

**APA560Hu02 10µg**  
**Active Epidermal Growth Factor (EGF)**  
**Organism Species: Homo sapiens (Human)**  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1st Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Asn971~Arg1023

**Tags:** N-terminal His-tag

**Purity:** >92%

**Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method).

**Buffer Formulation:** PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.

**Original concentration :** 1000ug/ml

**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:** 4.8

**Predicted Molecular Mass:** 9.9kDa

**Accurate Molecular Mass:** 10kDa as determined by SDS-PAGE reducing conditions.

## **[ USAGE ]**

Reconstitute in 10mM PBS (pH7.4) 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.

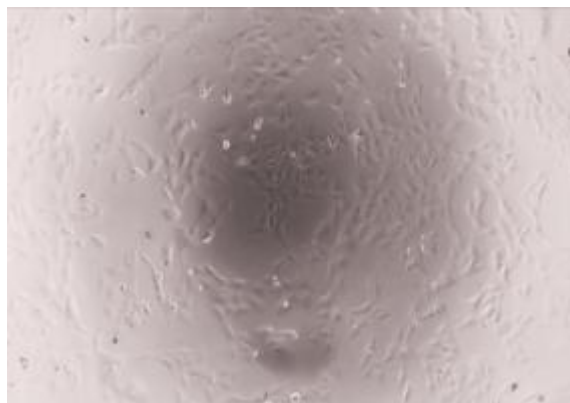
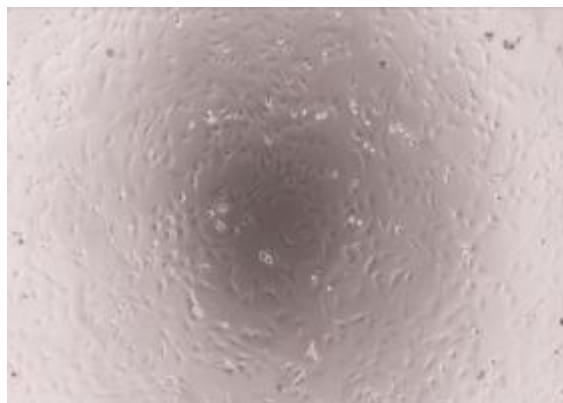
**Stability Test:** The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

## **[ SEQUENCE ]**

NSDSECPLSH DGYCLHDGVC MYIEALDKYA  
CNCVVG YIGE RCQYRDLKWW ELR

## **[ ACTIVITY ]**

Epidermal growth factor (EGF) is a growth factor that stimulates cell growth, proliferation, and differentiation by binding to its receptor EGFR. To test the effect of EGF on cell proliferation of 3T3 fibroblasts, Balb/c 3T3 cells were seeded into triplicate wells of 96-well plates at a density of 4,000 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 EGF. After incubated for 72h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10  $\mu$ l of CCK-8 solution was added to each well of the plate, then measure the absorbance at 450 nm using a microplate reader after incubating the plate for 1-4 hours at 37 °C. Cell proliferation of 3T3 cells after incubation with EGF for 72h observed by inverted microscope was shown in Figure 1.

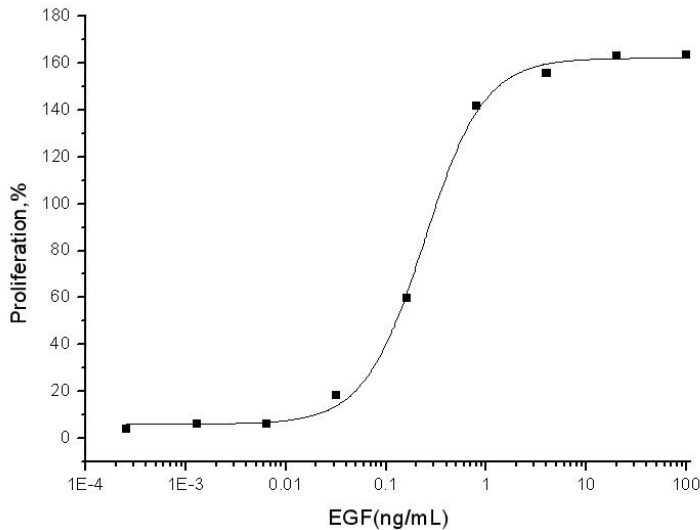


**A****B****Figure 1. Cell proliferation of 3T3 cells after stimulated with EGF .**

(A) Balb/c 3T3 cultured in DMEM, stimulated with 1ng/ml EGF 72h;

(B) Unstimulated Balb/c 3T3 cells cultured in serum-free DMEM for 72h.

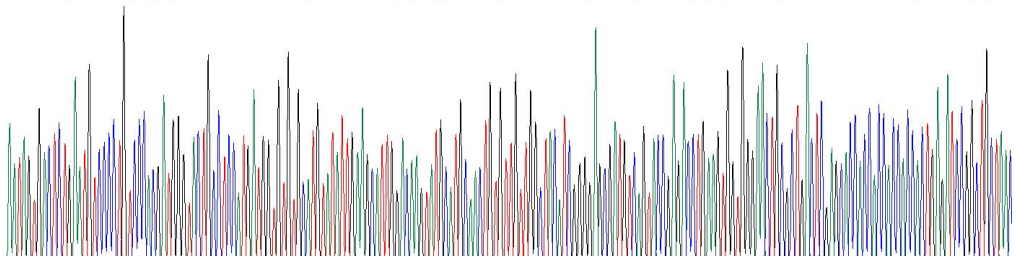
The dose-effect curve of EGF was shown in Figure2. It was obvious that EGF significantly promoted cell proliferation of 3T3 cells .The ED50 for this effect is typically 0.1ng/ml.



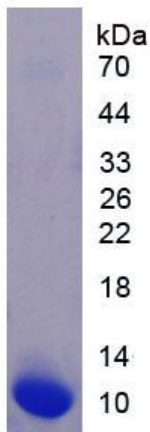
**Figure 2. The dose-effect curve of EGF on Balb/c 3T3 cells**

**[ IDENTIFICATION ]**

AATAGTGACTCTGTAATGTCCTCCG TG TCCGACGATGGG TAC TGCC TCCCATGATGG TG TCCCATATATA TTGAAGCAT TGG AGAAGTATGCATGCACTG TGTGTTGGC TGA TCGGGG AGCGATGT GATACCG GAGAC TGAAGTGTGGGAAC TCC GC TGACTGAGCCACCACCACCCACTGAGATCCGGCTGCTAAC  
 H S D S E C P L S H D D V C L E D G V C H I E R A L D K V A C R C V V G I G E R C Q V R D L E V V E L R \* L E R R R R R R R R \* D P A A H

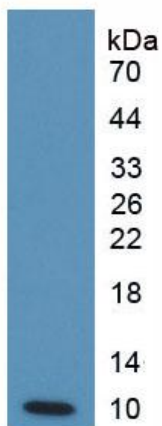


**Figure 3. Gene Sequencing (extract)**



**Figure 4. SDS-PAGE**

**Sample: Active recombinant EGF, Human**



**Figure 5. Western Blot**

**Sample: Recombinant EGF, Human;**

**Antibody: Rabbit Anti-Human EGF Ab (PAA560Hu02)**

### **[ 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.