ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Rat Recombinant VEGF152 (VEGF-C) Protein (Sf9), biologically active

Cat # VEGF31-R-10 Recombinant Rat VEGF152 protein **SIZE**: ,10 ug
FORM: Soln Lyophilized **Storage** : Store at -20oC.

Embryonic vascular system undergoes a series of complex, highly regulated series of events involving differentiation, migration and association of primitive endothelial cells. This process is termed vasculogenesis. Study of tumor angiogenesis has led to the identification of several proteins including basic fibroblast growth factor (bFGF) and vascular endothelial growth factor. VEGF acts by interacting with a family of largely endothelial-specific receptor tyrosine kinases that includes VEGFR-1 (flt-1), VEGFR-2 (flk-1/KDR), and VEGFR-3/Flt-4. Disruption of VEGFRs interferes with differentiation of endothelial cells and it is lethal for the embryo.

VEGF is a heparin-binding glycoprotein that is secreted as a homodimer of 45 kDa. There are several splice variants of VEGF-A. The major ones include: 121, 165, 189 and 206 amino acids (aa), each one comprising a specific exon addition. VEGF121 is acidic and freely secreted. VEGF165 is more basic, has heparin-binding properties and, although a signicant proportion remains cell-associated, most is freely secreted. VEGF189 is very basic; it is cell-associated after secretion and is bound avidly by heparin and the extracellular matrix, although it may be released as a soluble form by heparin, heparinase or plasmin. VEGF165 is the most predominant protein, but transcripts of VEGF121 may be more abundant. VEGF206 is rarely expressed and has been detected only in fetal liver. Recently, other splice variants of 145 and 183 aa have also been described. The 165, 189 and 206 aa splice variants have heparin-binding domains, which help anchor them in extracellular matrix and are involved in binding to heparin sulfate and presentation to VEGF receptors. This is a key factor for VEGF potency (i.e., the heparin-binding forms are more active). VEGF-A is regulated by growth factors, cytokines, gonadotropins, nitric oxide, hypoxia, hypoglycemia and oncogenic mutations.

VEGF-C152S is a point mutant generated by the replacement of the second conserved Cys residue of the recombinant processed VEGF-C by a Ser residue. VEGF-C 152S is analog to the human VEGF-C 156S mutant and only active toward VEGFR-3/FLT-4 but, unlike wild type VEGF-C, is unable to bind to and to activate signalling through VEGFR-2/KDR. VEGF-C152S was inactive in the vascular permeability assay and did not increase migration of the capillary endothelial cells, indicating that these VEGF-like effects of VEGF-C require VEGFR-2 binding. VEGF-C, also known as Vascular Endothelial Growth Factor Related Protein (VRP), is a recently discovered VEGF growth factor family member that is most closely related to VEGF-D. The rat VEGF-C cDNA encodes a pre-pro-protein of 416 amino acids residues. It is almost identical to the mouse VEGF-C protein. Similar to VEGF-D, VEGF-C has a VEGF homology domain spanning the middle third of the precursor molecule and long N- and C-terminal extensions. In adults, VEGF-C is highly expressed in heart, placenta, ovary and small intestine. Recombinant rat VEGF-C, lacking the N- and C-terminal extensions and containing only the middle VEGF homology domain, forms primarily non-covalently linked dimers. This protein is a ligand for both VEGFR-2/KDR and VEGFR-3/FLT -4. Since VEGFR-3 is strongly expressed in lymphatic endothelial cells, it has been postulated that VEGF-C is involved in the regulation of the growth and/or differentiation of lymphatic endothelium. Although recombinant rat VEGF-C is also a mitogen for vascular endothelial cells, it is much less potent than VEGF-A.

Form, Storage, and Reconstitution

recombinant rat VEGF-C 152 contains 152 amino acids residues and was fused to a His-tag (6x His) at the C-terminal end. As a result of glycosylation VEGF-C migrates as an 18-24 kDa protein in SDS-PAGE under reducing conditions.

Human VEGF165 Amino Acid Sequence

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APMAEGGGQN HHEVVKFMDV YQRSYCHPIE TLVDIFQEYP
DEIEYIFKPS CVPLMRCGGC CNDEGLECVP TEESNITMQI
MRIKPHQGQH IGEMSFLQHN KCECRPKKDR ARQENPCGPC
SERRKHLFVQ DPQTCKCSCK NTDSRCKARQ LELNERTCRC
DKPRR
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Human VEGF165 was produced in Sf9 and purified to >90% by SDS-PAGE and HPLC. Less than 1% as determined by silver-stained SDS-PAGE gel analysis. Endotoxin level is <0.1 ng/ug (<1 EU/ug) protein. It is supplied aslyophilized powder with 1 mg/ml BSA and no preservatives. The lyophilized protein is stable at room temperature but it is recommend to store desiccated at -20oC.

The lyophilized VEGF is soluble in water and buffers. It should be reconstituted in water or PBS at a concn of 0.1-1 mg/ml and stored in suitable aliquots at -20oC or below. Add 100 ul water or buffer (100 ug/ml concn), lightly vortex, and mix for 15 min at room temp. The vial should be centrifuged briefly to recover solution at the bottom. It is also possible to reconstitute the protein in PBS or other buffers containing 0.1% BSA as a carrier protein. The solution can be sterile filtered if necessary.

Biological activity

Measured by its ability to stimulate phosphorylation of the VEGFR-3/FLT-4 receptor in porcine aortic endothelial cells (PAE/FLT -4 cells). The ED50 for this effect is typically 150-300 ng/ml, corresponding to a specific activity of 5 x 103 Units/mg.

General References:

Keck PJ (1989) Science 246, 1309-1312; Leung DW (1989) Science 246, 1306-1309; Tischer E (1991) JBC 266, 11947-11954; Houck, KA (1991) Mol. Endocrinol. 5, 1806-1814; Poltorak Z (1997) JBC 272, 7151-7158; Lei J (1998) BBA 1443, 400-406; Whittle C (1999) Clin. CSci. 97, 303-312

*This product is for In vitro research use only.

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