Human PSGL-1/CD162 Antibody

Monoclonal Mouse IgG_{2B} Clone # 688124

Catalog Number: MAB9962

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human PSGL-1/CD162 in direct ELISAs.
Source	Monoclonal Mouse IgG_{2B} Clone # 688124
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Chinese hamster ovary cell line CHO-derived recombinant human PSGL-1/CD162 Gln42-Gly295 Accession # Q14242
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.

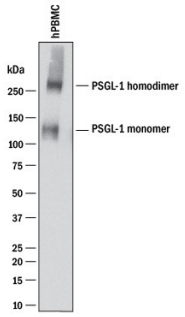
APPLICATIONS

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
Western Blot	2.0 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunocytochemistry	5-25 µg/mL	See Below
Immunohistochemistry	5-25 µg/mL	See Below
ELISA	This antibody functions as an ELISA capture antibody when paired with Sheep Anti-Human PSGL-1/CD162 Antigen Affinity-purified Polyclonal Antibody(Catalog # AF3345). *This product is intended for assay development on various assay platforms requiring antibody pairs. We recommend the Human PSGL-1/CD162 DuoSet ELISA (Catalog # DY3345-05) for convenient development of a sandwich ELISA.*	

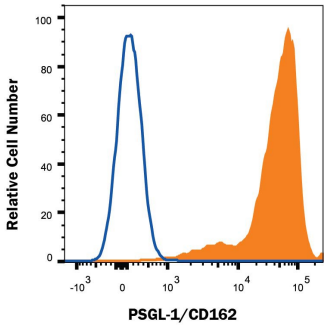
DATA

Western Blot



Detection of Human PSGL-1/CD162 by Western Blot. Western blot shows lysates of human peripheral blood mononuclear cells (PBMCs). PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human PSGL-1/CD162 Monoclonal Antibody (Catalog # MAB9962) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # Catalog # HAF018). A specific band was detected for PSGL-1/CD162 monomer at approximately 110-120 and PSGL-1/CD162 homodimer at approximately 250-260 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



Detection of PSGL-1/CD162 in Human Peripheral Blood Lymphocytes by Flow Cytometry. Human peripheral blood lymphocytes were stained with Mouse Anti-Human PSGL-1/CD162 Monoclonal Antibody (Catalog # MAB9962, filled histogram) or isotype control antibody (Catalog # Catalog # MAB0041, open histogram) followed by anti-Mouse IgG PE-conjugates secondary antibody (Catalog # Catalog # F0102B). View our protocol for Staining Membrane-associated Proteins.

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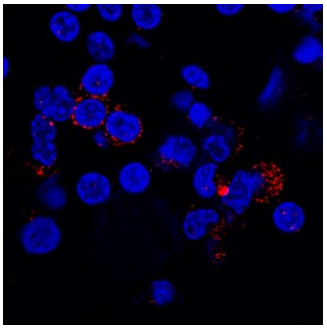
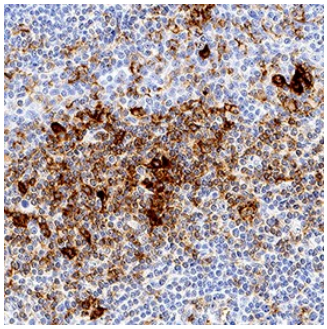
Immunocytochemistry



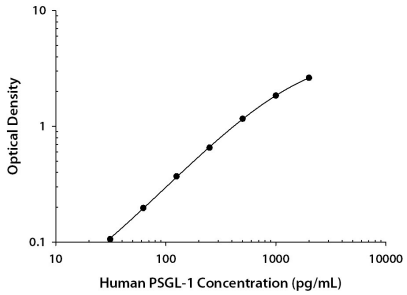
PSGL-1/CD162 in Human PBMCs. PSGL-1/CD162 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using Mouse Anti-Human PSGL-1/CD162 Monoclonal Antibody (Catalog # MAB9962) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Immunohistochemistry



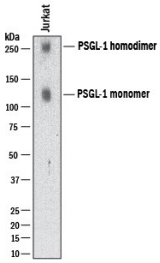
PSGL-1/CD162 in Human Tonsil. PSGL-1/CD162 was detected in immersion fixed paraffin-embedded sections of human tonsil using Mouse Anti-Human PSGL-1/CD162 Monoclonal Antibody (Catalog # MAB9962) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # Catalog # VC001). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

ELISA



Human PSGL-1/CD162 ELISA Standard Curve. Recombinant Human PSGL-1/CD162 protein was serially diluted 2-fold and captured by Mouse Anti-Human PSGL-1/CD162 Monoclonal Antibody (Catalog # MAB9962) coated on a Clear Polystyrene Microplate (Catalog # Catalog # DY990). Sheep Anti-Human PSGL-1/CD162 Antigen Affinity-purified Polyclonal Antibody(Catalog # Catalog # AF3345) was biotinylated and incubated with the protein captured on the plate. Detection of the standard curve was achieved by incubating Streptavidin-HRP (Catalog # Catalog # DY998) followed by Substrate Solution (Catalog # Catalog # DY999) and stopping the enzymatic reaction with Stop Solution (Catalog # Catalog # DY994).

Western Blot



Detection of Human PSGL-1/CD162 by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line and Ramos human Burkitt's lymphoma cell line. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human PSGL-1/CD162 Monoclonal Antibody (Catalog # MAB9962) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). Specific bands were detected for PSGL-1/CD162 at approximately 120, 250 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

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.** • 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.

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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:

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