

## DESCRIPTION

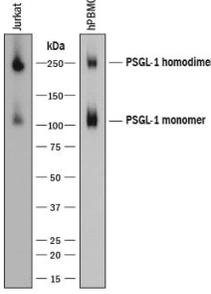
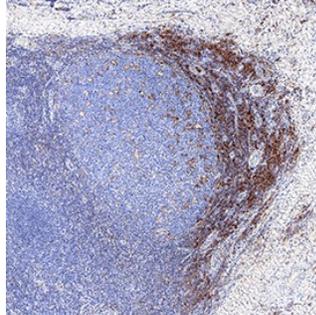
<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human PSGL-1/CD162 in direct ELISAs and Western blots.
<b>Source</b>	Polyclonal Sheep IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	Chinese hamster ovary cell line CHO-derived recombinant human PSGL-1/CD162 Thr44-Val295 Accession # NP_002997
<b>Formulation</b>	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
<b>Western Blot</b>	0.2 µg/mL	See Below
<b>Immunohistochemistry</b>	3-15 µg/mL	See Below

## DATA

<p><b>Western Blot</b></p> 	<p><b>Detection of Human PSGL-1/CD162 by Western Blot.</b> Western blot shows lysates of Jurkat human acute T cell leukemia cell line and human peripheral blood mononuclear cells (PBMCs). PVDF membrane was probed with 0.2 µg/mL of Sheep Anti-Human PSGL-1/CD162 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3345) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). Specific bands were detected for PSGL-1/CD162 homodimer at approximately 250 kDa and PSGL-1/CD162 monomer at approximately 110 kDa (as indicated). This experiment was conducted under reducing conditions and using <a href="#">Immunoblot Buffer Group 8</a>.</p>	<p><b>Immunohistochemistry</b></p>  <p><b>PSGL-1/CD162 in Human Tonsil.</b> PSGL-1/CD162 was detected in immersion fixed paraffin-embedded sections of human tonsil using Sheep Anti-Human PSGL-1/CD162 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3345) at 3 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell &amp; Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. View our protocol for <a href="#">Chromogenic IHC Staining of Paraffin-embedded Tissue Sections</a>.</p>
---	--	---

## PREPARATION AND STORAGE

<b>Reconstitution</b>	Sterile PBS to a final concentration of 0.2 mg/mL.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

**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 (#16) and three tyrosines (#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  $\beta$ 1,6 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 is 51%, 52% and 43% aa identical to 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.