

## **Synonym**

RP1-261G23.1,MGC70609,MVCD1,VEGFA,VPF

#### Source

Human VEGF165, premium grade(VE5-H4210) is expressed from human 293 cells (HEK293). It contains AA Ala 27 - Arg 191 (Accession # P15692-4). Predicted N-terminus: Ala 27

It is produced under our rigorous quality control system that incorporates a comprehensive set of tests including sterility and endotoxin tests. Product performance is carefully validated and tested for compatibility for cell culture use or any other applications in the early preclinical stage.

GMP-VE5H23 is the GMP version of this VE5-H4210. These two proteins display indistinguishable performance profiles, thereby ensuring a seamless transition for end users from early preclinical stag to later clinical phases.

#### **Molecular Characterization**

VEGF165(Ala 27 - Arg 191) P15692-4

This protein carries no "tag".

The protein has a calculated MW of 19.2 kDa. The protein migrates as 24 kDa±3 kDa when calibrated against <u>Star Ribbon Pre-stained Protein Marker</u> under reducing (R) condition (SDS-PAGE) due to glycosylation.

### Endotoxin

Less than 0.01 EU per μg by the LAL method / rFC method.

## **Host Cell Protein**

 $< 0.5 \text{ ng/}\mu\text{g}$  of protein tested by ELISA.

# **Host Cell DNA**

<0.02 ng/μg of protein tested by qPCR.

### **Sterility**

Negative

## Mycoplasma

Negative

#### **Purity**

>95% as determined by SDS-PAGE.

>95% as determined by SEC-MALS.

## **Formulation**

Lyophilized from  $0.22~\mu m$  filtered solution in PBS, pH7.4 with trehalose as protectant.

Contact us for customized product form or formulation.

#### Reconstitution

Please see Certificate of Analysis for specific instructions.

For best performance, we strongly recommend you to follow the reconstitution protocol provided in the CoA.

#### **Storage**

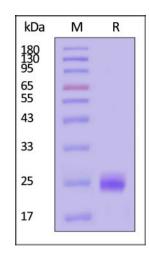
For long term storage, the product should be stored at lyophilized state at -20°C or lower.

Please avoid repeated freeze-thaw cycles.

This product is stable after storage at:

- -20°C to -70°C for 24 months in lyophilized state;
- -70°C for 3 months under sterile conditions after reconstitution.

#### **SDS-PAGE**



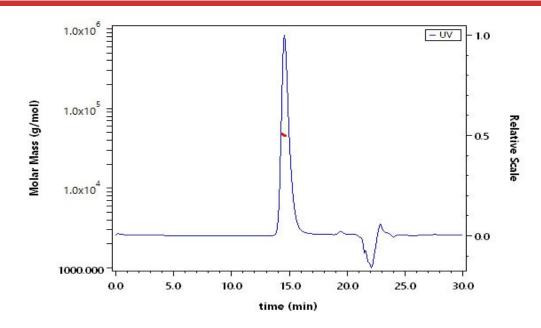
Human VEGF165, premium grade on SDS-PAGE under reducing (R) condition. The gel was stained with Coomassie Blue. The purity of the protein

#### **SEC-MALS**

Catalog # VE5-H4210



is greater than 95% (With Star Ribbon Pre-stained Protein Marker).

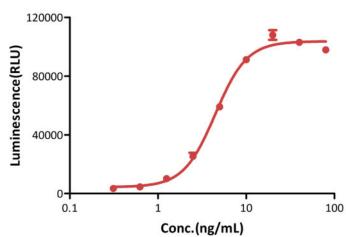


The purity of Human VEGF165, premium grade (Cat. No. VE5-H4210) is more than 95% and the molecular weight of this protein is around 42-60 kDa verified by SEC-MALS.

Report

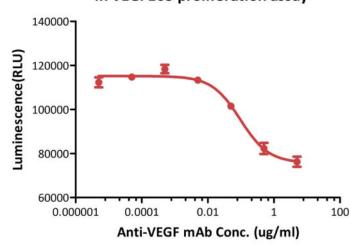
## **Bioactivity-CELL BASE**

# Human VEGF165, premium grade stimulates 293-NFAT/Luc-VEGFR2 cells



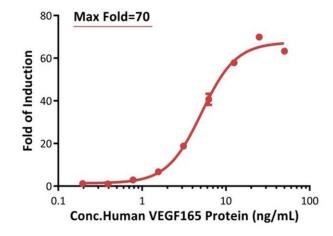
Human VEGF165, premium grade (Cat. No. VE5-H4210) stimulates 293-NFAT/Luc-VEGFR2 cells. The specific activity of Human VEGF165, premium grade is  $> 8.00 \times 10^5$  IU/mg, which is calibrated against human vascular endothelial growth factor 165 WHO International Standard (NIBSC code: 02/286) (QC tested).

# Inhibition effect of anti-VEGF mAb (Avastin) in VEGF165 proliferation assay



Inhibition assay shows that the proliferation effect of Human VEGF165, premium grade (Cat. No. VE5-H4210) is inhibited by increasing concentration of anti-VEGF mAb (Avastin). The concentration of VEGF165 used is 20 ng/mL. The ED50 is  $0.065\text{-}0.229~\mu\text{g/mL}$  (Routinely tested).

