Human $VEGF_{165}$ Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF-293-NA

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human VEGF in direct ELISAs and Western blots. In direct ELISAs, approximately 100% cross-reactivity with recombinant canine VEGF is observed, and less than 20% cross-reactivity with recombinant mouse $VEGF_{165}$ and recombinant rat $VEGF_{164}$ is observed, and less than 5% cross-reactivity with recombinant zebrafish VEGF is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*S. frugiperda* insect ovarian cell line *Sf* 21-derived recombinant human $VEGF_{165}$ Ala27-Arg191 Accession # NP_001165097.1
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

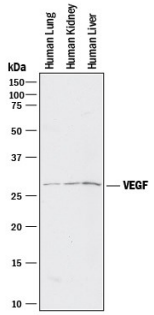
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	5-10 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	3-15 µg/mL	See Below
Neutralization	Measured by its ability to neutralize $VEGF_{165}$-induced proliferation in HUVEC human umbilical vein endothelial cells. Conn, G. *et al.* (1990) Proc. Natl. Acad. Sci. USA **87**:1323. The Neutralization Dose (ND_{50}) is typically 0.02-0.12 µg/mL in the presence of 10 ng/mL Recombinant Human $VEGF_{165}$.	

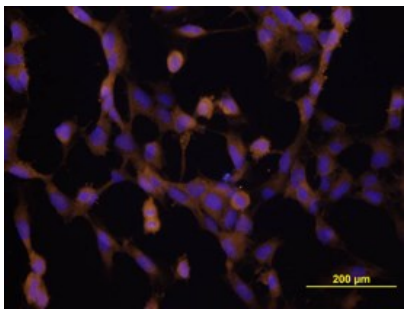
DATA

Western Blot



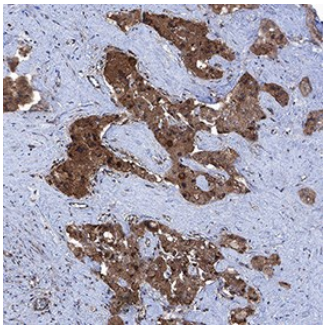
Detection of Human VEGF by Western Blot. Western blot shows lysates of human lung tissue, human kidney tissue, and human liver tissue. PVDF membrane was probed with 5-10 µg/mL of Goat Anti-Human $VEGF_{165}$ Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-293-NA) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for VEGF at approximately 27 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



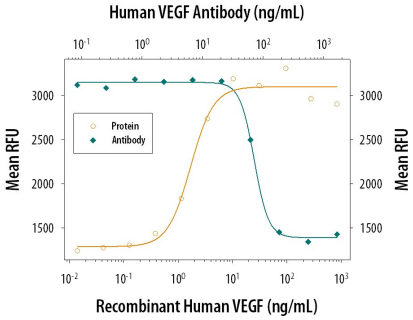
VEGF in HUVEC Cells. VEGF was detected in immersion fixed human umbilical vein endothelial cells (HUVECs) using Goat Anti-Human $VEGF_{165}$ Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-293-NA) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (yellow; Catalog # NL001) and counter-stained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Immunohistochemistry



VEGF in Human Liver Cancer Tissue. VEGF was detected in immersion fixed paraffin-embedded sections of human liver cancer tissue using Goat Anti-Human VEGF 165 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-293-NA) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm in cancer cells. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Neutralization



Cell Proliferation Induced by $VEGF_{165}$ and Neutralization by Human VEGF Antibody. Recombinant Human $VEGF_{165}$ (Catalog # 293-VE) stimulates proliferation in HUVEC human umbilical vein endothelial cells in a dose-dependent manner (orange line). Proliferation elicited by Recombinant Human $VEGF_{165}$ (10 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human $VEGF_{165}$ Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-293-NA). The ND_{50} is typically 0.02-0.12 µg/mL.

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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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. It is a member of the PDGF family that is characterized by the presence of eight conserved cysteine residues and a cystine knot structure. VEGF165 appears to be the most abundant and potent isoform, followed by VEGF121 and VEGF189. Human VEGF165 is an approximately 44 kDa molecular weight homodimer sharing 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. Although VEGF affinity is highest for binding to VEGF R1, VEGF R2 appears to be the primary mediator of VEGF angiogenic activity. VEGF165 binds the Semaphorin receptor, Neuropilin-1 and promotes complex formation with VEGF R2. VEGF is required during embryogenesis and functions to regulate the proliferation, migration, and survival of endothelial cells. In adults, VEGF functions mainly in wound healing and the female reproductive cycle. Pathologically, it is involved in tumor angiogenesis and vascular leakage. Circulating VEGF levels correlate with disease activity in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus. VEGF is induced by hypoxia and cytokines such as IL-1, IL-6, IL-8, Oncostatin M (OSM) and TNF-alpha.

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