Human JAM-C Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1189

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human JAM-C in direct ELISAs and Western blots. In direct ELISAs and Western blots, approximately 75% cross-reactivity with recombinant mouse (rm) JAM-C is observed and less than 1% cross-reactivity with recombinant human JAM-1 and rmJAM-1 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human JAM-C Val32-Asn241 (Ala149Pro) Accession # Q9BX67
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.

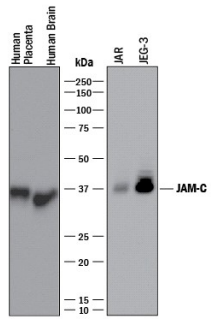
APPLICATIONS

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 µg/mL	See Below
Flow Cytometry	2.5 µg/10^6 cells	Human platelets
Simple Western	20 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Neutralization	Measured by its ability to neutralize JAM-C-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 2-8 µg/mL in the presence of 0.2 µg/mL Recombinant Human JAM-B Fc Chimera.	

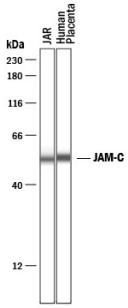
DATA

Western Blot



Detection of Human JAM-C by Western Blot. Western blot shows lysates of human placenta tissue, human brain tissue, JAR human choriocarcinoma cell line, and JEG-3 human epithelial choriocarcinoma cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human JAM-C Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1189) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for JAM-C at approximately 36-38 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Simple Western



Detection of Human JAM-C by Simple Western™. Simple Western lane view shows lysates of JAR human choriocarcinoma cell line and human placenta, loaded at 0.2 mg/mL. A specific band was detected for JAM-C at approximately 53 and 54 kDa (as indicated) using 20 µg/mL of Goat Anti-Human JAM-C Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1189) 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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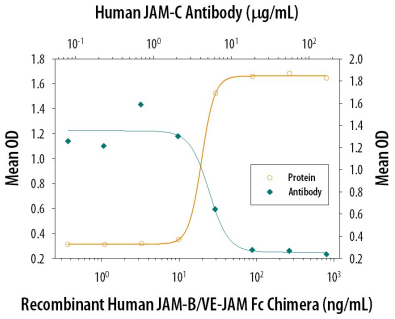
Neutralization



Cell Adhesion Mediated by JAM-C and Neutralization by Human JAM-C 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 Fc Chimera (0.2 µg/mL) is neutralized (green line) by increasing concentrations of Human JAM-C Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1189). The ND_{50} is typically 2-8 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. Human JAM-C cDNA predicts a 310 amino acid (aa) residue precursor protein with a putative 31 aa signal peptide, a 210 aa extracellular region containing two Ig domains, a 23 aa transmembrane domain and a 46 aa cytoplasmic domain containing a PDZ-binding motif and a PKC phosphorylation site (3, 4). Human JAM-C shares 86% aa sequence identity with its mouse homologue. It also shares approximately 36% and 32% aa sequence homology with human JAM-B and JAM-A, respectively (3-5). Human JAM-C shows widespread tissue expression and the highest levels are found in the placenta, brain, kidney and heart. JAM-C is expressed on endothelial cells of high endothelial venules in human tonsil. It is also expressed on platelets, T-cells and NK cells (3-5). Unlike other JAM family members, JAM-C forms only weak homotypic interactions. JAM-C binds to JAM-B to facilitate the interactions between JAM-B and the integrin alpha4beta1 (6). This heterotypic interaction between leukocyte JAM-C and endothelial JAM-B may play a role in regulating leukocyte transmigration (5). On platelets, JAM-C is a counter-receptor for the leukocyte integrin Mac-1(CD11b/CD18) (7). JAM-C has also been identified as a strong candidate gene for hypoplastic left heart syndrome (8).

The nomenclature used for the JAM family proteins is confusing. VE-JAM has been referred to in the literature variously as JAM-B or JAM-C. Until further clarification, R&D Systems has adopted the nomenclature where both mouse and human VE-JAM are referred to as JAM-B. Under this system, JAM-C refers to the protein encoded by the gene localized to human chromosome 11.

References:

1. Chavakis, T. *et al.* (2003) Thromb. Haemost. **89**:13.
2. Aurand-Lions, M. *et al.* (2001) Blood **98**:3699.
3. Arrate, M.P. *et al.* (2001) J. Biol. Chem. **276**:45826.
4. Liang, T. *et al.* (2002) J. Immunol. **168**:1618.
5. Johnson-Leger, C. *et al.* (2002) Blood **100**:25793.
6. Cunningham, A. *et al.* (2002) J Biol. Chem. **277**:27589.
7. Santoso, S. *et al.* (2002) J. Exp. Med. **196**:679.
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