

Product Information

Anti-Vascular Endothelial Growth Factor Receptor-2

produced in goat, affinity isolated antibody

Catalog Number **V1014**

Product Description

Anti-Vascular Endothelial Growth Factor Receptor-2 is produced in goat using purified recombinant mouse vascular endothelial cell growth factor receptor 2 (VEGF R2)¹ extracellular domain expressed in mouse NSO cells as immunogen. Affinity isolated antigen specific antibody is obtained from goat anti-VEGF R2 antiserum by immunospecific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Vascular Endothelial Growth Factor Receptor-2 recognizes recombinant mouse VEGF R2 by various immunochemical techniques, including neutralization, immunoblotting, and immunohistochemistry. Based on immunoblotting, this antibody shows ~40% cross-reactivity with recombinant human VEGF R2, 2% cross-reactivity with recombinant mouse VEGF R1, and no cross-reactivity with recombinant human VEGF R1 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.² The mitogenic activity of VEGFs appears to be mediated by specific VEGF receptors.

Vascular Endothelial Growth Factor Receptor 2 (VEGF R2, KDR, Flk-1) is one of the five receptor tyrosine kinases (RTKs), VEGF R1/Flt1, tek/tie-2, VEGF R2/KDR/Flk-1, VEGF R3/Flt-4, and tie-1, whose expression is almost exclusively restricted to endothelial cells. These RTKs play central roles in vasculogenesis and angiogenesis. Tie-1 and tek/tie-2 are a class of RTKs containing two immunoglobulin-like domains, three EGF homology domains, and three fibronectin type III domains in their extracellular regions. VEGF R1/Flt-1, VEGF R2/KDR/Flk-1, and VEGF R3/Flt-4 are members of the class III subfamily of RTKs containing seven immunoglobulin-like repeats in their extracellular domains.

VEGF R1 and VEGF R2 are both expressed in an endothelial cell-specific manner. They are detectable in virtually all tissues in adults and embryos. Monocytes express VEGF R1 and VEGF R2. VEGF R2 is also expressed in pancreatic duct cells, hematopoietic stem cells, megakaryocytes, specific tumor cell types such as malignant melanoma cells, and retinal progenitor cells.³ In the retina, two functional VEGF R2 forms are expressed as a result of alternative splicing.⁴ VEGF R2 is a key marker for pluripotent hematopoietic stem cells.⁵

VEGF R1 and VEGF R2 are closely related in their putative roles in angiogenesis. VEGF R2 is involved in commitment of endothelial-cell lineages and to cell proliferation, while VEGF R1 seems to be responsible for guiding endothelial cells into the proper spatial organization of the lumen-containing vessels. VEGF R1 binds both PlGF and VEGF with high affinity; whereas, VEGF R2 binds VEGF with high affinity, but does not bind PlGF. Recombinant soluble VEGF R2/Fc chimera binds VEGF with high affinity and is a potent VEGF antagonist.⁶

Reagent

Lyophilized from 0.2 µm-filtered solution in phosphate buffered saline containing carbohydrates.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To one vial of lyophilized powder, add 1 mL of 0.2 µm filtered phosphate buffered saline (PBS) 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 $2-8^{\circ}\text{C}$ for up to one month. For prolonged storage, freeze in working aliquots at -20°C . Avoid repeated freezing and thawing. Do not store in frost-free freezer.

Product Profile

Neutralization: Anti-Mouse VEGF R2 has the ability to neutralize the biological activity of recombinant mouse vascular endothelial growth factor receptor-2 on HUVE (human umbilical vein endothelial) cells in the presence of recombinant mouse VEGF R2/Fc (50 ng/mL) and recombinant mouse VEGF (5 ng/mL). Recombinant mouse VEGF R2 is added to various concentrations of the antibody for 1 hour at 37°C in a 96 well plate. Following this pre-incubation, recombinant mouse VEGF is added to the mixture and incubated for another 1 hour at 37°C . HUVE cells are then added to the mixture. The assay mixture in a total volume of 100 μL , containing antibody at concentrations of 5-10,000 $\mu\text{g/mL}$, recombinant mouse VEGF R2 at 50 $\mu\text{g/mL}$, recombinant mouse VEGF at 5 ng/mL, and cells at 5×10^4 cells/mL, is incubated at 37°C for 72 hours in a humidified CO_2 incubator. The mixture is pulsed with ^3H -thymidine during the final 20 hours. The cells are detached and harvested onto glass fiber filters, and the ^3H -thymidine incorporated into the DNA is measured.⁷

The Neutralization Dose₅₀ (ND₅₀) for anti-mouse VEGF R2 is 0.1-0.3 $\mu\text{g/mL}$ in the presence of 50 $\mu\text{g/mL}$ of recombinant mouse VEGF R2 and 5 ng/mL of recombinant mouse VEGF₁₆₅ using the HUVE cell line.

The ND₅₀ 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 R2 activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

Immunoblotting: a working antibody concentration of 0.1-0.2 $\mu\text{g/mL}$ is recommended. The detection limit for recombinant mouse VEGF R2 is ~ 20 ng/lane under non-reducing and reducing conditions.

Immunohistochemistry: a working antibody concentration of 5-15 $\mu\text{g/mL}$ is recommended to detect VEGF R2 in cells and tissues using a chromogenic detection system.

Flow cytometry: a working antibody concentration of 3-10 $\mu\text{g/mL}$ is recommended.

Note: In order to obtain the best results in various techniques and preparations, determination of optimal working dilutions by titration test is recommended.

Endotoxin: <0.1 EU (endotoxin units) per 1 μg of the antibody by the LAL (Limulus amoebocyte lysate) method.

References

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TD,KAA,PHC,TMS,MAM 06/16-1