

Reagents Provided

Peridinin-Chlorophyll-Protein-Complex (PerCP)-conjugated mouse monoclonal anti-human VEGF R2: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 89106

Isotype: mouse IgG₁

Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

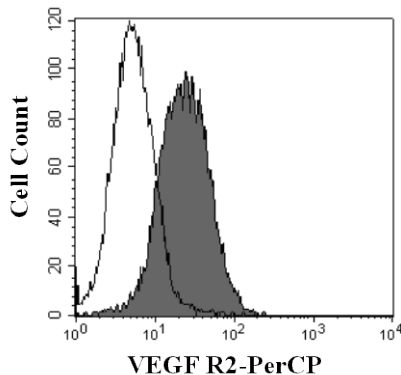
Designed to quantitatively determine the percentage of cells bearing VEGF R2 within a population and qualitatively determine the density of VEGF R2 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PerCP-labeled monoclonal antibody, which binds to cells expressing VEGF R2. Unbound PerCP-conjugated antibody is then washed from the cells. Cells expressing VEGF R2 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of VEGF R2. Cell surface expression of VEGF R2 is determined by flow cytometry. PerCP has a maximum absorption of 482 nm and 564 nm and a maximum emission of 675 nm.

Reagent Preparation

PerCP-conjugated mouse anti-human VEGF R2: Use as is; no preparation necessary.



HUVEC cells were stained with PerCP-conjugated anti-human VEGF R2 (Catalog # FAB357C, filled histogram) or PerCP-conjugated isotype control (Catalog # IC002C, open histogram).

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) followed by centrifugation at 500 x g for 5 minutes. 50 µL of packed cells should then be transferred to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA) to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10⁶ cells/mL and 25 µL of cells (1 x 10⁵) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

- 1) Cells should be Fc-blocked by treatment with 1 µg of human IgG/10⁵ cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
- 2) Transfer 25 µL of the Fc-blocked cells (1 x 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PerCP-conjugated VEGF R2 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted VEGF R2 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
- 6) Finally, resuspend the cells in 200 - 400 µL of PBS buffer for analysis by flow cytometry.
- 7) As a control for analysis, cells in a separate tube should be treated with PerCP-labeled mouse IgG₁ antibody.

This procedure may need modification, depending upon final utilization.

Background Information

Vascular Endothelial Growth Factor (VEGF) family members are major mediators of vasculogenesis and angiogenesis.¹⁻³ Specific biological activities attributed to VEGFs include: mitogenic activity on endothelial cells, increased vascular permeability, stimulation of monocyte migration across endothelial cells, and angiogenic activity. Three VEGF family receptors have been described: Flt-1 (*fms*-like tyrosine kinase) also known as VEGF R1,⁴ KDR (kinase-insert domain-containing receptor) also known as Flk-1 and VEGF R2,⁵ and Flt-4 also known as VEGF R3.⁶ The three receptors contain seven extracellular immunoglobulin-like domains and share substantial sequence homology. In addition, neuropilin-1, best known as a neuronal receptor,⁷ also acts as a co-receptor for VEGF when expressed on vascular endothelial cells, endothelial cell progenitors, and monocytes.⁷⁻⁹ VEGF R2 is considered the major signal transducing receptor on endothelial cells upon binding VEGF, VEGF-C, VEGF-D, or the viral paralog VEGF-E.³ Through its endothelial mitogenic and hyperpermeability activities, VEGF influences a variety of immune functions related to wound healing and blood protein traffic across endothelial barriers.

References

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3. Karkkainen, M.J. and T.V. Petrova (2000) *Oncogene* **19**:5598.
4. DeVries, C. *et al.* (1992) *Science* **255**:989.
5. Terman, B.I. *et al.* (1992) *Biochem. Biophys. Res. Commun.* **187**:1579.
6. Galland, F. *et al.* (1993) *Oncogene* **8**:1233.
7. Shimizu, M. *et al.* (2000) *J. Cell Biol.* **148**:1283.
8. Bernatchez, P.N. *et al.* (2002) *J. Cell. Biochem.* **85**:629.
9. Schuch, G. *et al.* (2002) *Blood* **100**:4622.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.