Animal-Free™ Recombinant Human VEGF 165

Catalog Number: BT-VEGF-AFL

DESCRIPTION

Source	*E. coli*-derived human VEGF protein Ala27-Arg191 Accession # NP_001165097.1 Produced using non-animal reagents in an animal-free laboratory.
N-terminal Sequence Analysis	Met & Pro28
Structure / Form	Disulfide-linked homodimer
Predicted Molecular Mass	19.2 kDa (monomer)

SPECIFICATIONS

SDS-PAGE	19-21 kDa, under reducing conditions.
Activity	Measured in a cell proliferation assay using HUVEC human umbilical vein endothelial cells. Conn, G. *et al.* (1990) Proc. Natl. Acad. Sci. USA **87**:1323. The ED_{50} for this effect is 1.50-12.0 ng/mL. The specific activity of recombinant human VEGF165 is >8.0 x 10^5 units/mg, which is calibrated against the human VEGF165 WHO standard (NIBSC code: 02/286).
Endotoxin Level	<0.01 EU per 1 µg of the protein by the LAL method.
Purity	>97%, by SDS-PAGE with quantitative densitometry by Coomassie® Blue Staining.
Formulation	Lyophilized from a 0.2 µm filtered solution in Sodium Acetate. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 500 µg/mL in sterile deionized water.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

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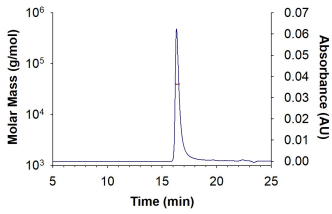
DATA

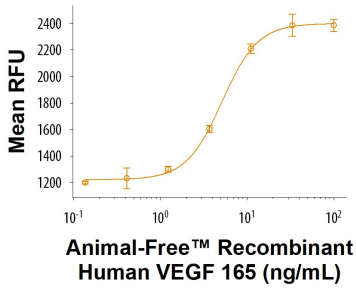
SEC-MALS



SEC-MALS Data	Result
Retention Time	16.2 - 16.6 min
MW - Predicted (Monomer)	19.2 kDa
MW - MALS	39.8 kDa
Polydispersity	1.000
System Suitability: BSA Monomer 66.4 ± 3.32 kDa	Pass

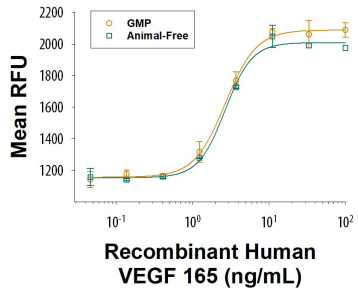
Animal-Free™ Recombinant Human VEGF 165 Protein SEC-MALS. Recombinant Human VEGF 165 (Catalog # BT-VEGF-AFL) has a molecular weight (MW) of 39.8 kDa as analyzed by SEC-MALS in non-reducing conditions, suggesting that this protein is a disulfide-linked homodimer.

Bioactivity



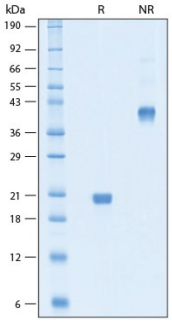
Animal-Free™ Recombinant Human VEGF 165 Protein Bioactivity. Animal-Free™ Recombinant Human VEGF 165 (Catalog # BT-VEGF-AFL) stimulates proliferation in HUVEC human umbilical vein endothelial cells. The ED_{50} for this effect is 1.50-12.0 ng/mL.

Bioactivity



Equivalent Bioactivity of GMP and Animal-Free grades of Recombinant Human VEGF 165. Equivalent bioactivity of GMP (Catalog # BT-VEGF-GMP) and Animal-Free (Catalog # BT-VEGF-AFL) grades of Recombinant Human VEGF 165 as measured in a cell proliferation assay using HUVEC human umbilical vein endothelial cells (orange and green, respectively).

SDS-PAGE



Recombinant Human VEGF 165 Protein SDS-PAGE. 2 µg/lane of Recombinant Human VEGF 165 Protein (Catalog # BT-VEGF-AFL) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 19-21 kDa.

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BACKGROUND

Vascular endothelial growth factor (VEGF or VEGF-A), also known as vascular permeability factor (VPF), is a potent mediator of both angiogenesis and vasculogenesis in the fetus and adult (1-3). It is a member of the PDGF family that is characterized by the presence of eight conserved cysteine residues and a cystine knot structure (4). Humans express alternately spliced isoforms of 121, 145, 165, 183, 189, and 206 amino acids (aa) in length (4). $VEGF_{165}$ appears to be the most abundant and potent isoform, followed by $VEGF_{121}$ and $VEGF_{189}$ (3, 4). Isoforms other than $VEGF_{121}$ contain basic heparin-binding regions and are not freely diffusible (4). Human $VEGF_{165}$ shares 88% aa sequence identity with corresponding regions of mouse and rat, 96% with porcine, 95% with canine, and 93% with feline, equine and bovine VEGF, respectively. VEGF binds the type I transmembrane receptor tyrosine kinases VEGF R1 (also called Flt-1) and VEGF R2 (Flk-1/KDR) on endothelial cells (4). Although VEGF affinity is highest for binding to VEGF R1, VEGF R2 appears to be the primary mediator of VEGF angiogenic activity (3, 4). $VEGF_{165}$ binds the semaphorin receptor, Neuropilin-1 and promotes complex formation with VEGF R2 (5). VEGF is required during embryogenesis to regulate the proliferation, migration, and survival of endothelial cells (3, 4). In adults, VEGF functions mainly in wound healing and the female reproductive cycle (3). Pathologically, it is involved in tumor angiogenesis and vascular leakage (6, 7). Circulating VEGF levels correlate with disease activity in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus (8). VEGF is induced by hypoxia and cytokines such as IL-1, IL-6, IL-8, oncostatin M and TNF-α (3, 4, 9).

Due to its role in angiogenesis of blood vessels, tumor and stroma cells use VEGF to stimulate formation of blood vessels and the proliferation and survival of endothelial cells. Specific immunotherapies targeting the VEGF signaling pathway include the recombinant antibody against VEGF (Bevacizumab), antibodies targeting the main VEGF receptor (VEGFR2), and small molecule inhibitors against VEGF receptor tyrosine kinases (10). Immune checkpoint inhibitors are an important tool in cancer therapies as tumor cells can hijack immune checkpoint signals to evade detection by immune cells. In addition to stimulating the formation of tumor blood vessels, VEGF has immunosuppressive effects by acting on dendritic cells to block their antigen-presenting and T cell stimulatory functions. Targeting VEGF in combination with other immune checkpoint ligands or receptors may prove more effective in immunotherapy approaches to certain cancer types (11). Because of its role in the formation of blood vessels, VEGF is also an important factor in skeletal development where blood supply and vascularization are crucial. This has made VEGF an important molecule in regenerative studies for bone repair as sustained release of VEGF has been shown to improve the efficiency of bone regeneration (12).

In differentiation protocols for stems cells, VEGF is a commonly added growth factor for the transformation of induced pluripotent stem cells into hematopoietic progenitor cells used to make Natural Killer cells (13, 14). VEGF has also been used to transform intermediate mesoderm into kidney glomerular podocytes or stem cell-derived liver spheres (15, 16). VEGF may also be used in assistance of stem cell transplantations by supporting angiogenesis at sites of stem cell transplants or as a homing tool for adipose-derived mesenchymal stem cells or bone marrow stem cells to migrate to (17, 18).

References:

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MANUFACTURING SPECIFICATIONS

Animal-Free Manufacturing Conditions
Our dedicated controlled-access animal-free laboratories ensure that at no point in production are the products exposed to potential contamination by animal components or byproducts. Every stage of manufacturing is conducted in compliance with R&D Systems' stringent Standard Operating Procedures (SOPs). Production and purification procedures use equipment and media that are confirmed animal-free.

Production

- All molecular biology procedures use animal-free media and dedicated labware.
- Dedicated fermentors are utilized in committed animal-free areas.

Purification

- Protein purification columns are animal-free.
- Bulk proteins are filtered using animal-free filters.
- Purified proteins are stored in animal-free containers in a dedicated cold storage room.

Quality Assurance

- Low Endotoxin Level.
- No impairment of biological activity.
- High quality product obtained under stringent conditions.
- For *ex vivo* research or bioproduction, **additional documentation** can be provided.

Please read our complete Animal-Free Statement

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