Monoclonal Anti-human P-Selectin (CD62P)-Fluorescein

Catalog Number: BBA34
Lot Number: LAE03
100 Tests

Reagents Provided

Fluorescein-conjugated mouse monoclonal anti-human P-Selectin: Supplied as 25 μg of antibody in 1 mL PBS containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 9E1

Isotype: mouse IgG_1

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

Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

Intended Use

Designed to quantitatively determine the percentage of cells bearing P-Selectin (CD62P) within a population and qualitatively determine the density on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody which binds to the cells expressing the CD62P. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells CD62P structure are fluorescently stained, with the intensity of staining directly proportional to the density of CD62P. Cell surface expression of CD62P is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated mouse anti-human P-Selectin: Use as is; no preparation 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. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Whole blood cells 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 4×10^6 cells/mL and 25 μL of cells (1×10^5) are 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 to be used for staining with the antibody may be first Fc-blocked by treatment with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of fluorescein-conjugated anti-CD62P reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-P-Selectin reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a 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 final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with fluorescein-labeled mouse IgG_1 antibody.

This procedure may need to be modified, depending upon final utilization.

R&D Systems Inc.
1-800-343-7475

Background Information

P-Selectin, also known as CD62P, GMP-140 and PADGEM, is a member of the selectin family of adhesion molecules and mediates interactions between vascular endothelial cells and leukocytes. P-selectin has a molecular weight of 140 kD and is expressed on the surface of endothelial cells and activated platelets following cellular activation. The ligand for P-Selectin appears to be PSGL-1 (P-Selectin glycoprotein ligand-1) (1). A variety of cells have been shown to bind P-Selectin through the PSGL-1 structure including myeloid cells, lymphocytes, dendritic cells, $CD34^+$ stem cells and some tumor cell lines (2, 3). A soluble form of P-Selectin has been reported and it has been shown to inhibit the adhesion of activated neutrophils to endothelium (4). CD62P appears to be stored in secretory granules of platelets and endothelial cells and can be rapidly translocated to the plasma membrane following cellular activation (5).

References

1. Sako, D. *et al.* (1993) Cell **75**:1179.
2. Moore, K. L. *et al.* (1992) J. Cell Biol. **118**:445.
3. Laszik, Z. *et al.* (1996) Blood **88**:3010.
4. Gamble, J.R. *et al.* (1990) Science **249**:414.
5. McEvers, R.P. *et al.* (1989) J. Clin. Invest. **84**:92.

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.