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APA560Hu02 100µg Active Epidermal Growth Factor (EGF) 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: Prokaryotic expression.

Host: E. coli

Residues: His365~Phe487

Tags: N-terminal His-tag

Purity: >92%

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

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: 6.0

Predicted Molecular Mass: 14.3kDa

Accurate Molecular Mass: 18kDa 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.

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[<u>USAGE</u>]

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.

[<u>SEQUENCE</u>]

HGCTLG CKNTPGSYYC TCPVGFVLLP DGKRCHQLVS CPRNVSECSH DCVLTSEGPL CFCPEGSVLE RDGKTCSGCS SPDNGGCSQL CVPLSPVSWE CDCFPGYDLQ LDEKSCAASG PQPFLLF

[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, 3T3 cells were seeded into triplicate wells of 96-well plates at a density of 2, 000cells/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, 10μ 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.

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Cell proliferation of 3T3 cells after incubation with EGF for 72h observed by inverted microscope was shown in Figure 1.



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

- (A) 3T3 cells cultured in DMEM, stimulated with 1ng/mL EGF 72h;
- (B) Unstimulated 3T3 cells cultured in serum-free DMEM for 72h.

The dose-effect curve of EGF was shown in Figure 2. It was obvious that EGF significantly promoted cell proliferation of 3T3 cells. The ED50 for this effect is typically 0.9848 to 2.958ng/mL.

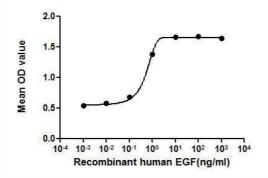


Figure 2. The dose-effect curve of EGF on 3T3 cells.

[IDENTIFICATION]

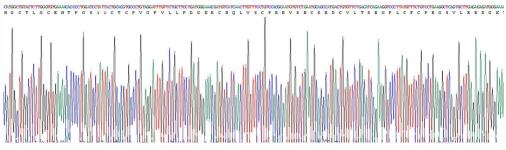
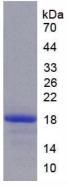


Figure 3. Gene Sequencing (extract)





Sample: Active recombinant EGF, Human

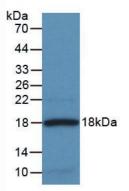


Figure 5. Western Blot

Sample: Recombinant EGF, Human;

Antibody: Rabbit Anti-Human EGF Ab (PAA560Hu02)