**APA542Hu61 1mg**

**Active Endostatin (ES)**

**Organism Species: *Homo sapiens* (Human)**

***Instruction manual***

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

## [ PROPERTIES ]

**Source:** Eukaryotic expression.

**Host:** 293F cell

**Residues:** His1154~Ser1335

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

**Purity:** >98%

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

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

**Original Concentration:** 250μg/mL

**Applications:** Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

**Predicted isoelectric point:** 9.1

**Predicted Molecular Mass:** 21.6kDa

**Accurate Molecular Mass:** 21kDa 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$^{o}$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 ]

HSHRDFQ PVLHLVALNS PLSGGMRGIR GADFQCFQQA RAVGLAGTFR
AFLSSRLQDL YSIVRRADRA AVPIVNLKDE LLFPSWEALF SGSEGPLKPG
ARIFSFDGKD VLRHPTWPQK SVWHGSDPNG RRLTESYCET WRTEAPSATG
QASSLLGGRL LGQSAASCHH AYIVLCIENS FMTAS

## [ ACTIVITY ]

Endostatin (ES) is a naturally occurring, 20kDa C-terminal fragment derived from type XVIII collagen. It is reported to serve as an anti-angiogenic agent, similar to angiostatin and thrombospondin. Endostatin is a broad-spectrum angiogenesis inhibitor and may interfere with the pro-angiogenic action of growth factors such as basic fibroblast growth factor (bFGF/FGF-2) and vascular endothelial growth factor (VEGF). To test the effect of ES on inhibit the VEGF basic-dependent proliferation of ECV304 endothelium cell line, cells were seeded into triplicate wells of 96-well plates at a density of 5, 000 cells/well when the cell attached, replaced with serum-free standard DMEM overnight. Then the medium was replaced with 2% serum standard DMEM which contain 10ng/mL of VEGFA and various concentrations of ES. 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℃. The result was shown in Figure 1. It was obvious that ES (100ng/mL) significantly inhibit cell proliferation of EV304 cells.

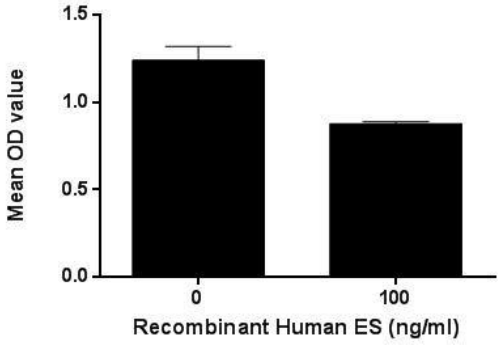


**Figure 1. ES inhibit proliferation of EV304 cells**

# [ IDENTIFICATION ]

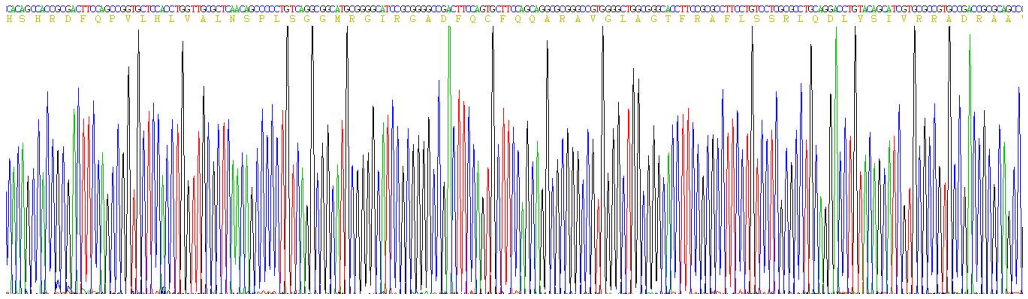

**Figure 2. Gene Sequencing (extract)**

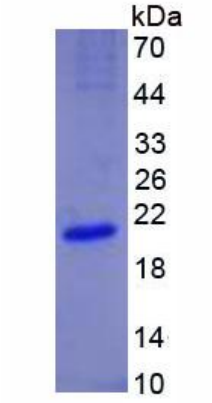


**Figure 3. SDS-PAGE**

**Sample: Active recombinant ES, Human**

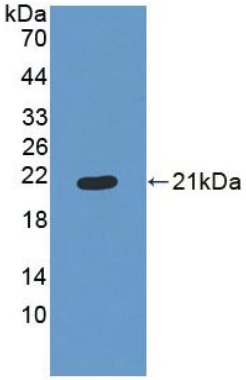


**Figure 4. Western Blot**

**Sample: Recombinant ES, Human;**

**Antibody: Rabbit Anti-Human ES Ab (PAA542Hu06)**

## [ 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.