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ProductInformation

Anti-Vascular Endothelial Growth Factor Receptor-1
Antibody produced in goat
Affinity Isolated Antibody

Product Number **V 1139**

Product Description

Anti-Vascular Endothelial Growth Factor Receptor-1 is produced in goat using purified recombinant mouse vascular endothelial cell growth factor receptor 1 (VEGF R1) extracellular domain expressed in mouse NSO cells as immunogen. Affinity isolated antigen specific antibody is obtained from goat anti-VEGF R1 antiserum by immuno-specific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Vascular Endothelial Growth Factor Receptor-1 recognizes recombinant mouse VEGF R1 by various immunochemical techniques including immunoblotting, ELISA capture, neutralization, flow cytometry, and blockade of receptor-ligand interaction. The antibody has the ability to neutralize the biological activity of recombinant mouse VEGF R1. Based on ELISA, this antibody shows <7% cross-reactivity with recombinant human VEGF R1, 4% cross-reactivity with recombinant mouse $VEGF_{164}$, 1% cross-reactivity with recombinant rat $VEGF_{164}$, and 0.2% cross-reactivity with recombinant mouse VEGF R2 and VEGF R3.

Vascular endothelial growth factors (VEGFs) are a family of closely related growth factors having a conserved pattern of eight cysteine residues and sharing common VEGF receptors. VEGFs stimulate the proliferation of endothelial cells, induce angiogenesis, and increase vascular permeability in both large and small vessels.[1] The mitogenic activity of VEGFs appears to be mediated by specific VEGF receptors. Vascular Endothelial Growth Factor Receptor 1 (VEGF R1, Flt-1)[2, 3] is one of the five receptor tyrosine kinases (RTKs) (VEGF R1/Flt1, VEGF R2/KDR/Flk-1, VEGF R3/Flt-4, tie-1, and tek/tie-2) whose expression is almost exclusively restricted to endothelial cells. These RTKs play central roles in vasculogenesis and angiogenesis.

VEGF R1 is responsible for guiding endothelial cells into the proper spatial organization of lumen-containing vessels. Hypoxia induces endothelial cell expresssion of VEGF R1 but not VEGF R2. Alternative splicing of VEGF R1 pre-mRNA is important in the regulation of VEGF activity in angiogenesis.[4] VEGF B binds to VEGF R1 and regulates plasminogen activator activity in endothelial cells.[5] VEGF R1 binds both PIGF and VEGF with high affinity, whereas VEGF R2 binds VEGF with high affinity but does not bind PIGF.[6]

The human VEGF R1 (Flt-1) gene has been mapped to chromosome 13q12.

Reagent

The antibody is supplied as ~100 µg of antiserum lyophilized from a 0.2 µm filtered solution of phosphate buffered saline with 5% trehalose.

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of sterile phosphate buffered saline to produce a 0.1 mg/mL stock solution of antibody.

Storage/Stability

Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots. Avoid repeated freezing and thawing. Do not store in frost-free freezer.

Product Profile
Neutralization: The antibody has the ability to neutralize the bioactivity of recombinant mouse VEGF R1 using HUVE (human umbilical vein endothelial) cells in the presence of 5 ng/mL of recombinant mouse VEGF. HUVE cells (5 x 10^4 cells/mL) are added to the wells of a 96 well plate containing various concentrations (0.1-100 µg/mL) of the antibody and preincubated for 1 hour at 37 °C. Following this preincubation, recombinant mouse VEGF (5 ng/mL) is added to the mixture. The assay mixture in a total volume of 100 µL, containing antibody at concentrations of 0.1-100 µg/mL, recombinant mouse VEGF at 5 ng/mL, and cells at 5 x 10^4 cells/mL, is incubated at 37 °C for 72 hours in a humidified CO_2 incubator. The mixture is pulsed with ^{3}H-thymidine during the final 20 hours. The cells are detached and harvested onto glass fiber filters, and the ^{3}H-thymidine incorporated into the DNA is measured.[7]

The ND_{50} is the concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when the cytokine is present at a concentration just high enough to elicit a maximum response.

The exact concentration of antibody required to neutralize mouse VEGF R1 mediated response is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

For ELISA capture, the antibody can be used as a capture antibody in a mouse VEGF R1 ELISA in combination with biotinylated mouse VEGF R1 affinity purified polyclonal detection antibody. Using plates coated with 100 µL/well of the capture antibody at 0.8 µg/mL, in combination with 100 µL/well of the detection antibody, an ELISA for sample volumes of 100 µl can be obtained. Titrate each preparation of the recombinant protein for standard preparation to arrive at the most suitable dose range. A two-fold dilution series starting at 10 ng/mL is recommended.

For immunoblotting, a working antibody concentration of 0.1-0.2 µg/ml is recommended. The detection limit for recombinant mouse VEGF R1 is ~20 ng/lane under non-reducing and reducing conditions.

By flow cytometry, a working antibody concentration of 3-10 µg/mL/10^6 cells is recommended using with an appropriate secondary antibody for indirect immunofluorescence staining of cells.

For neutralization of receptor-ligand interaction, a working antibody concentration of 1-4 µg/mL will block 50% of the binding of 10 ng/mL of recombinant mouse P*l*GF-2 to immobilized recombinant mouse Flt-1/Fc chimera (100 µL of a 1 µg/mL solution coated in each well) in a functional ELISA.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining the optimal working dilutions by titration.

References

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