

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human PSGL-1/CD162. Recognizes 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 (1). This antibody stains human and canine leukocytes but does not recognize monkey, mouse, rabbit, porcine, feline or bovine leukocytes.
Source	Monoclonal Mouse IgM Clone # CHO131
Purification	IgM-specific Affinity-purified from hybridoma culture supernatant
Immunogen	CHO Chinese hamster ovary cell line transfected with human PSGL-1/CD162
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Flow Cytometry	2.5 µg/10 ⁶ cells	Human whole blood monocytes and granulocytes
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Adhesion Blockade	The adhesion of U937 human histiocytic lymphoma cells (5 x 10 ⁴ cells/well, 30 minute preincubation with the antibody) to immobilized Recombinant Human P-Selectin/CD62P (Catalog # ADP3, 10 µg/mL, 100 µL/well) was inhibited by 50 µg/mL of the antibody.	

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Human PSGL-1 (P-Selectin Glycoprotein Ligand-1; also CD162), is a 120 kDa mucin-type glycoprotein that plays a key role in leukocyte adhesion (1-3). It is synthesized as a 412 amino acid (aa) preproprecursor that contains a 17 aa signal sequence, a 24 aa propeptide, a 279 aa extracellular domain (ECD), a 21 aa transmembrane segment and a 71 aa cytoplasmic region (4, 5). Following cleavage of the pre- and prosegments, it is expressed as a 240 kDa disulfide-linked homodimer. The extreme N-terminus (aa 1-16 of the mature molecule) contains one threonine (aa 16) and three tyrosines (aa 5, 7, and 10) that are involved in ligand binding. The Thr residue allows for O-linked glycosylation in the form of a core-2 structure (GalNAc-Gal) linked in a β1,6 bond to a sialylated Lewis X motif (GlcNAc linked to both Fuc and Gal with a terminal sialic acid residue) (1, 2, 5, 6, 7). The three tyrosine residues allow for sulfation (8, 9). When binding to P-selectin, Tyr sulfation and glycosylation are essential. Tyr7 provides the most efficient sulfate moiety, while Fuc and sialic acid are essentially mandatory (7). When binding to E-selectin, only carbohydrate is needed, while both carbohydrate and Tyr10 are used for L-selectin binding (6, 8). There are 16 decameric aa repeats in the ECD of the longform of PSGL-1. This form is referred to as the A allele, and represents 65 - 80% of the population. Alleles B and C show deletions of decameric repeats #2 (aa 132-141) plus #9 and 10 (aa 222-241), respectively. Shorter forms may show weaker binding to P-selectin (9, 10). Soluble forms of PSGL-1 are also known. Neutrophil elastase will cleave somewhere within repeats #5-9, while cathepsin G cleaves after Tyr7 (11). The loss of Tyr5 and 7 should impact binding affinity. PSGL-1 is found on virtually all leukocytes and macrophages/DC's (1). Although there is similarity in the organization of the ECD between species, there is little aa identity. Human PSGL-1 ECD shares 51%, 52% and 43% aa sequence identity with equine, canine and mouse ECD, respectively.

References:

1. Yang, J. *et al.* (1999) *Thromb. Haemost.* **81**:1.
2. Cummings, R.D. (1999) *Braz. J. Med. Biol. Res.* **32**:519.
3. McEver, R.P. and R.D. Cummings (1997) *J. Clin. Invest.* **100**:485.
4. Sako, D. *et al.* (1993) *Cell* **75**:1179.
5. Veldman, G.M. *et al.* (1995) *J. Biol. Chem.* **270**:16470.
6. Bernimoulin, M.P. *et al.* (2003) *J. Biol. Chem.* **278**:37.
7. Leppanen, A. *et al.* (2000) *J. Biol. Chem.* **275**:39569.
8. Sako, D. *et al.* (1995) *Cell* **83**:323.
9. Afshar-Kharghan, V. *et al.* (2001) *Blood* **97**:3306.
10. Lozano, M.L. *et al.* (2001) *Br. J. Haematol.* **115**:969.
11. Gardiner, E.E. *et al.* (2001) *Blood* **98**:1440.