



Recombinant ov-Vascular Endothelial Growth Factor-E



FOR RESEARCH ONLY! NOT FOR HUMAN USE!

Cat.-no:	300-045
Size:	20 µg
Lot. No.:	According to product label
Country of origin:	Germany

Scientific Background

Gene:	<i>n.k.</i>
Synonyms:	orf virus VEGF, VEGF-E

A DNA sequence encoding the mature variant of ov-VEGF-E isolate D1701 (GenBank accession No. AF106020) was expressed in *E. coli* as a 132 amino acid residue fusion protein with an N-terminal His-tag sequence and a thrombin cleavage site. Recombinant VEGF-E homodimer was dimerized in vitro and has a predicted mass of approximately 35 kDa.

Based on sequence similarity to VEGF-A, a gene encoding a VEGF homologue has recently been discovered in the genome of Orf virus (OV) (Lyttle et al., 1994). Different isolates of Orf virus show significant amino acid sequence similarity to VEGF-A and described as a viral virulence factor that appears to be derived from captured host genes. All eight cysteine residues of the central cysteine knot motif characteristic of members of the VEGF family are conserved among other residues in the VEGF-E proteins (Dehio et al., 1999; Wise et al., 1999). Alignment of all mammalian VEGF sequences indicated that VEGF-E is distinct from the previously described VEGFs but most closely related to VEGF-A. Like VEGF-A, VEGF-E was found to bind with high affinity to VEGF receptor-2 (KDR) resulting in receptor autophosphorylation, whilst in contrast to VEGF-A, VEGF-E cannot bind to VEGF receptor-1 (Flt-1). Furthermore VEGF-E can also not bind to VEGF receptor-3 (FLT-4). Therefore VEGF-E is a potent angiogenic factor selectively binding to VEGF receptor -2/KDR.

References

1. Dehio et al., 1999 EMBO J. 18:363-374
2. Lyttle et al., 1994 J. Virol 68:84-92
3. Wise et al., 1999 Proc. Natl. Acad. Sci USA 96:3071-3076

Sequence

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MGSSHHHHHHSSGLVPRGSHDSTKTKWSEVFENSGCKPRPMVFRVHD  
EHPELTSQRFNPPCVTLMRGCGCCNDESLECVPTEEANVTMQLMGA  
SVSGGNGMQHLSFVEHKKCDCKPPLTTTPPTTRPPRRRR
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Database References

Protein RefSeq:	NP_005420.1
Uniprot ID:	Q2F842
mRNA RefSeq:	NM_005429.2

Product Specifications

Expressed in	<i>E. coli</i>
Purity	> 90% by SDS-PAGE & silver stain
Endotoxin level	< 0.1ng per µg of ov-VEGF-E
Buffer	PBS
Stabilizer	None
Formulation	lyophilized
Length (aa):	132
MW:	35 kDa (Dimer)
Result by N-terminal sequencing	UNDER WORK!

Stability: Lyophilised samples are stable for greater than six months at -20°C to -70°C. Reconstituted VEGF-E should be stored in working aliquots at -20°C.

Reconstitution: The lyophilised ovVEGF-E should be reconstituted in water or medium to a concentration not lower than 50 µg/ml. For long term storage we would recommend to add at least 0.1% human or bovine serum albumin.



AVOID REPEATED FREEZE AND THAW CYCLES!

Biological Activity: The biological activity was determined (i) by the ability to induce VEGFR-2/KDR receptor phosphorylation in PAE/KDR cells and (ii) in a cell proliferation assay using primary HUVECs. The ED₅₀ for this effect is typically 1 - 5 ng/mL.



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Handling/Applications

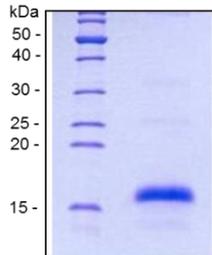


Fig. 1: SDS-PAGE analysis of recombinant ov-VEGF-E. Sample was loaded in 15% SDS-polyacrylamide gel under reducing conditions and stained with Coomassie blue.

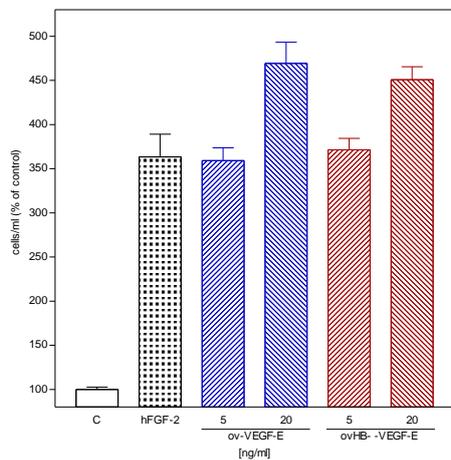


Fig. 2: Stimulation of cell proliferation in primary human umbilical vein endothelial cells (HUVEC) by recombinant ov-VEGF-E and ov-HB-VEGF-E. Values are the means (\pm SD) of triplicate determinations and expressed as percentage of control.

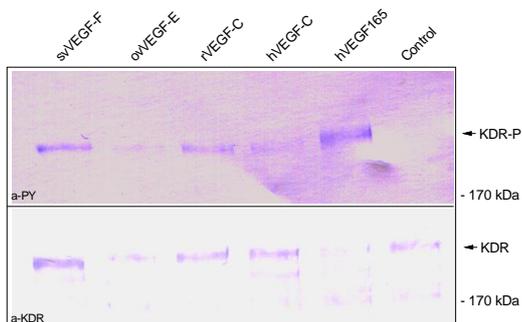


Fig. 3: Confluent PAE/KDR cells were stimulated with 50ng/ml human VEGF₁₆₅ (#300-036), human VEGF-C (#300-079), rat VEGF-C (#R20-015), Orf virus VEGF-E (#300-045) and snake venom VEGF-F (#300-097) for 10 min at 37°C. The IP was performed using an anti-human KDR antibody (#101-M32), WB with an anti-human KDR antibody (#101-M34) and an anti-Phosphotyrosine antibody.