Recombinant Snake Venom Vascular Endothelial Growth Factor-F

FOR RESEARCH ONLY! NOT FOR HUMAN USE!

Cat.-no:	**300-096**
Size:	5 µg
Lot. No.:	According to product label
Country of origin:	Germany

Scientific Background

Gene:	*ND*
Synonyms:	svVEGF, VEGF-F

Vascular endothelial growth factor (VEGF-A) and its family proteins are crucial regulators of blood vessel formation and vascular permeability. Snake venom has recently been shown to be an exogenous source of unique VEGF (known as VEGF-F), and two types of VEGF-F with distinct biochemical properties have been reported. VEGF-Fs (venom type VEGFs) are highly variable in structure and function among species, in contrast to endogenous tissue-type VEGFs (VEGF-As) of snakes. Although the structures of tissue-type VEGFs are highly conserved among venomous snake species and even among all vertebrates, including humans, those of venom-type VEGFs are extensively variegated, especially in the regions around receptor-binding loops and C-terminal putative coreceptor-binding regions, indicating that highly frequent variations are located around functionally key regions of the proteins.
The svVEGF-F was identified from the Viperidae snake Bothrops insularis venom glands. The deduced primary sequence, after complete sequencing of the longest snake venom VEGF (svVEGF) cDNA, displayed similarity with vertebrate VEGFs and with the hypotensive factor from Vipera aspis venom. The mature svVEGF appears to be ubiquitously distributed throughout snake venoms. The produced recombinant protein dimerizes after refolding processes and was biologically characterized, showing ability to increase vascular permeability. These results established that svVEGF is a novel and important active toxin during the early stages of bothropic snake bite envenoming and represents a new member of the VEGF family of proteins.

References

1. Junqueira de Azevedo et al, JBC 276, 2001
2. Gasmi et al, JBC 276, 2002
3. Yamazaki et al, JBC 278, 2003
4. Takahashi et al, JBC 279, 2004
5. Yamazaki et al, JBC 280, 2005
6. Yamazaki et al, JBC 284, 2009

Sequence

MGQVMPFMEVYRHSVCQTRETLVSILEEHPDEVSHIFRPSCVTALR
CGGCCTDESLKCTATGKRSVGREIMRVDPHKGTSKTEVMQFTEHTD
CECRPRSASGVNSRKHKRNPEEGEPRAKFPFV

Database References

Protein RefSeq:	AAK52102.1
Uniprot ID:	Q90X24
mRNA RefSeq:	AY033151.1

Product Specifications

Expressed in	E.coli
Purity	> 95% by SDS-PAGE & silver stain
Endotoxin level	= 0.13 ng per µg of sv-VEGF-F
Buffer	50 mM acidic acid
Stabilizer	None
Formulation	lyophilized
Length (aa):	124
MW:	27,6 kDa (Dimer)
Result by N-terminal sequencing	MGQVMP

Stability: Lyophilized samples are stable for greater than six months at –20°C to –70°C. Reconstituted svVEGF-F should be stored in working aliquots at -20°C.

Reconstitution: The lyophilized svVEGF-F should be reconstituted in water or medium to a concentration not lower than 50 µg/ml. For long term storage we 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) by the dose-dependent stimulation of the proliferation of human umbilical vein endothelial cells (HUVEC) and human dermal lymphatic endothelial cells (HDLEC) using a concentration range of 2-50 ng/ml.

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Handling/Applications

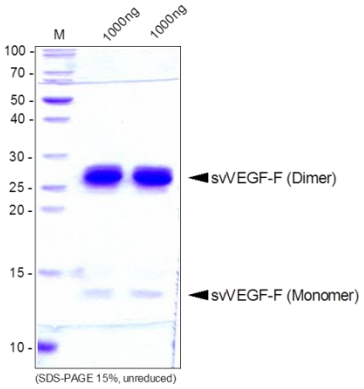

Fig. 1. SDS-PAGE analysis of recombinant human VEGF-F. Samples were loaded in 15% SDS-polyacrylamide gel under non-reducing conditions and stained with Coomassie blue.

