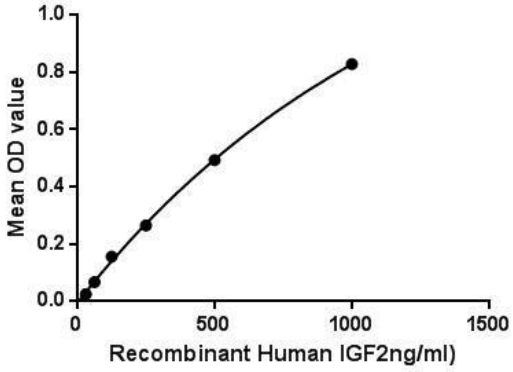
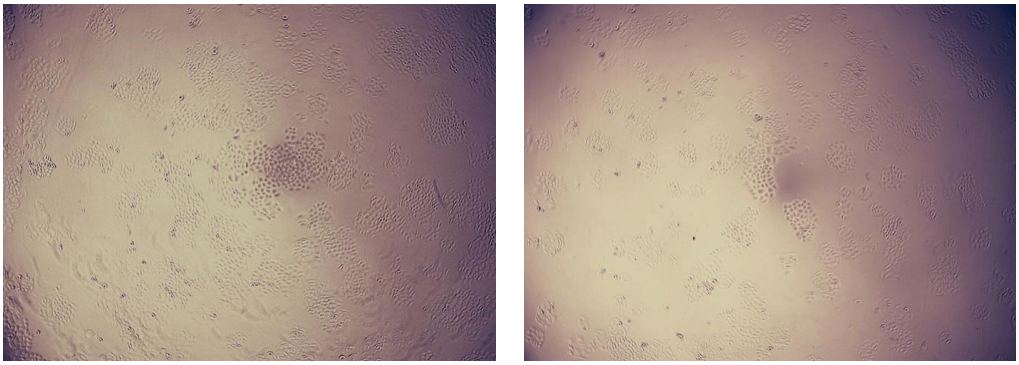


Figure 1. The binding activity of IGF2 with EMMPRIN.

To test the effect of IGF2 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 human IGF2. 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 IGF2 for 96h observed by inverted microscope was shown in Figure 2. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant IGF2 for 96h. The result was shown in Figure 3. It was obvious that IGF2 significantly increased cell viability of MCF-7 cells.



A B

Figure 2. Cell proliferation of MCF-7 cells after stimulated with IGF2.

(A) MCF-7 cells cultured in DMEM, stimulated with 1ng/mL IGF2 for 96h;

(B) Unstimulated MCF7 cells cultured in DMEM for 96h.

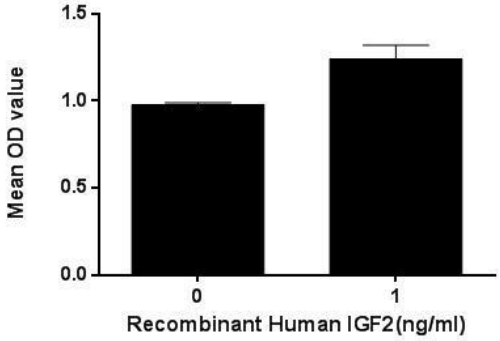


Figure 3. Cell proliferation of MCF-7 cells after stimulated with IGF2.

[IDENTIFICATION]

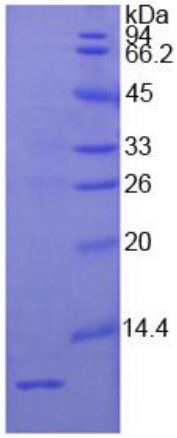


Figure 4. SDS-PAGE

Sample: Active recombinant IGF2, Human

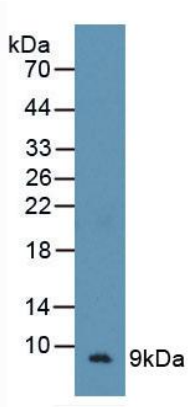


Figure 5. Western Blot

Sample: Recombinant IGF2, Human;

Antibody: Rabbit Anti-Human IGF2 Ab (PAA051Hu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.