

APA143Ra61 100µg

Active Vascular Endothelial Growth Factor A (VEGFA)

Organism Species: Rattus norvegicus (Rat)

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: Ala27~Arg190

Tags: N-terminal His-tag

Purity: >98%

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

Buffer Formulation: PBS, pH7.4, containing 1mM DTT, 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: 7.9

Predicted Molecular Mass: 20.8kDa

Accurate Molecular Mass: 22kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 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]

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APTT EGEQKAHEVV KFMDVYQRSY  
CRPIETLVDI FQEYPDEIEY IFKPSCVPLM RCAGCCNDEA LECVPTSESN  
VTMQIMRIKP HQSQHIGEMS FLQHSRCECR PKKDRTKPEN HCEPCSERRK  
HLFVQDPQTC KCSCCKNTDSR CKARQLELNE RTCRCDKPRR
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[ACTIVITY]

Vascular endothelial growth factor A (VEGF-A), a glycosylated mitogen, is known to be a vascular permeability factor and an endothelial cell growth factor secreted by the smooth muscle and endothelial cells. It has been reported that VEGF-A induces vascular permeability and growth, promotes monocyte/macrophage migration, and inhibits cell apoptosis and so on. To test the effect of VEGF-A on cell proliferation of ECV304 endothelium 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 VEGFA. 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°C .

Cell proliferation of ECV304 cells after incubation with VEGFA for 72h observed by inverted microscope was shown in Figure 1.



Figure 1. Cell proliferation of ECV304 cells after stimulated with VEGFA.

(A) ECV304 cells cultured in serum-free DMEM, stimulated with 10ng/mL VEGFA for 72h;

(B) Unstimulated ECV304 cells cultured in serum-free DMEM for 72h.

The dose-effect curve of VEGFA was shown in Figure 2. It was obvious that VEGFA significantly promoted cell proliferation of ECV304 cells. The ED50 for this effect is typically 5.58 to 9.98 ng/mL.

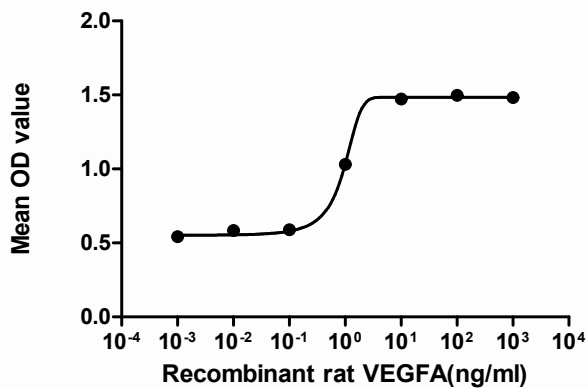


Figure 2. The dose-effect curve of VEGFA on ECV304 cells.

[IDENTIFICATION]

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YDLCCTAGACGAGGGGGGCGAAACCCDAGAAAGTGGTGGAGTTCAGTGCATGACGGGGGCTATTCGGGTGCAATTGAGAGCTGCTGGTGGAGACTTTCGGAGCTGGCCCGAGTGGAGATGGAGATGGAGCTATCTCAAGCGTCTCTGGTGGTGGCCDAGTGGGGTGGGGGGCTCTGGCAATGATGAACCCCTGGAGTGGCTG  
A P T T E G E Q K A H E V V K F M D V Y Q R S Y C R P I E T L V D I F Q E Y P D E I E Y I F K P S C V P L M R C A G C C N D E A L E C V
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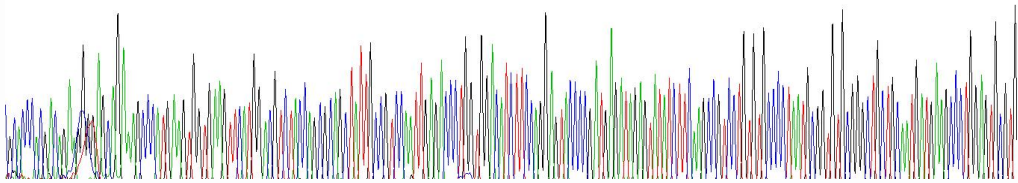


Figure 1. Gene Sequencing (extract)

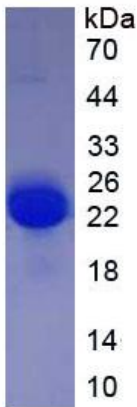


Figure 2. SDS-PAGE

Sample: Active recombinant VEGFA, Rat

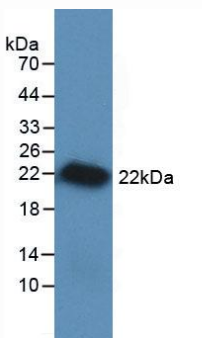


Figure 3. Western Blot

Sample: Recombinant VEGFA, Rat;

Antibody: Rabbit Anti-Rat VEGFA Ab (PAA143Ra06)