

Monoclonal Anti-human PSGL-1-Fluorescein

Catalog Number: FAB996F

Lot Number: ABDH01

100 Tests

Reagents Provided

Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human PSGL-1: Supplied as 50 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: CHO131

Isotype: mouse IgM

Reagents Not Provided

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

Storage

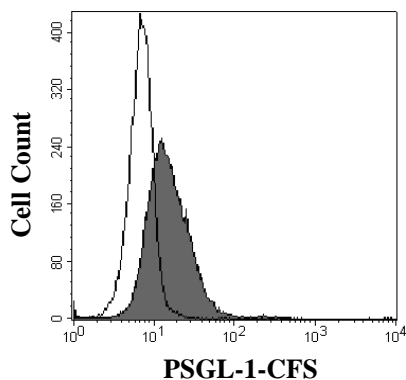
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing PSGL-1 within a population and qualitatively determine the density of PSGL-1 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 CHO cells expressing transfected P-selectin glycoprotein ligand-1 (PSGL-1), α1,3-fucosyl-transferase VII, and core 2 β1,6-N-acetylglucosaminyl-transferase1. The IgM fraction of the tissue culture supernatant was purified by ion exchange chromatography. The purified antibody was then conjugated to CFS fluorochrome. Cell surface expression of PSGL-1 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.



Whole blood granulocytes were stained with CFS-conjugated anti-human PSGL-1 (Catalog # FAB996F, filled histogram) or isotype control (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 and macrophages/DCs.¹ This antibody was selected for its ability to recognize sLe^x-bearing core 2 O-glycan structures. 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

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

Flow Cytometry Validation

This antibody has been tested for flow cytometry using whole blood granulocytes.

- Cells may be Fc-blocked with 1 µg of human IgG/10⁵ cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10 µL of conjugated antibody was added to 1 - 2.5 x 10⁵ cells and incubated for 30 minutes at room temperature.
- 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).
- 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 IgM 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.