Product Information

Monoclonal Anti-Vascular Endothelial Growth Factor, Clone 26503

Produced in mouse, purified immunoglobulin

V4758

Product Description

Monoclonal Anti-Vascular Endothelial Growth Factor (VEGF; IgG2b isotype) is purified from a hybridoma produced by the fusion of mouse myeloma cells and B cells from a mouse immunized with recombinant human VEGF expressed in Sf21 insect cells used as immunogen. The 165 amino acid isoform of recombinant human VEGF was used as the immunogen to prepare the antibody.[3] The antibody is purified by Protein G affinity chromatography.

Monoclonal Anti-Vascular Endothelial Growth Factor recognizes human $VEGF_{165}$ and $VEGF_{121}$. The antibody may be used in various immunochemical techniques, including immunoblotting, capture ELISA, and neutralization.

Vascular Endothelial Growth Factor (VEGF) also known as vasculotropin, is an angiogenic growth factor. VEGF is a heparin-binding, dimeric glycoprotein (~46 kDa), which is heat and acid stable.[1] VEGF is a basic protein with an isoelectric point of 8.5.[2] Four cDNA clones have been identified. These clones arise through alternative splicing and encode mature, monomeric human VEGF having 121, 165, 189, or 206 amino acids.

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 PBS to produce a 0.5 mg/mL stock solution.

If aseptic technique is used, no further filtration should be necessary for use in cell culture environments.

Storage/Stability

Prior to reconstitution, store at –20 °C.

Reconstituted product may be stored at 2-8 °C for up to one month. For extended storage, freeze in working aliquots at –20 °C. Repeated freezing and thawing, or storage in frost-free freezers, is not recommended.

Product Profile

Neutralization: The antibody is tested for its ability to neutralize the biological activity of recombinant human $VEGF_{165}$ on HUVEC cells. It also recognizes $VEGF_{121}$. In this bioassay, recombinant human VEGF is incubated with various dilutions of the antibody for 1 hour at 22 °C in a 96-well plate. After the incubation, HUVEC cells were added to the antigen-antibody mixture. The assay mixture, which contains a total volume of 0.1 mL and recombinant human VEGF at 10 ng/mL, was incubated at 37 °C for 48 hours in a humidified CO_2 incubator and then pulsed for 24 hours with ^{3}H-thymidine. Cells were harvested onto glass filters and the ^{3}H-thymidine incorporation into DNA was measured.

The Neutralization $Dose_{50}$ (ND_{50}) for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response.

Immunoblotting: a working concentration of 1-2 µg/mL is recommended. Using a colorimetric detection system, the detection limit for rhVEGF is ~2 ng/lane under non-reducing conditions. Use of this antibody under reducing conditions is not recommended.

Capture ELISA: This antibody can be used as a capture antibody in a human VEGF ELISA in combination with a biotinylated, anti-human VEGF affinity purified polyclonal detection antibody. An ELISA for sample volumes of 100 µL can be obtained using plates coated with 100 µL/well of the capture antibody at 2-8 µg/mL, in combination with 100 µL/well of the detection antibody. The suggested coating concentration should be titrated to determine the optimal concentration. The recommended concentration of the detection reagent is 0.1-0.4 µg/mL.

Note: In order to obtain the best results using various techniques and preparations, determination of optimal working dilutions by titration test is recommended. Endotoxin: < 0.1 EU/µg antibody as determined by the LAL method.

References

1. Ferrara, N., et al., Biochem. Biophys. Res. Commun., 161, 851 (1989).
2. Ferrara, N., et al., Endocrine Reviews, 13, 18 (1992).
3. Leung, D. W., et al., Science, 246, 1306 (1989)

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