**Human PSGL-1/CD162 Antibody**

**Antigen Affinity-purified Polyclonal Sheep IgG**

**Catalog Number:** AF3345

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**DESCRIPTION**

**Species Reactivity:** Human

**Specificity:** Detects human PSGL-1/CD162 in direct ELISAs and Western blots.

**Source:** Polyclonal Sheep IgG

**Purification:** Antigen Affinity-purified

**Immunogen:** Chinese hamster ovary cell line CHO-derived recombinant human PSGL-1/CD162 Thr44-Val295

**Accession #:** NP_002997

**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.*

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**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**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Western Blot</th>
<th>Immunohistochemistry</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2 µg/mL</td>
<td>3-15 µg/mL</td>
<td>This antibody functions as an ELISA detection antibody when paired with Mouse Anti-Human PSGL-1/CD162 Monoclonal Antibody (Catalog # MAB9962). 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.</td>
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**DATA**

**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 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 Immunoblot Buffer Group 8.

**Immunohistochemistry**

PSGL-1/CD162 in Human Tonsil. 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 & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

**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 # DY990). Sheep Anti-Human PSGL-1/CD162 Antigen Affinity-purified Polyclonal Antibody (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 # DY988) followed by Substrate Solution (Catalog # DY996) and stopping the enzymatic reaction with Stop Solution (Catalog # DY994).

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PREPARATION AND STORAGE

**Reconstitution**
Sterile PBS to a final concentration of 0.2 mg/mL.

**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.

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 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 is 51%, 52% and 43% aa identical to equine, canine and mouse ECD, respectively.

References: