# Recombinant Human VEGF 165 GMP

Catalog Number: BT-VEGF-GMP

## DESCRIPTION

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|---|---|
| **Source** | *E. coli*-derived human VEGF protein<br>Ala27 - Arg191<br>Accession # NP_001165097.1<br>Produced using non-animal reagents in an animal-free laboratory.<br>Manufactured and tested under cGMP guidelines. |
| **N-terminal Sequence Analysis** | Met-Ala27-Pro-Met-Ala-Glu-Gly-Gly-Gly-Gln<br>Pro28-Met-Ala-Glu-Gly-Gly-Gly-Gln-Asn-His |
| **Structure / Form** | Disulfide-linked homodimer |
| **Predicted Molecular Mass** | 19.2 kDa (monomer) |

## SPECIFICATIONS

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|---|---|
| **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.<br>The $ED_{50}$ for this effect is 1.50-12.0 ng/mL.<br>The specific activity of recombinant human $VEGF_{165}$ is > 8.0 x $10^5$ units/mg, which is calibrated against the human $VEGF_{165}$ 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. |
| **Mass Spectrometry** | The molecular weight by TCEP reduced mass spectrometry is 19282 ± 10 Da and 19080 Da ± 10 Da, and is free of unexpected mass peaks. |
| **Host Cell Protein** | <0.100 ng per µg of protein when tested by ELISA. |
| **Mycoplasma** | Negative for Mycoplasma. |
| **Host Cell DNA** | <0.00150 ng per µg of protein when tested by PCR. |
| **Formulation** | Lyophilized from a 0.2 µm filtered solution in Sodium Acetate. See Certificate of Analysis for details. |

## PREPARATION AND STORAGE

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|---|---|
| **Reconstitution** | Reconstitute at 500 µg/mL in sterile deionized water. |
| **Shipping** | The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below. |
| **Stability & Storage** | **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**<br>• A minimum of 12 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date.<br>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.<br>• 3 months, ≤ -20 °C under sterile conditions after reconstitution. |



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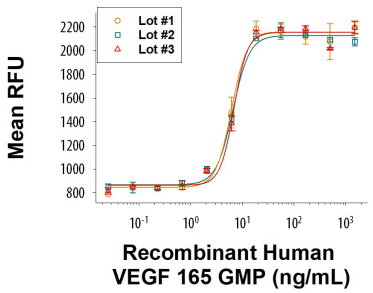
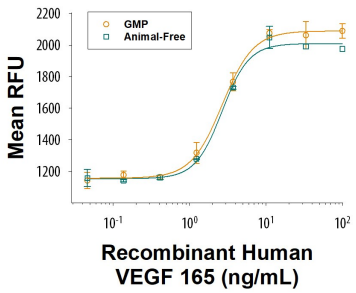
## DATA

### Bioactivity



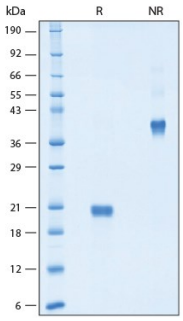

**Recombinant Human VEGF 165 GMP Protein Bioactivity.** GMP-grade Recombinant Human VEGF 165 (Catalog # BT-VEGF-GMP) as measured in a cell proliferation assay using HUVEC human umbilical vein endothelial cells. The $ED_{50}$ for this effect is 1.50-12.0 ng/mL. Three independent lots were tested for activity and plotted on the same graph to show lot-to-lot consistency of GMP VEGF 165.

### Bioactivity



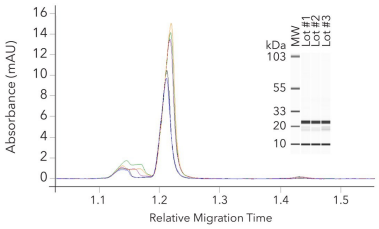

**Equivalent Bioactivity of GMP and Animal-Free grades of Recombinant Human VEGF 165.** Equivalent bioactivity of GMP (Catalog # BT-VEGF-GMP) and Animal-Free (Catalog # BT-VEGF-AFL) grades of Recombinant Human VEGF 165 as measured in a cell proliferation assay using HUVEC human umbilical vein endothelial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

### SDS-PAGE



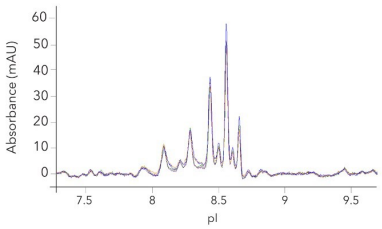

**Recombinant Human VEGF 165 GMP Protein SDS-PAGE** 2 µg/lane of Recombinant Human VEGF 165 GMP Protein (Catalog # BT-VEGF-GMP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 19-21 kDa.

### Data




**Recombinant Human VEGF 165 GMP Protein Purity Analysis by Size Separation.** Three independent lots of Recombinant Human VEGF 165 GMP Protein (Catalog # BT-VEGF-GMP) were analyzed by Maurice CE-SDS PLUS (IS is an Internal Standard). A gel-like representation of the purity analysis data (inset) can be obtained from the Lane View feature in Compass software for iCE. Profiles from the three runs were superimposed, showing excellent manufacturing consistency.

### Data




**Recombinant Human VEGF 165 GMP Protein Purity Analysis by Charge Variation.** Three independent lots of Recombinant Human VEGF 165 GMP Protein (Catalog # BT-VEGF-GMP) were analyzed by Maurice icIEF using native fluorescence detection (Mkr 5.85 and 9.99 are pI Markers). Profiles from the three runs were superimposed, showing excellent manufacturing consistency.



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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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- Post-bottling lot-specific bioassay results (compliance with an established range) and results of microbial testing according to USP
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

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