Coud-Clone Corp.

APA024Hu01 100µg Active Active Endocrine Gland Derived Vascular Endothelial Growth Factor (EG-VEGF) 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: Met1~Phe105 Tags: Two N-terminal Tags, His-tag and GST-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: 8.1 Predicted Molecular Mass: 41.7kDa

Accurate Molecular Mass: 44kDa 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.

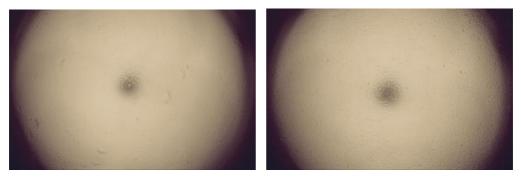
# [<u>SEQUENCE</u>]

MRGATRVSIM LLLVTVSDCA VITGACERDV QCGAGTCCAI SLWLRGLRMC TPLGREGEEC HPGSHKVPFF RKRKHHTCPC LPNLLCSRFP DGRYRCSMDL KNINF

# [ACTIVITY]

Endothelial gland-derived VEGF (EG-VEGF) is an angiogenic protein that is structurally unrelated to VEGF. It is expressed in steroidogenic tissues such as adrenal gland, ovary, testis, and placenta. Like VEGF it can induce fenestrae in endothelial cells. To test the effect of EG-VEGF on cell proliferation of ECV-304 endothelium cell line, cells were seeded into triplicate wells of 96-well plates at a density of 5,000 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 EG-VEGF. After incubated for 72h, 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 measure the absorbance at 450nm using a microplate reader after incubating the plate for 1-4 hours at  $37^{\circ}$ C.

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А

В

Figure 1. Cell proliferation of ECV-304 cells after stimulated with EG-VEGF.

(A) Unstimulated ECV-304 cells cultured in 1640 for 96h;

(B) ECV-304 cells cultured in 1640, stimulated with 10ng/mL VEGF121 for 96h.

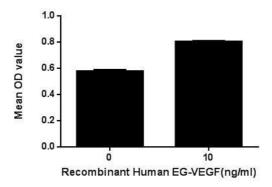


Figure 2. Cell proliferation of ECV-304 cells after stimulated with EG-VEGF



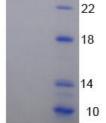


Figure 3. SDS-PAGE

Sample: Active recombinant EG-VEGF, Human

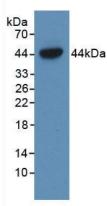


Figure 4. Western Blot

Sample: Recombinant EG-VEGF, Human;

Antibody: Rabbit Anti-Human EG-VEGF Ab (PAA024Hu01)

#### [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.