

APA047Hu02 100µg
Active Active Hepatocyte Growth Factor (HGF)
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: Gln32~Asn291

Tags: N-terminal GST-tag

Purity: >95%

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: 8.5

Predicted Molecular Mass: 54.5kDa

Accurate Molecular Mass: 55kDa 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 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]

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QRKRRNTIH EFKKSAKTTL
IKIDPALKIK TKKVNTADQC ANRCTRNKGL PFTCKAFVFD KARKQCLWFP
FNSMSSGVKK EFGHEFDLYE NKDYIRNCII GKGRSYKGTV SITKSGIKCQ
PWSSMIPHEH SFLPSSYRGK DLQENYCRNP RGEEGPWCF TSNPEVRYEV
CDIPQCSEVE CMTNGESYR GLMDHTESGK ICQRWDHQTP HRHKFLPERY
PDKGFDDNYC RNPDGQPRPW CYTLDPHTRW EYCAIKTCAD N
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[ACTIVITY]

Hepatocyte growth factor (HGF) is a paracrine cellular growth, motility and morphogenic factor. It is secreted by mesenchymal cells and targets and acts primarily upon epithelial cells and endothelial cells, but also acts on haemopoietic progenitor cells and T cells. It has been shown to have a major role in embryonic organ development, specifically in myogenesis, in adult organ regeneration, and in wound healing. Besides, Heparan sulfate proteogly (HSPG) can has been identified as an interactor of HGF, thus a binding ELISA assay was conducted to detect the interaction of recombinant human HGF and recombinant human HSPG. Briefly, HGF were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to HSPG-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-HGF 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°C. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of HGF and HSPG was shown in Figure 1, and this effect was in a dose dependent manner.

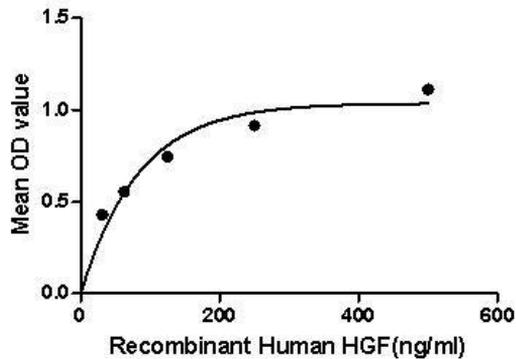


Figure 1. The binding activity of HGF with HSPG.

To test the effect of HGF on cell proliferation of HepG2 cell line, 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 HGF. 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 $^{\circ}$ C .



A

B

Figure 2. Cell proliferation of HepG2 cells after stimulated with HGF.

(A) Unstimulated HepG2 cells cultured in DMEM for 72h;

(B) HepG2 cells cultured in DMEM, stimulated with 0.01ng/mL HGF for 72h.

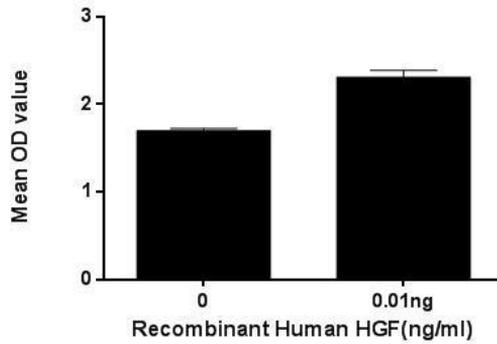


Figure 3. Cell proliferation of HepG2 cells after stimulated with HGF.

[IDENTIFICATION]

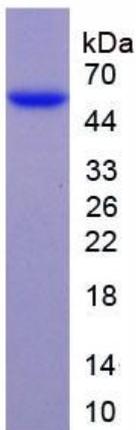


Figure 4. SDS-PAGE

Sample: Active recombinant HGF, Human

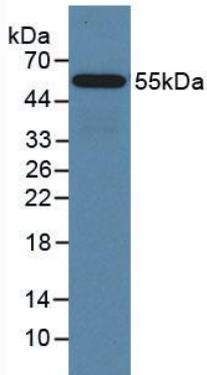


Figure 5. Western Blot

Sample: Recombinant HGF, Human;

Antibody: Rabbit Anti-Human HGF Ab (PAA047Hu02)

[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.