# **Human VEGF165 Protein Stimulation (Fold)**



#### Response to human VEGF165 protein (Fold).

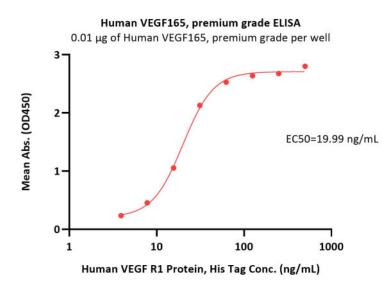
The VEGFR2 (Luc) HEK293 Reporter Cell was stimulated with serial



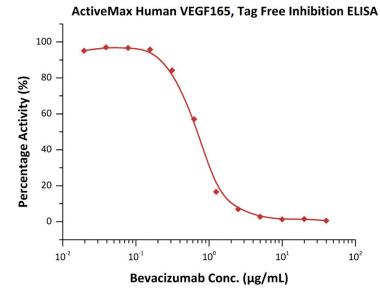


dilutions of human VEGF165 protein (AcroBiosystems, Cat. No. VE5-H4210). The max induction fold was approximately 70 (Routinely tested).

## **Bioactivity-ELISA**

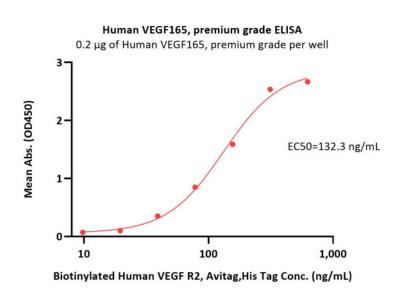


Immobilized Human VEGF165, premium grade (Cat. No. VE5-H4210) at 0.1  $\mu$ g/mL (100  $\mu$ L/well) can bind Human VEGF R1 Protein, His Tag (Cat. No. VE1-H52H9) with a linear range of 4-31 ng/mL (QC tested).



Immobilized Human VEGF165, premium grade (Cat. No. VE5-H4210) at 2  $\mu$ g/mL (100  $\mu$ L/well) can bind pre-mixed increasing concentrations of Bevacizumab and 0.5  $\mu$ g/mL (100  $\mu$ L/well) Biotinylated Human VEGF R2, Avitag,His Tag (Cat. No. KDR-H82E5) with a half maximal inhibitory concentration (IC50) of 0.70  $\mu$ g/mL (Routinely tested).

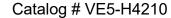
# **Bioactivity-SPR**



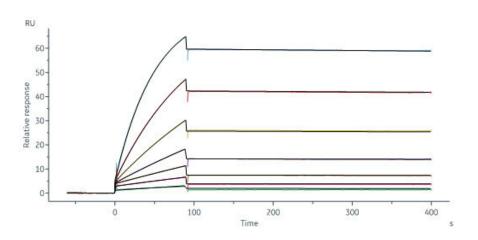
Immobilized Human VEGF165, premium grade (Cat. No. VE5-H4210) at 2  $\mu$ g/mL (100  $\mu$ L/well) can bind Biotinylated Human VEGF R2, Avitag,His Tag (Cat. No. KDR-H82E5) with a linear range of 10-156 ng/mL (Routinely tested).



## **Human VEGF165 Protein, premium grade**

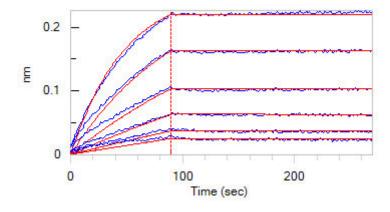




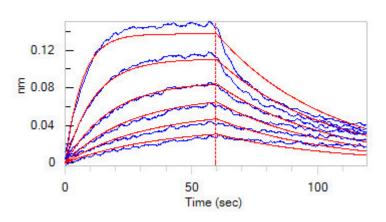


Anti-VEGFA Antibody, Human IgG1 captured on Protein A Chip can bind Human VEGF165, premium grade (Cat. No. VE5-H4210) with an affinity constant of 0.103 nM as determined in a SPR assay (Biacore 8K) (Routinely tested).

## **Bioactivity-BLI**

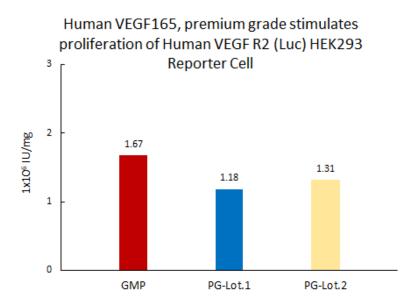


Loaded bind Anti-VEGFA Antibody, Human IgG1 on AHC2 Biosensor, can bind Human VEGF165, premium grade (Cat. No. VE5-H4210) with an affinity constant of 53.8 pM as determined in BLI assay (ForteBio Octet Red96e) (Routinely tested).



Loaded bind Human NRP1, Fc Tag (Cat. No. NR1-H5252) on Protein A Biosensor, can bind Human VEGF165, premium grade (Cat. No. VE5-H4210) with an affinity constant of 17.3 nM as determined in BLI assay (ForteBio Octet Red96e) (Routinely tested).

## **Bioactivity-Stability**



The Cell based assay shows batch-to-batch consistency between Acro's GMP and PG VEGF165.



## **Human VEGF165 Protein, premium grade**

Catalog # VE5-H4210



## **Background**

VEGF165 is the most abundant splice variant of VEGF-A. VEGF165 is produced by a number of cells including endothelial cells, macrophages and T cells. VEGF165 is involved in angiogenesis, vascular endothelial cell survival, growth, migration and vascular permeability. VEGF gene expression is induced by hypoxia, inflammatory cytokines and oncogenes. VEGF165 binds to heparan sulfate and is retained on the cell surface and in the extracellular matrix. VEGF165 binds to the receptor tyrosine kinases, VEGFR1 and VEGFR2. VEGF165 is the only splice variant that binds to co-receptors NRP-1 and NRP-2 that function to enhance VEGFR2 signaling. Binding of VEGF165 to VEGFR1 and VEGFR2 leads to activation of the PI3K/AKT, p38 MAPK, FAK and paxillin. VEGF plays a key role in tumor angiogenesis in many cancers.

