Human/Mouse JAM-B/VE-JAM Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1074

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human JAM-B/VE-JAM in direct ELISAs. Detects human and mouse JAM-B/VE-JAM in Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human JAM-B/VE-JAM Phe29-Asn236 Accession # P57087
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.

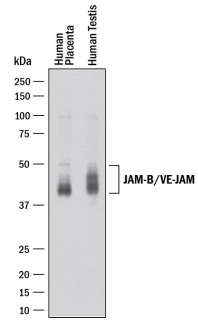
APPLICATIONS

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
Western Blot	1-2 µg/mL	See Below
Immunohistochemistry	3-25 µg/mL	See Below
Simple Western	20 µg/mL	See Below
Neutralization	Measured by its ability to neutralize JAM-B/VE-JAM-mediated adhesion of the J45.01 human acute lymphoblastic leukemia T lymphocyte cell line. Fong, S. *et al.* (2002) J. Immunol. **168**:1618. The Neutralization Dose (ND_{50}) is typically 0.2-0.8 µg/mL in the presence of 0.2 µg/mL Recombinant Human JAM-B/VE-JAM Fc Chimera.	

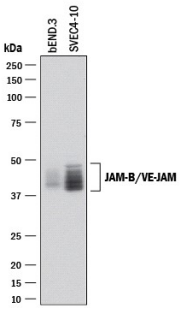
DATA

Western Blot



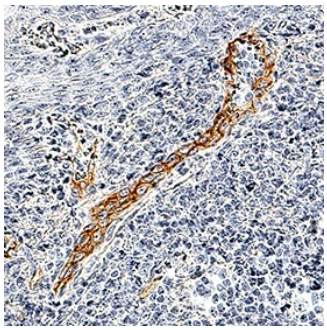
Detection of Human JAM-B/VE-JAM by Western Blot. Western blot shows lysates of human placenta tissue and human testis tissue. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human/Mouse JAM-B/VE-JAM Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1074) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for JAM-B/VE-JAM at approximately 43-50 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Western Blot



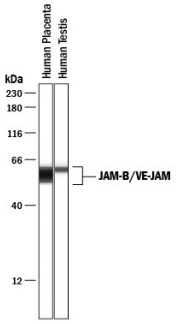
Detection of Mouse JAM-B/VE-JAM by Western Blot. Western blot shows lysates of bEnd.3 mouse endothelioma cell line and SVEC4-10 mouse vascular endothelial cell line. PVDF membrane was probed with 2 µg/mL of Goat Anti-Human/Mouse JAM-B/VE-JAM Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1074) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for JAM-B/VE-JAM at approximately 40-48 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



JAM-B/VE-JAM in Human Tonsil. JAM-B/VE-JAM was detected in immersion fixed paraffin-embedded sections of human tonsil using Goat Anti-Human/Mouse JAM-B/VE-JAM Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1074) at 3 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 plasma membrane in endothelial cells. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Simple Western



Detection of Human JAM-B/VE-JAM by Simple Western™. Simple Western lane view shows lysates of human placenta tissue and human testis tissue, loaded at 0.2 mg/mL. A specific band was detected for JAM-B/VE-JAM at approximately 58-61 kDa (as indicated) using 20 µg/mL of Goat Anti-Human JAM-B/VE-JAM Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1074) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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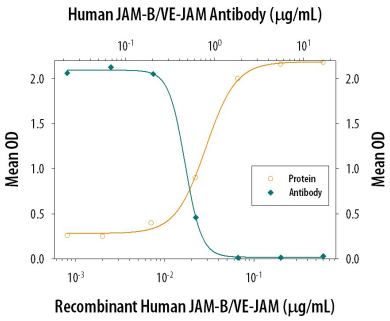
Neutralization



Cell Adhesion Mediated by JAM-B/VE-JAM and Neutralization by Human JAM-B/VE-JAM Antibody. Recombinant Human JAM-B/VE-JAM Fc Chimera (Catalog # 1074-VJ), immobilized onto a microplate previously coated with Goat Anti-Human IgG Fc (Catalog # G-102-C), supports the adhesion of the J45.01 human acute lymphoblastic leukemia T lymphocyte cell line in a dose-dependent manner (orange line), as measured by endogenous cellular lysosomal acid phosphatase activity. Adhesion elicited by Recombinant Human JAM-B/VE-JAM Fc Chimera (0.2 µg/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human/Mouse JAM-B/VE-JAM Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1074). The ND_{50} is typically 0.2-0.8 µg/mL.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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.

BACKGROUND

The family of juctional adhesion molecules (JAM), comprising at least three members, are type I transmembrane receptors belonging to the immunoglobulin (Ig) superfamily (1, 2). These proteins are localized in the tight junctions between endothelial cells or epithelial cells. Some family members are also found on blood leukocytes and platelets. JAM-B, alternatively named vascular endothelial JAM (VE-JAM), is expressed prominently on high endothelial venules of lymphoid organs where it is localized to the intercellular boundaries of high endothelial cells. It is also expressed on the endothelium of a variety of non-lymphoid organs, especially the heart and placenta (3, 5). Human JAM-B cDNA predicts a 298 amino acid residue (aa) precursor protein with a putative 28 aa signal peptide, a 209 aa extracellular region containing two Ig domains, a 23 aa transmembrane domain and a 38 aa cytoplasmic domain containing a PDZ-binding motif and a PKC phosphorylation site. Human JAM-B shares approximately 79% aa sequence homology with its mouse homologue. It also shares approximately 35% aa sequence homology with human JAM-A or JAM-C. JAM-B exhibits homotypic interactions, as well as heterotypic interactions with JAM-C, but not JAM-A (4, 5, 7). It is also a ligand for the integrin $\alpha_4\beta_1$. However, the JAM-B/$\alpha_4\beta_1$ interaction is facilitated only after prior adhesion of JAM-B to JAM-C (6). Through its heterotypic interactions with JAM-C, JAM-B is an adhesive ligand for T, NK, and dendritic cells, and may play a role in regulating leukocyte transmigration (5).

The nomenclature used for the JAM family proteins is confusing. VE-JAM has been referred in the literature variously as JAM-B or JAM-3. Until further clarification, R&D Systems has adopted the nomenclature where both mouse and human VE-JAM are referred to as JAM-B.

References:

1. Chavakis, T. *et al.* (2003) Thromb. Haemost. **89**:13.
2. Aurand-Lions, M. *et al.* (2001) Blood **98**:3699.
3. Palmeri, A. *et al.* (2000) J. Biol. Chem. **275**:19139.
4. Cunnigham, S. *et al.* (2000) J. Biol. Chem. **275**:34750.
5. Liang, T. *et al.* (2002) J. Immunol. **168**:1618.
6. Cunningham, A. *et al.* (2002) J Biol. Chem. **277**:27589.
7. Arrate, M. *et al.* (2001) J. Biol. Chem. **276**:45826.

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