ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Human Recombinant VEGF121 Protein (Sf9), biologically active

Cat # VEGF24-R-10 Recombinant Human VEGF121 protein **SIZE**: 10 ug

Cat # VEGF24-R-100 Recombinant Human VEGF121 protein **SIZE**: 100 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.

Summary of Various human VEGF Isoforms

Human VEGF-A (VPF) precursor 232 aa (#P15692)
Signal sequence 1-26 aa; mature chain 27-232 aa

Name	Accession #	Features
VEGF206	P15692-1	
VEGF189	P15692-2	166-182 missing
VEGF183	P15692-3	160-182 missing
VEGF165	P15692-4	141K->N; 142-182 missing
VEGF148	P15692-5	141K->N; 142-182 missing; 215 A->M; 216-232 missing
VEGF145	P15692-6	166-226 missing;
VEGF121	P15692-7	141K->N; 142-226 missing
VEGF165B	P15692-8	141K->N; 142-182 missing; 227-232 (CDKPRR->SLTRKD)
VEGF121B	P15692-9	142-226 missing

Form, Storage, and Reconstitution

Recombinant human VEGF121 is a 28.4 kDa disulfide-linked homodimeric protein consisting of two 121 amino acids (mol 28423 dalton) polypeptide chains. VEGF-121 circulates more freely than other VEGF forms, which bind more tightly with vascular heparin sulfates. Glycosylation is not required for efficient secretion of VEGF.

The sequence of the first five N-terminal amino acids was determined and was found to be Ala-Pro-Met-Ala-Glu. Less than 1% as determined by silver-stained SDS-PAGE gel analysis.

Human VEGF121 Amino Acid Sequence

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APMAEGGGQN HHEVVKFMDV YQRSYCHPIE TLVDIFQEYP
DEIEYIFKPS CVPLMRCGGC CNDEGLECVP TEESNITMQI
MRIKPHQGQH IGEMSFLQHN KCECRPKKDR ARQENCDKPR
R
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Human VEGF121 was produced in Sf9 insect cells and purified to >98% by SDS-PAGE and HPLC. Endotoxin level is <0.1 ng/ug (<1 EU/ug) protein. It has been sterile filtered and lyophilized with 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, 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

The biological activity was determined by its mitogenic activity on human umbilical vein endothelial cells (HUVEC) using a concentration range of 0.2-0.4 ng/ml corresponding to a specific activity of $5x10^5$ IU/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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