Monoclonal Anti-human E-Selectin (CD62E)/P-Selectin (CD62P)-Fluorescein

Catalog Number: FAB6169F
Lot Number: ABLQ01
100 Tests

Reagents Provided

Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human E-Selectin/P-Selectin: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: BBIG-E6 (13D5)

Isotype: mouse IgG_1

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

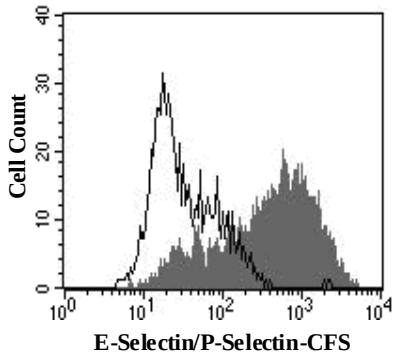
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

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

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with activated HUVECs (human umbilical vein endothelial cells). The IgG fraction of the ascites fluid was purified by Protein A affinity chromatography.The purified antibody was then conjugated to CFS fluorochrome. Cell surface expression of E-Selectin/P-Selectin is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515 - 545 nm.



TNF-α-stimulated HUVEC cells were stained with CFS-conjugated anti-human E-Selectin/P-Selectin (Catalog # FAB6169F, filled histogram) or isotype control (Catalog # IC002F, open histogram).

Background Information

E-Selectin, also known as Endothelial Leukocyte Adhesion Molecule 1 (ELAM-1), and Leukocyte Endothelial Cell Adhesion Molecule 2 (LECAM-2), has been designated CD62E. It is a cell surface glycoprotein expressed by activated endothelium and it mediates the adhesion of blood neutrophils. E-Selectin and P-Selectin are related (44% amino acid sequence identity). P-Selectin occurs on activated platelets and endothelial cells.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using HUVEC cells stimulated with TNF-α.

1. Cells may be Fc-blocked 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. After blocking, 10 μL of conjugated antibody was added to 1 - 2.5 x 10^5 cells and incubated for 30 minutes at room temperature.
3. Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Human Lyse Buffer (Catalog # FC002).
4. The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with CFS-labeled mouse IgG_1 antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

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.

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