



Recombinant Human Vascular Endothelial Growth Factor₁₆₅

20200305BB



FOR RESEARCH ONLY! NOT FOR HUMAN USE!

Cat.-no:	300-035S
Size:	2 µg
Lot. No.:	According to product label
Country of origin:	Germany

Scientific Background

Gene:	<i>vegf</i>
Synonyms:	VEGF-A, VPF

Human Vascular Endothelial Growth Factor VEGF₁₆₅, a 23kDa protein consisting of 165 amino acid residues, is produced as a homodimer. VEGF is a polypeptide growth factor and a member of the platelet-derived growth factor family. It is a specific mitogen for vascular endothelial cells and a strong angiogenic factor in vivo. Two high-affinity tyrosine kinase receptors for VEGF₁₆₅ have been identified, VEGFR-1 (FLT-1), and VEGFR-2 (KDR). Consistent with the endothelial cell-specific action of VEGF₁₆₅, expression of both receptor genes has been found predominantly but not exclusively on endothelial cells. Expression of VEGFR-1 was also found on human monocytes, neutrophils (PMNs), bovine brain pericytes and villous and extra villous trophoblast. In addition to its action as a mitogen it is a potent vascular permeability factor (VPF) in vivo. VEGF₁₆₅ is also a chemo attractant molecule for monocytes and endothelial cells. 5 different proteins are generated by differential splicing: VEGF₁₂₁, VEGF₁₄₅, VEGF₁₆₅, VEGF₁₈₉ and VEGF₂₀₆. The most abundant form is VEGF₁₆₅. Whereas VEGF₁₂₁ and VEGF₁₆₅ are secreted proteins, VEGF₁₄₅, VEGF₁₈₉ and VEGF₂₀₆ are strongly cell-associated. The isoforms VEGF₁₄₅, VEGF₁₆₅ and VEGF₁₈₉ bind to heparin with high affinity. VEGF₁₆₅ is apparently a homo-dimer, but preparations of VEGF₁₆₅ show some heterogeneity on SDS gels, depending on the secretion of different glycosylation patterns. All dimeric forms have similar biological activities but their bioavailability is very different. There is good evidence that different cells and tissues express different VEGF isoforms. The other members of this increasing growth factor family are VEGF-B, -C, -D and -E. Another member is the Placenta growth factor PIGF.

References

1. Breier et al., Dev 114:521, 1992
2. Fiebig et al., Eur J Biochem 211:19, 1993
3. Flamme et al., Dev Biol 162:699, 1995
4. Kremer et al., Cancer Res 57:3852, 1997

Sequence

APMAEGGQNHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCV
PLMRCGGCCNDEGLECVPTESNITMQIMRIKPHQGQHI GEMSFLOHNKCEC
RPKKDRARQENPCGPCSEERRKHLFVQDPQTCKCCKNTDSRCKARQLELNER
TCRCDKPRR

Database References

Protein RefSeq:	NP 001165097
Uniprot ID:	P15692-4
mRNA RefSeq:	NM 001171626

Product Specifications

Expressed in	Insect cells
Purity	> 95% by SDS-PAGE
Buffer	50 mM acetic acid
Stabilizer	None
Formulation	lyophilized
Length (aa):	165
MW:	45 kDa
Result by N-terminal sequencing	APMAEGG

Stability: Lyophilized samples are stable for greater than six months at -20°C to -70°C. Reconstituted VEGF₁₆₅ should be stored in working aliquots at -20°C.

Reconstitution: Centrifuge the vial prior to opening! The lyophilized VEGF₁₆₅ should be reconstituted in 50 mM acetic acid 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 ED₅₀ for stimulation of cell proliferation by human umbilical vein endothelial cells for VEGF₁₆₅ has been determined to be in the range of 1-4 ng/ml.



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Handling/Application

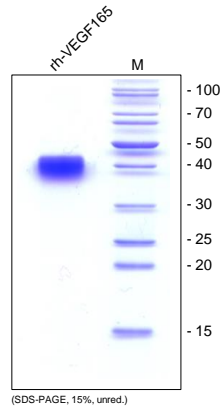


Fig. 1: SDS-PAGE analysis of recombinant human VEGF₁₆₅. Sample was loaded in 15% SDS-polyacrylamide gel under non-reducing condition and stained with Coomassie blue.

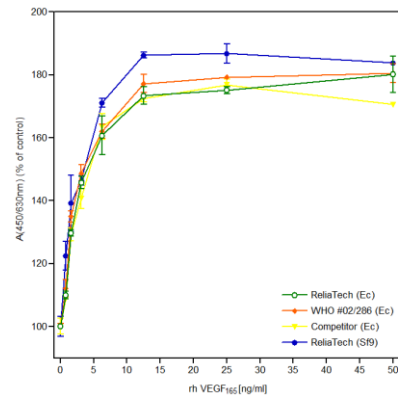


Fig. 4: Proliferation assay with primary human dermal lymphatic endothelial cells (HDLEC). The cells were stimulated using recombinant human VEGF₁₆₅ and the WHO standard #02/286. Values are the means (\pm SD) of triplicate determinations and expressed as percentage of control.

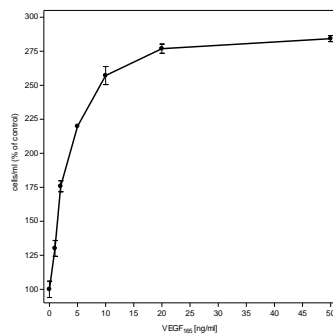


Fig. 2: VEGF₁₆₅-induced proliferation of HUVEC cells. HUVECs were stimulated with increasing amounts of human VEGF₁₆₅.

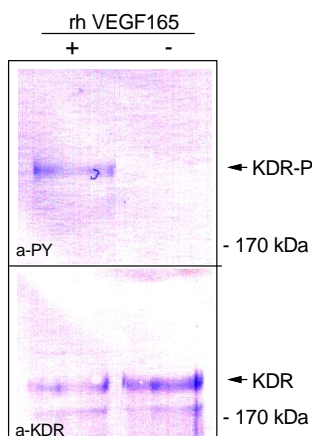


Fig. 3: Confluent PAE/KDR cells were stimulated with 50ng/ml human VEGF₁₆₅ for 10 min at 37°C. Cells were lysed and an IP was performed using a mouse anti-human KDR antibody (Cat# 101-M32). WB was performed with a mouse anti-human KDR antibody (Cat# 101-M34) and an anti-Phosphotyrosine antibody.