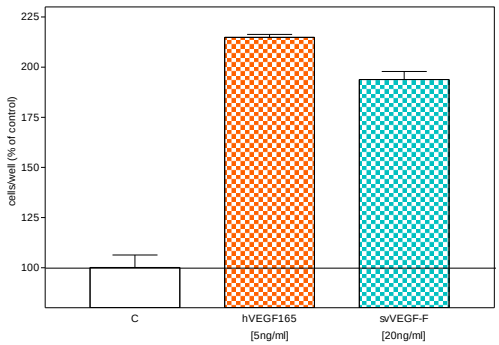

Fig. 2. Proliferation assay with HUVECs. Human $VEGF_{165}$ was used as positive control. Although svVEGF-F (20 ng/ml) had a significant lower activity as human $VEGF_{165}$ (5 ng/ml) it clearly induced the proliferation of HUVECs. Values are the means (±SD) of triplicate determinations and expressed as percentage of control.

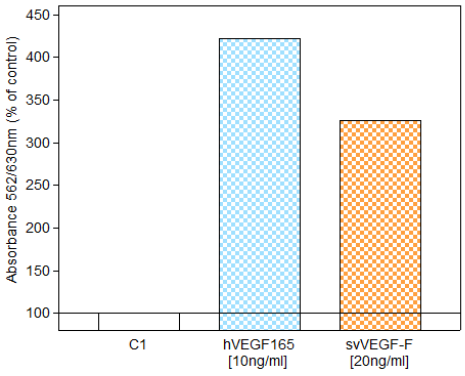

Fig 3. Transwell migration assay of svVEGF-F with $hVEGF_{165}$ as control.

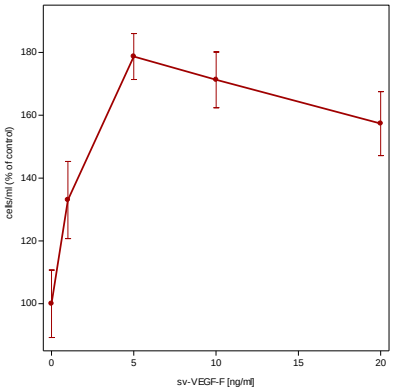

Fig 4. Dose-dependent stimulation of proliferation in primary HUVECs by sv-VEGF-F. Values are the means (±SD) of triplicate determinations and expressed as percentage of control.

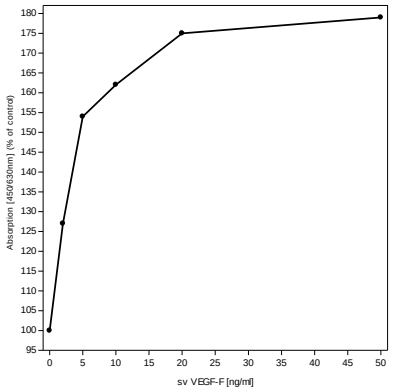

Fig. 5. Dose-dependent stimulation of cell proliferation in primary human dermal lymphatic endothelial cells (HDLEC) by recombinant snake venom VEGF-F. Values are the means (±SD) of triplicate determinations and expressed as percentage of control.

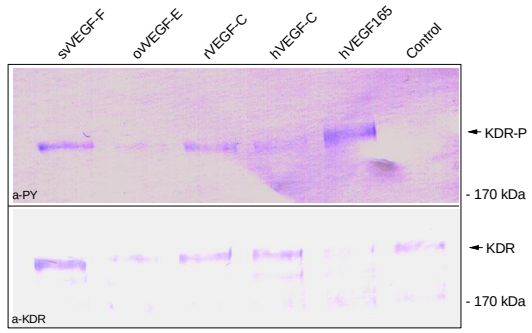

Fig. 6: Confluent PAE/KDR cells were stimulated with 50ng/ml human $VEGF_{165}$ (**#300-036**), human VEGF-C (**#300-079**), rat VEGF-C (**#R20-015**), Orf virus VEGF-E (**#300-045**) and snake venom VEGF-F (**#300-097**) for 10 min at 37°C. The IP was performed using an anti-human KDR antibody (**#101-M32**), WB with an anti-human KDR antibody (**#101-M34**) and an anti-Phosphotyrosine antibody.

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