

## DESCRIPTION

<b>Species Reactivity</b>	Human/Mouse/Rat
<b>Specificity</b>	Detects mouse CD31/PECAM-1 in direct ELISAs and Western blots. In direct ELISAs and Western blots, approximately 10% cross-reactivity with recombinant human CD31 and recombinant porcine CD31 is observed. Detects mouse CD31 and rat CD31 in flow cytometry.
<b>Source</b>	Polyclonal Goat IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived recombinant mouse CD31/PECAM-1 Glu18-Lys590 Accession # Q08481
<b>Endotoxin Level</b>	<0.10 EU per 1 µg of the antibody by the LAL method.
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose.

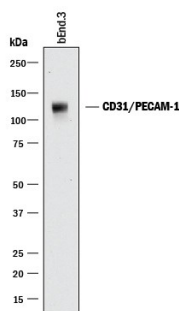
## APPLICATIONS

**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>	0.5 µg/mL	See Below
<b>Flow Cytometry</b>	0.25 µg/10 <sup>6</sup> cells	See Below
<b>Immunocytochemistry</b>	5-15 µg/mL	See Below
<b>Immunohistochemistry</b>	3-15 µg/mL	See Below
<b>CyTOF-ready</b>	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

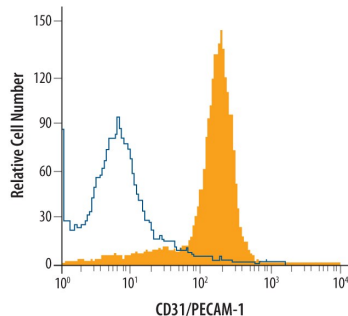
## DATA

### Western Blot



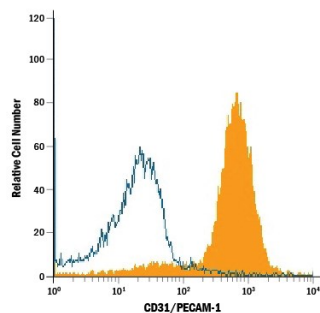
**Detection of Mouse CD31/PECAM-1 by Western Blot.** Western blot shows lysates of bEnd.3 mouse endothelioma cell line. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human/Mouse/Rat CD31/PECAM-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3628) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for CD31/PECAM-1 at approximately 130 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

### Flow Cytometry



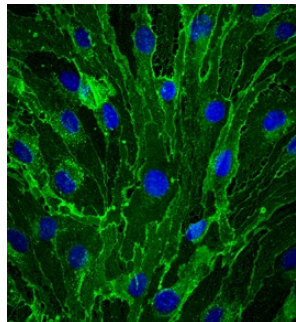
**Detection of CD31/PECAM-1 in Mouse Splenocytes by Flow Cytometry.** Mouse splenocytes were stained with Goat Anti-Human/Mouse/Rat CD31/PECAM-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3628, filled histogram) or control antibody (Catalog # AB-108-C, open histogram), followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108).

### Flow Cytometry



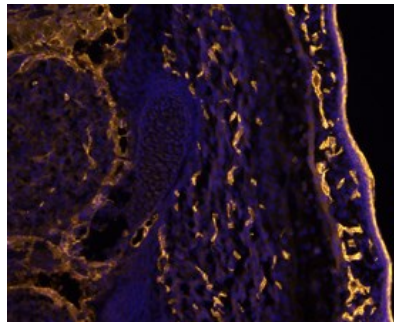
**Detection of CD31/PECAM-1 in Rat Splenocytes by Flow Cytometry.** Rat splenocytes were stained with Goat Anti-Human/Mouse/Rat CD31/PECAM-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3628, filled histogram) or isotype control antibody (Catalog # AB-108-C, open histogram), followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108).

