Monoclonal Anti-human VEGF Receptor Phycoerythrin Sampler Pack

Catalog Number: FABSP3P
75 Total Tests

Reagent Information

Phycoerythrin-conjugated mouse monoclonal anti-human VEGF R1 (Flt-1): Contains 250 µL of ready to use PE-labeled antibody (sufficient for 25 tests) clone # 49560 (Catalog # FAB321P). Mouse IgG_1 isotype.

Phycoerythrin-conjugated mouse monoclonal anti-human VEGF R2 (KDR): Contains 250 µL of ready to use PE-labeled antibody (sufficient for 25 tests) clone # 89106 (Catalog # FAB357P). Mouse IgG_1 isotype.

Phycoerythrin-conjugated mouse monoclonal anti-human VEGF R3 (Flt-4): Contains 250 µL of ready to use PE-labeled antibody (sufficient for 25 tests) clone # 54733 (Catalog # FAB3492P). Mouse IgG_1 isotype.

Isotypes: mouse IgG_1

Storage: 2 - 8° C

Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

Intended Use

Designed to quantitatively determine the percentage of cells bearing VEGF R1 (Flt-1), VEGF R2 (KDR) or VEGF R3 (Flt-4) within a population and qualitatively determine the density of these VEGF receptors on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing the VEGF receptor. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing the VEGF receptor are fluorescently stained, with the intensity of staining directly proportional to the density of VEGF receptor. Cell surface expression of VEGF receptors is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Phycoerythrin-conjugated mouse anti-human VEGF R1, VEGF R2, and VEGF R3: Use as is; no preparation is necessary.

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) by centrifugation at 500 x g for 5 minutes. Transfer 50 µL of packed cells 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), as described above, 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 10^6 cells/mL and 25 µL of cells (1 x 10^5) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate 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 or mouse IgG/10^5 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 10^5 cells) or 50 µL of packed whole blood to a 5 mL tube.
3) Add 10 µL of PE-conjugated anti-VEGF receptor reagent.
4) Incubate for 30 - 45 minutes at 2 - 8° C.
5) Following this incubation, remove unreacted anti-VEGF receptor 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' Human Erythrocyte Lysing Kit, Cat. # WL1000.*
6) Resuspend the cells in 200 - 400 µL of PBS buffer for final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with the appropriate PE-labeled isotype control antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

Vascular Endothelial Growth Factor (VEGF) family members are major mediators of vasculogenesis and angiogenesis (1 - 3). Specifically, biological activities attributed to VEGFs include: mitogenic activity on endothelial cells, increased permeability of endothelial cells to proteins, 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, 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 R1 is expressed primarily on endothelial cells but is also found on human peripheral blood monocytes (10). VEGF R2 is considered the major signal transducing receptor on endothelial cells upon binding either VEGF, VEGF-C, VEGF-D or the viral homolog VEGF-E (3). VEGF R3 is expressed primarily on endothelial cells of lymphatic vessels and is also found on tumor blood vessels during neovascularization (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.
10. Sawana, A. *et al*. (2001) Blood **97**:785.

Note: 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.