Recombinant Human VEGF 165 GMP

Catalog Number: 293-GMP

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 9 (baculovirus)-derived human VEGF protein Met1-Arg191 Accession # NP_001165097 Produced in an animal component free process (ACFP). Manufactured and tested under cGMP guidelines.
N-terminal Sequence Analysis	Ala_{27}-Pro-Met-Ala-Glu-Gly-Gly-Gly-Gln-Asn
Structure / Form	Disulfide-linked homodimer
Predicted Molecular Mass	19.2 kDa (monomer)

SPECIFICATIONS

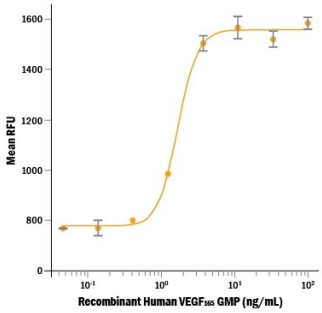
SDS-PAGE	19-21 kDa, reducing conditions
Activity	Measured in a cell proliferation assay using HUVEC human umbilical vein endothelial cells. Conn, G. *et al*. (1990) Proc. Natl. Acad. Sci. USA **87**:1323. The ED_{50} for this effect is 1-6 ng/mL. The specific activity of recombinant human $VEGF_{165}$ is >1.0 x 10^6 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 silver staining.
Mycoplasma	Negative when tested in a ribosomal RNA hybridization assay.
Formulation	Lyophilized from a 0.2 µm filtered solution in HCl. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile PBS. Alternatively, reconstitute at 100-500 µg/mL in sterile 4 mM HCl.
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.** • A minimum of 12 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 3 months, ≤ -20 °C under sterile conditions after reconstitution.

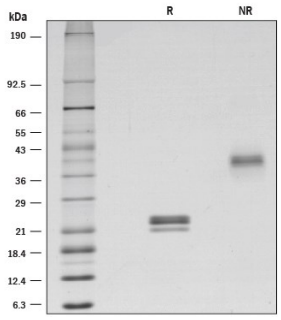
DATA

Bioactivity



GMP-grade Recombinant Human VEGF165 (Catalog # 293-GMP) stimulates proliferation in HUVEC human umbilical vein endothelial cells. The ED_{50} for this effect is 1-6 ng/mL.

SDS-PAGE



1 µg/lane of GMP-grade Recombinant Human VEGF 165 (Catalog # 293-GMP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by silver staining, showing bands at 21 and 23 kDa and 39 kDa, respectively.

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Recombinant Human VEGF 165 GMP

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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).

References:

1. Leung, D.W. *et al.* (1989) Science **246**:1306.
2. Keck, P.J. *et al.* (1989) Science **246**:1309.
3. Byrne, A.M. *et al.* (2005) J. Cell. Mol. Med. **9**:777.
4. Robinson, C.J. and S.E. Stringer (2001) J. Cell. Sci. **114**:853.
5. Pan, Q. *et al.* (2007) J. Biol. Chem. **282**:24049.
6. Weis, S.M. and D.A. Cheresh (2005) Nature **437**:497.
7. Thurston, G. (2002) J. Anat. **200**:575.
8. Carvalho, J.F. *et al.* (2007) J. Clin. Immunol. **27**:246.
9. Angelo, L.S. and R. Kurzrock (2007) Clin. Cancer Res. **13**:2825.

MANUFACTURING SPECIFICATIONS

GMP Proteins

R&D Systems, a Bio-Techne Brand's GMP proteins are produced according to relevant sections of the following documents: WHO TRS, No. 822, 1992 Annex 1, Good Manufacturing Practices for Biological Products; USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and USP Chapter 92, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.

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- Material review process for variances
- Monitoring of stability over product shelf-life

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- N-terminal amino acid analysis, SDS-PAGE analysis, and endotoxin level (as determined by LAL assay) performed on each bulk QC lot, not on individual bottlings of each QC lot
- Post-bottling lot-specific bioassay results (compliance with an established range) and results of microbial testing according to USP
- Mycoplasma testing by ribosomal RNA hybridization assay

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