### Immunocytochemistry



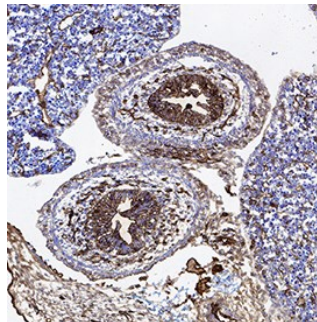
**CD31/PECAM-1 in bEnd.3 Mouse Cell Line.** CD31/PECAM-1 was detected in immersion fixed bEnd.3 mouse endothelioma cell line using Goat Anti-Human/Mouse/Rat CD31/PECAM-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3628) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 493-conjugated Anti-Goat IgG Secondary Antibody (green; Catalog # NL003) and counterstained with DAPI (blue). Specific staining was localized to cell membrane. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

### Immunohistochemistry



**CD31/PECAM-1 in Mouse Embryo.** CD31/PECAM-1 was detected in immersion fixed frozen sections of mouse embryo (E13.5) using Goat Anti-Human/Mouse/Rat CD31/PECAM-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3628) at 10 µg/mL overnight at 4 °C. Tissue was stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (yellow; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to developing endothelium. View our protocol for [Fluorescent IHC Staining of Frozen Tissue Sections](#).

### Immunohistochemistry



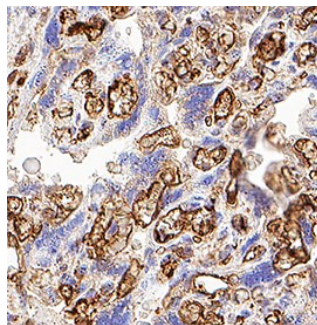
**CD31/PECAM-1 in Mouse Embryo.** CD31/PECAM-1 was detected in immersion fixed frozen sections of mouse embryo (14 d.p.c.) using Goat Anti-Human/Mouse/Rat CD31/PECAM-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3628) at 10 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to developing guts. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

### Immunohistochemistry



**CD31/PECAM-1 in Rat Heart.** CD31/PECAM-1 was detected in immersion fixed paraffin-embedded sections of rat heart using Goat Anti-Human/Mouse/Rat CD31/PECAM-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3628) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to endothelial cells in vasculature.

### Immunohistochemistry



**Detection of CD31/PECAM-1 in Human Placenta.** CD31/PECAM-1 was detected in immersion fixed paraffin-embedded sections of Human Placenta using Goat Anti-Human/Mouse/Rat CD31/PECAM-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3628) at 15 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to endothelial cells in chorionic villi. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

### PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <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

PECAM-1 (Platelet-Endothelial Cell Adhesion Molecule-1), also known as CD31, is a 130 kDa type I transmembrane glycoprotein adhesion molecule in the immunoglobulin superfamily (1, 2). Expression is restricted to cells involved in circulation, especially endothelial cells, platelets, monocytes, neutrophils and lymphocyte subsets. PECAM-1 is concentrated at cell-cell junctions and is required for Transendothelial Migration (TEM) (1-3). The Extracellular Domain (ECD) of PECAM-1 has ten potential N-linked glycosylation sites and six C2-type Ig-like domains, the first of which is critical for adhesion and extravasation (3, 4). The cytoplasmic domain contains Immunoregulatory Tyrosine-based Inhibitory and Switch Motifs (ITIM, ITSM) that mediate both inhibition and activation via phosphotyrosine-mediated engagement of SH2-containing signaling molecules (1, 5). Metalloproteinase-mediated ectodomain shedding occurs during apoptosis (6) but increased serum PECAM-1 ectodomain in HIV and active multiple sclerosis occurs independent of apoptosis (7, 8). In humans, expression of six isoforms with exon deletions in the cytoplasmic domain is tissue- and stage-specific, but full-length PECAM-1 is predominant. A form lacking the ITSM predominates in mouse (9). Mouse PECAM-1 ECD shows 77%, 63%, 63%, 63%, and 61% amino acid (aa) identity with rat, human, canine, porcine, and bovine PECAM-1, respectively. PECAM-1 participates with other adhesion molecules in some functions, but is the critical molecule for TEM. Homotypic PECAM-1 adhesion in trans, combined with cycling of PECAM-1 to and from surface-connected endothelial cell vesicles, leads leukocytes across endothelial tight junctions (3, 10). Homotypic adhesion and signaling functions also strongly suppress mitochondria-dependent apoptosis (11). In platelets, PECAM-1 is necessary for limiting thrombus formation (12) and promoting integrin-mediated clot retraction and platelet spreading (13), but mechanisms for these phenomena are unclear. PECAM<sup>-/-</sup> mice are deficient in chemokine-mediated chemotaxis (14).

## References:

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