Sterile

#5874

Rat Vascular Endothelial Growth Factor-164 ($rVEGF_{164}$)

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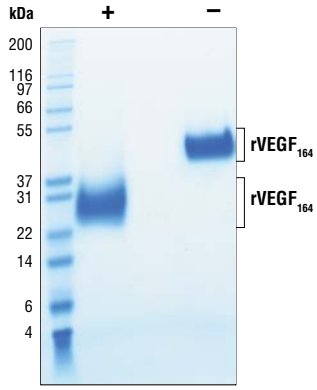
For Research Use Only. Not For Use In Diagnostic Procedures.

Source: Recombinant rat $VEGF_{164}$ ($rVEGF_{164}$) Ala27-Arg190 (Accession #NP_001103803) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant $rVEGF_{164}$ contains no "tags" and the nonglycosylated protein has a calculated MW of 19,234. DTT-reduced protein migrates as a 24-31 kDa polypeptide. Lower mobility in SDS-PAGE is due to glycosylation. The non-reduced cystine-linked homodimer migrates as a 46-53 kDa protein. The expected amino-terminal APTTE of recombinant $rVEGF_{164}$ was verified by amino acid sequencing.

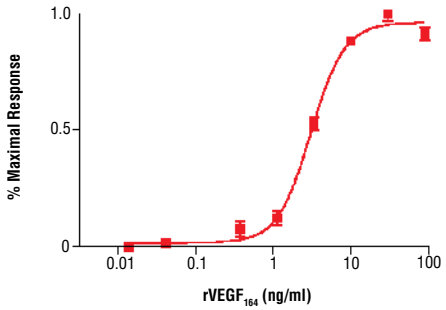
Endotoxin: Less than 0.01 ng endotoxin/1 µg $rVEGF_{164}$.

Purity: >98% as determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant $rVEGF_{164}$. All lots are greater than 98% pure.

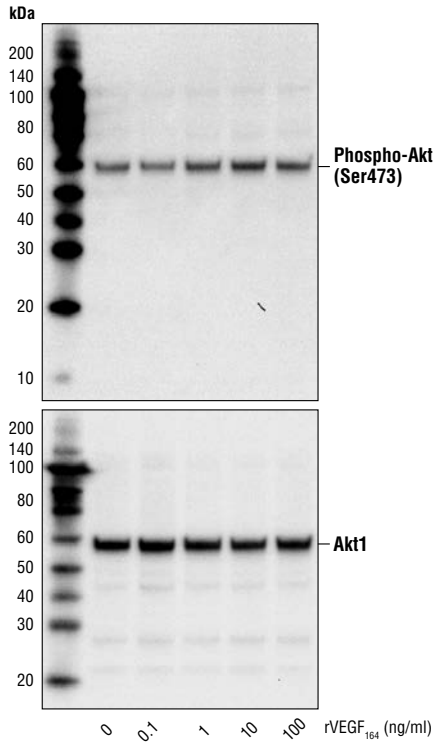


The purity of recombinant $rVEGF_{164}$ was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant $rVEGF_{164}$ and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant $rVEGF_{164}$ was determined in a cell proliferation assay using HUVEC. The ED_{50} of each lot is between 1-5 ng/ml.



◀ *The proliferation of HUVEC treated with increasing concentrations of $rVEGF_{164}$ was assessed. After 72-hour treatment with $rVEGF_{164}$, 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 $rVEGF_{164}$ for 15 minutes, using Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb #4060 (upper) or Akt1 (C73H10) Rabbit mAb #2938 (lower).

Formulation: With carrier: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2 containing 20 µg BSA per 1 µg $rVEGF_{164}$.

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 $rVEGF_{164}$ 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 $rVEGF_{164}$ to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock $rVEGF_{164}$ 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: $VEGF_{164}$ is one of many splice variants of the VEGF-A gene, and is one amino acid shorter than its human counterpart, $VEGF_{165}$ (1, 2). $VEGF_{164}$ is produced by a number of cells including endothelial cells, macrophages, and T cells (1, 2). $VEGF_{164}$ is involved in angiogenesis, vascular endothelial cell survival, growth, migration, and vascular permeability (1,2). Gene expression is induced by hypoxia, inflammatory cytokines, and oncogenes (1, 2). $VEGF_{164}$ binds to heparan sulfate and is retained on the cell surface and in the extracellular matrix (1-3). VEGFR1 and VEGFR2 are the receptor tyrosine kinases for $VEGF_{164}$ (2). NRP-1 and NRP-2 may function as co-receptors and enhance VEGFR2 signaling (2-3). Binding of $VEGF_{164}$ to VEGFR1 and VEGFR2 leads to activation of the PI3K/AKT, p38 MAPK, FAK, and Paxillin (2).

Background References:

(1) Haigh, J.J. (2008) *Organogenesis* 4, 247-56.

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

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