Sterile

#8065

Human Vascular Endothelial Growth Factor-165 ($hVEGF_{165}$)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 03/10/20

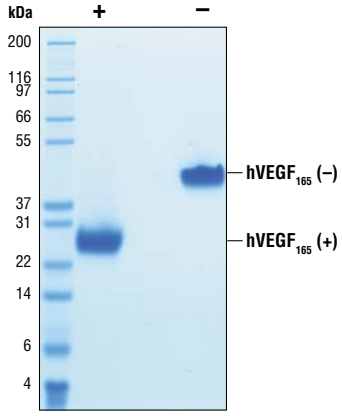
For Research Use Only. Not For Use In Diagnostic Procedures.

Source: Recombinant human $VEGF_{165}$ ($hVEGF_{165}$) Ala207-Arg371 (Accession #NP_001020539) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant $hVEGF_{165}$ contains no "tags" and has a calculated MW of 19,165. DTT-reduced protein migrates as a 24 kDa polypeptide and the non-reduced cystine-linked homodimer migrates as a 40 kDa protein. The expected amino-terminal APMAE of recombinant $hVEGF_{165}$ was verified by amino acid sequencing.

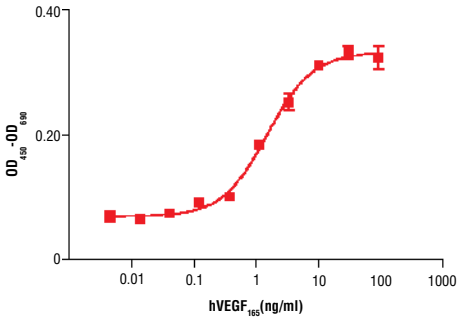
Endotoxin: Less than 0.01 ng endotoxin/1μg $hVEGF_{165}$.

Purity: >98% as determined by SDS-PAGE of 6 μg reduced (+) and non-reduced (-) recombinant $hVEGF_{165}$. All lots are greater than 98% pure.

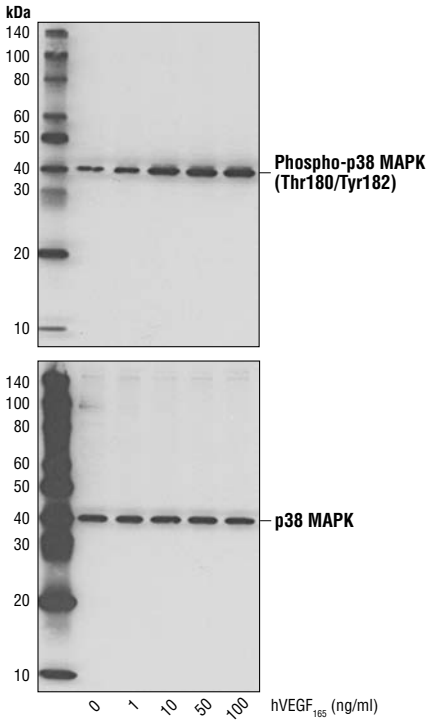


The purity of recombinant $hVEGF_{165}$ was determined by SDS-PAGE of 6 μg reduced (+) and non-reduced (-) recombinant $hVEGF_{165}$ and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant $hVEGF_{165}$ was determined in a cell proliferation assay using HUVEC. The ED_{50} of each lot is between 1-6 ng/ml.



◀ *The proliferation of HUVEC treated with increasing concentrations of $hVEGF_{165}$ was assessed. After 72-hour treatment with $hVEGF_{165}$ cells were incubated with a tetrazolium salt and the OD_{450} - OD_{650} was determined.*



Western blot analysis of extracts from HUVEC untreated or treated with $hVEGF_{165}$ for 15 minutes, using Phospho-p38 MAPK (Thr180/Tyr182) (3D7) Rabbit mAb #9215 (upper) and p38 MAPK Antibody #9212 (lower).

Formulation: With carrier: Lyophilized from a 0.22 μm filtered solution of PBS, pH 7.2 containing 20 μg BSA per 1 μg $hVEGF_{165}$.

Carrier free: Lyophilized from a 0.22 μm filtered solution of PBS, pH 7.2.

Reconstitution:
With carrier: Add sterile PBS or PBS containing 1% bovine or human serum albumin or 5-10% FBS to a final $hVEGF_{165}$ concentration of greater than 50 μg/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile PBS or PBS containing protein to minimize absorption of $hVEGF_{165}$ to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock $hVEGF_{165}$ should be greater than 50 μg/ml.

Storage: Stable in lyophilized state at -20°C for 1 year after receipt. Sterile stock solutions reconstituted with carrier protein are stable at 4°C for 2 months and at -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Maintain sterility. Storage at -20°C should be in a manual defrost freezer.

Applications: Optimal concentration for the desired application should be determined by the user.

Background: $VEGF_{165}$ is the most abundant splice variant of VEGF-A (1,2). $VEGF_{165}$ is produced by a number of cells including endothelial cells, macrophages and T cells. $VEGF_{165}$ is involved in angiogenesis, vascular endothelial cell survival, growth, migration and vascular permeability (1). VEGF gene expression is induced by hypoxia, inflammatory cytokines and oncogenes (1,2). $VEGF_{165}$ binds to heparan sulfate and is retained on the cell surface and in the extracellular matrix (2,3). $VEGF_{165}$ binds to the receptor tyrosine kinases, VEGFR1 and VEGFR2 (1). $VEGF_{165}$ is the only splice variant that binds to co-receptors NRP-1 and NRP-2 (1-3) that function to enhance VEGFR2 signaling (1). Binding of $VEGF_{165}$ to VEGFR1 and VEGFR2 leads to activation of the PI3K/AKT, p38 MAPK, FAK and paxillin (1). VEGF plays a key role in tumor angiogenesis in many cancers (2).

Background References:

(1) Takahashi, H. and Shibuya, M. (2005) *Clin Sci (Lond)* 109, 227-41.

(2) Neufeld, G. et al. (1999) *FASEB J* 13, 9-22.

(3) Robinson, C.J. and Stringer, S.E. (2001) *J Cell Sci* 114, 853-65.