Monoclonal
Anti-human E-Selectin-Fluorescein (CD62E)

Catalog Number: BBA21
Lot Number: LAB04
100 Tests

Reagents Provided

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

Clone #: BBIG-E5 (10C10)

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

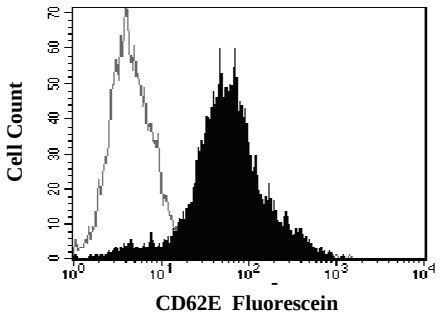
Designed to quantitatively determine the percentage of cells bearing E-Selectin (CD62E) within a population and qualitatively determine the density on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody which binds to the cells expressing CD62E. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells with the CD62E structure are fluorescently stained, with the intensity of staining directly proportional to the density of CD62E. Cell surface expression of CD62E is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated mouse anti-human E-Selectin/CD62E: Use as is; no preparation necessary.



Human umbilical cord endothelial cells cultured for 4 hours in the presence of 10 ng/mL of rhTNF-α were stained with Fluorescein-conjugated anti-human CD62E (Catalog # BBA21, filled histogram) or with isotype control (Catalog # IC002F, open histogram).

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anti-coagulant. 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 x 10^6 cells/mL and 25 μL of cells (1 x 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 x 10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of fluorescein-conjugated anti-CD62E reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-L-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.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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

Background Information

E-Selectin (Endothelial Leukocyte Adhesion Molecule-1, ELAM-1, CD62E) is a 115 kDa type-I membrane glycoprotein expressed only on endothelial cells and only after activation by inflammatory cytokines such as IL-1β and TNF-α or endotoxin (1 - 4). Expression is transitory reaching a maximum within 6 hours of stimulation and then declining with the generation of a soluble form of E-Selectin (1 - 5). Expression of E-Selectin on cell surfaces facilitates the rolling attachment of leukocytes to the endothelium which is an important step in the extravasation of leukocytes at sites of inflammation (1 - 6). E-Selectin is thought to play a prominent role in inflammatory precesses of the skin (4).

References

1. Bevilacqua, M.P. and R.M. Nelson (1993) J. Clin. Invest. **91**:379.
2. McEver, R.P. (1994) Curr. Opin. Immunol. **6**:75.
3. Tedder, T.F. *et al*. (1995) FASEB J. **9**:866.
4. Kansas, G.S. (1996) Blood **88**:3259.
5. Wyble, C.W. *et al*. (1997) J. Surg. Res. **73**:107.
6. Kunkel, E.J. and K. Levy (1996) Circ. Res. **79**:1196.

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.