APB367Hu01 100μg
Active Vascular Endothelial Growth Factor Receptor 2 (VEGFR2)
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: Asn46~Thr320
Tags: N-terminal His-tag
Purity: >98%
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: 7.6
Predicted Molecular Mass: 32.1kDa
Accurate Molecular Mass: 33kDa 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 37ºC 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]

NTTLQ
ITCRGQRDLD WLWPNNQSGS EQRVEVTECS DGLFCKTLTI PKVIGNDTGA
YKCFYRETDL ASVIYVYVQD YRSPFIASVS DQHGVVYITE NKNKTVVIPC
LGSISNLNVS LCARYPEKRF VPDGNRISWD SKKGFTIPSY MISYAGMVFC
EAKINDESYQ SIMYIVVVVG YRIYDVVLSP SHGIELSVGE KLVLNCTART
ELNVGIDFNW EYPSSKHQHK KLVNRDLKTQ SGSEMKKFLS TLTIDGVTRS
DQGLYTCAAS SGLMTKKNST

[ACTIVITY]

Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) also known as kinase insert domain receptor is a VEGF receptor. There are three main subtypes of VEGFR, numbered 1, 2 and 3. All members of the VEGF family stimulate cellular responses by binding to VEGF receptors on the cell surface, causing them to dimerize and become activated through transphosphorylation. VEGFR2 appears to mediate almost all of the known cellular responses to VEGF. Besides, Vascular Endothelial Growth Factor C (VEGFC) has been identified as an interactor of VEGFR2, thus a binding ELISA assay was conducted to detect the interaction of recombinant human VEGFR2 and recombinant human VEGFC. Briefly, VEGFR2 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to VEGFC-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-VEGFR2 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50μL stop

solution to the wells and read at 450nm immediately. The binding activity of VEGFR2 and VEGFC was shown in Figure 1, and this effect was in a dose dependent manner.

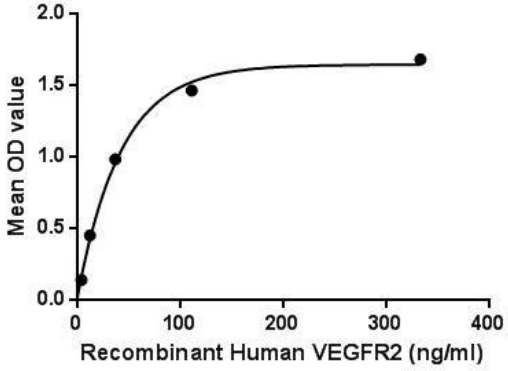
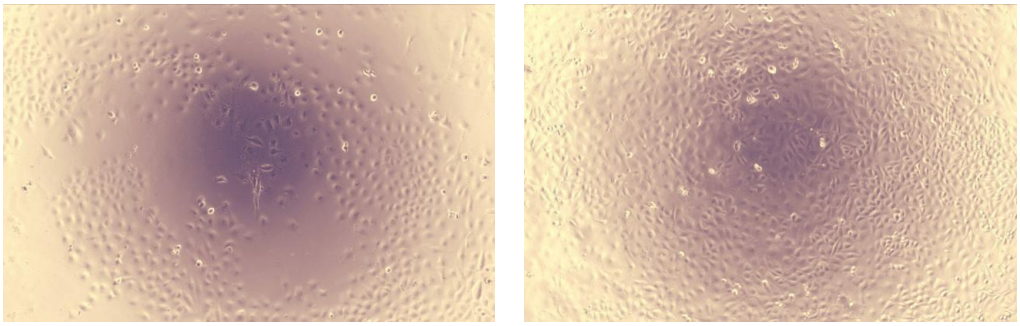


Figure 1. The binding activity of VEGFR2 with VEGFC.

Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) also known as kinase insert domain receptor acts as a cell-surface receptor for VEGFA, VEGFC and VEGFD. VEGFR2 functions as the primary mediator of vascular endothelial growth factor activation in endothelial cells. Regulation of VEGFR-2 expression appears critical in mitogenesis, differentiation, and angiogenesis. To test the effect on inhibit the VEGF-dependent proliferation of endothelium cells, ECV-304 cells were seeded into triplicate wells of 96-well plates at a density of 5,000 cells/well and allowed to attach, replaced with serum-free overnight, then the medium was replaced with 2% serum standard DMEM including 1μg/mL Vascular Endothelial Growth Factor C (VEGFC) and various concentrations of recombinant human VEGFR2. After incubated for 96h, 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 the absorbance at 450nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37℃. Proliferation of ECV-304 cells after incubation with VEGFR2 for 96h observed by inverted microscope was shown in Figure 2. Cell viability was

assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant VEGFR2 for 96h. The result was shown in Figure 2. It was obvious that VEGFR2 significantly inhibit cell viability of ECV-304.



A B

Figure 2. Cell proliferation of ECV-304 cells inhibit by VEGFR2.

(A) ECV-304 cells cultured in DMEM, stimulated with 10ng/mL VEGFR2 for 96h;

(B) Unstimulated ECV-304 cells cultured in DMEM for 96h.

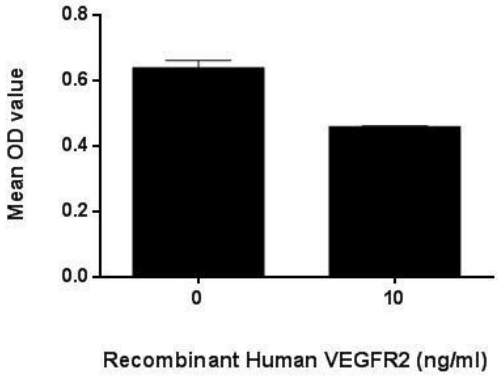


Figure 3. VEGFR2 inhibit VEGF-dependent proliferation of ECV-304 cells.

[IDENTIFICATION]

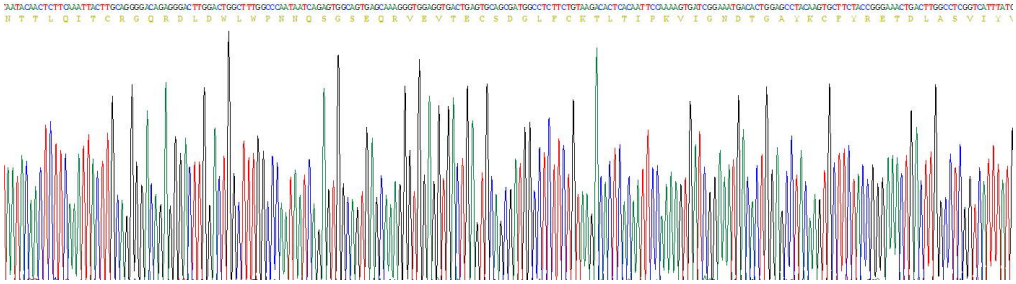


Figure 4. Gene Sequencing (extract)

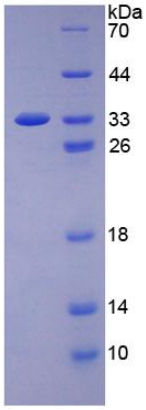


Figure 5. SDS-PAGE

Sample: Active recombinant VEGFR2, Human

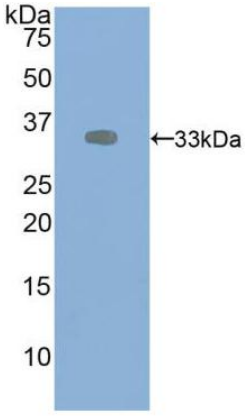


Figure 6. Western Blot

Sample: Recombinant VEGFR2, Human;

Antibody: Rabbit Anti-Human VEGFR2 Ab (PAB367Hu01)

[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.