Human/Mouse/Rat/Canine ALCAM/CD166 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1172

DESCRIPTION

Species Reactivity	Human/Mouse/Rat/Canine
Specificity	Detects human, mouse and rat ALCAM/CD166 in Western blots. In direct ELISAs, less than 10% cross-reactivity with rhBCAM, recombinant mouse (rm) OCAM, and rmMAdCAM-1 is observed. Detects canine ALCAM/CD166 in flow cytometry.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse ALCAM/CD166 Trp28-Lys527 Accession # AAC06342
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.

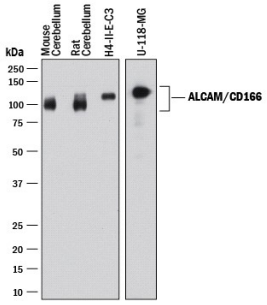
APPLICATIONS

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	0.2 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	12.5 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.	

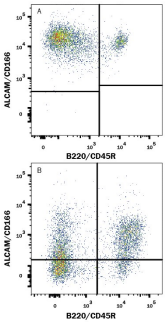
DATA

Western Blot



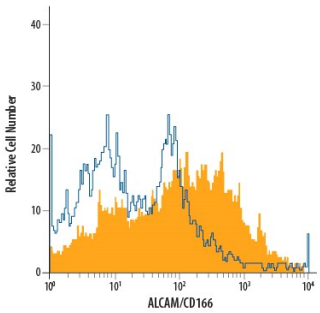
Detection of Human, Mouse, and Rat ALCAM/CD166 by Western Blot. Western blot shows lysates of mouse brain (cerebellumn) tissue, rat brain (cerebellum) tissue, H4-II-E-C3 rat hepatoma cell line, and U-118-MG human glioblastoma/astrocytoma cell line. PVDF membrane was probed with 0.2 µg/mL of Goat Anti-Mouse/Rat/Canine ALCAM/CD166 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1172) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for ALCAM/CD166 at approximately 90-120 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



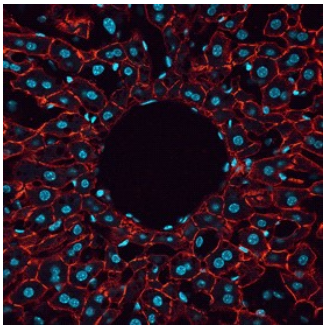
Detection of ALCAM/CD166 in Mouse Splenocytes by Flow Cytometry. Mouse splenocytes either (A) activated or (B) resting were stained with Goat Anti-Mouse/Rat/Canine ALCAM/CD166 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1172) followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107) and Rat Anti-Mouse B220/CD45R APC-conjugated Monoclonal Antibody (Catalog # FAB1217A). Quadrant markers were set based on control antibody staining (Catalog # AB-108-C).

Flow Cytometry



Detection of ALCAM/CD166 in Canine PBMCs by Flow Cytometry. Canine peripheral blood mononuclear cells (PBMCs) treated with PMA and Calcium Ionomycin for 24 hours were stained with Goat Anti-Mouse/Rat/Canine ALCAM/CD166 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1172, filled histogram) or isotype control antibody (Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107).

Immunohistochemistry



ALCAM/CD166 in Mouse Liver. ALCAM/CD166 was detected in perfusion fixed frozen sections of mouse liver using Goat Anti-Mouse/Rat/Canine ALCAM/CD166 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1172) at 1.7 µg/mL overnight at 4 °C. Tissue was stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). View our protocol for Fluorescent IHC Staining of Frozen Tissue Sections.

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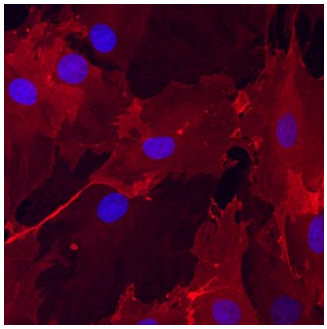
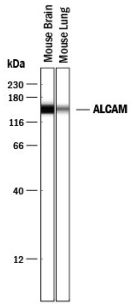
Immunocytochemistry



ALCAM/CD166 in Rat Mesenchymal Stem Cells. ALCAM/CD166 was detected in immersion fixed undifferentiated rat mesenchymal stem cells using Goat Anti-Mouse/Rat/Canine ALCAM/CD166 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1172) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Simple Western



Detection of Mouse ALCAM/CD166 by Simple Western™. Simple Western lane view shows lysates of mouse brain tissue and mouse lung tissue loaded at 0.2 mg/mL. A specific band was detected for ALCAM/CD166 at approximately 149 kDa (as indicated) using 12.5 µg/mL of Goat Anti-Mouse/Rat/Canine ALCAM/CD166 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1172) 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.

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

ALCAM, activated leukocyte cell adhesion molecule, is a type I membrane glycoprotein and a member of the immunoglobulin supergene family. It is also known as CD166, MEMD, SC-1/DM-GRASP/BEN in the chicken, and KG-CAM in the rat. ALCAM is expressed on thymic epithelial cells, activated B and T cells, and monocytes. ALCAM can bind itself homotypically and is also capable of binding CD6, NgCAM, and other, as of yet, unidentified brain proteins. ALCAM/CD6 interaction may be involved in T cell development and T cell regulation. Additionally, ALCAM/CD6 and ALCAM/NgCAM interactions may play roles in the nervous system. ALCAM has also been observed to be upregulated on highly metastasizing melanoma cell lines and may play a role in tumor migration. ALCAM is a 583 amino acid (aa) protein consisting of a 27 aa signal peptide, a 500 aa extracellular domain, a 24 aa transmembrane domain, and a 32 aa cytoplasmic domain. The extracellular domain of ALCAM contains 5 Ig-like domains of which the amino-terminal V1 domain is essential for ligand binding and ALCAM-mediated cell aggregation (1-4). The ECD of mouse ALCAM shares 97.5% and 93.2% aa sequence identity with rat and canine ALCAM ECD, respectively.

References:

1. Bowen, M.A. *et al.* (1995) J. Exp. Med. **181**:2213.
2. Aruffo, A. *et al.* (1997) Immunol. Today **18**:498.
3. Degen, W.G. *et al.* (1998) Am. J. Pathol. **152**:805.
4. Van Kempen, L. *et al.* (2001) J. Biol. Chem. **276**:25783.

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