Monoclonal
Anti-human PSGL-1/CD162-Phycoerythrin

Catalog Number: FAB9961P
Lot Number: ACDX01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human PSGL-1/CD162: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 688101

Isotype: mouse IgG_{2A}

Reagents Not Provided

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

Storage

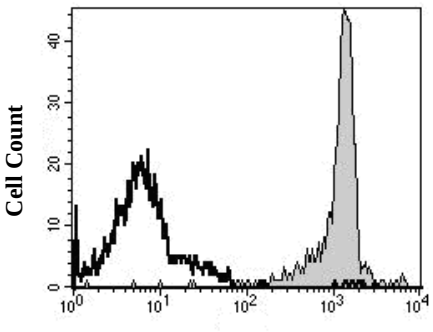
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing PSGL-1/CD162 within a population and qualitatively determine the density of PSGL-1/CD162 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 purified, CHO-S-derived, recombinant human PSGL-1 (rhPSGL-1; aa 42 - 295; Accession # NP_002997) extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of PSGL-1/CD162 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.



Human peripheral blood monocytes were stained with PE-conjugated anti-human PSGL-1/CD162 (Catalog # FAB9961P, filled histogram) or PE-conjugated isotype control (Catalog # IC003P, open histogram).

Background Information

Human PSGL-1 (P-Selectin Glycoprotein Ligand-1; also known as CD162), is a 120 kDa mucin-type glycoprotein that plays a key role in leukocyte adhesion.[1, 2] PSGL-1 is found on virtually all leukocytes, including macrophages/DCs.[1] This antibody was selected for its ability to recognize sLe^X-bearing core 2 O-glycan stuctures. It does not recognize sLe^x on an extended core 1 O-glycan. The sLe^X-bearing core 2 O-glycan structure decorates the P-Selectin ligand PSGL-1, and the presence of this glycan structure is required for high affinity P-Selectin binding.[3] This antibody stains human and canine leukocytes, but does not recognize monkey, mouse, rabbit, porcine, feline, or bovine leukocytes.

References

1. Yang, J. *et al*. (1999) Thromb. Haemost. **81**:1.
2. McEver, R.P. & R.D. Cummings (1997) J. Clin. Invest. **100**:485.
3. Walcheck, B. *et al*. (2002) Blood **99**:4063.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using human peripheral blood monocytes.

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 up to 1 x 10^6 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 PE-labeled mouse IgG_{2A} 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.

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