

## DESCRIPTION

|                           |  |
|---------------------------|--|
| <b>Species Reactivity</b> | Mouse/Rat/Canine   |
| <b>Specificity</b>        | Detects mouse ALCAM in direct ELISAs and Western blots. In direct ELISAs, approximately 40% cross-reactivity with recombinant human (rh) ALCAM is observed, and less than 10% cross-reactivity with rhBCAM, recombinant mouse (rm) OCAM, and rmMAdCAM-1 is observed. |
| <b>Source</b>             | Polyclonal Goat IgG  |
| <b>Purification</b>       | Antigen Affinity-purified  |
| <b>Immunogen</b>          | Mouse myeloma cell line NS0-derived recombinant mouse ALCAM/CD166<br>Trp28-Lys527<br>Accession # AAC06342  |
| <b>Formulation</b>        | Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.<br>*Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.  |

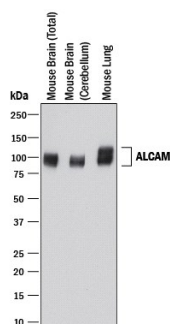
## APPLICATIONS

**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    |
|-----------------------------|--|-----------|
| <b>Western Blot</b>         | 0.25 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> | 5-15 µg/mL   | See Below |
| <b>Simple Western</b>       | 12.5 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. |           |

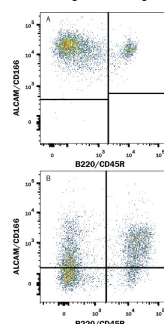
## DATA

### Western Blot



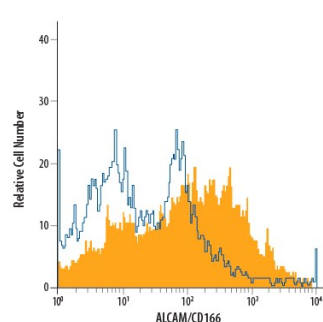
**Detection of Mouse ALCAM/CD166 by Western Blot.** Western blot shows lysates of mouse brain (total) tissue, mouse brain (cerebellum) tissue, and mouse lung tissue. PVDF membrane was probed with 0.25 µg/mL of Goat Anti-Mouse/Rat/Canine ALCAM/CD166 Antigen Affinity-purified Polyclonal Antibody (Catalog # 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



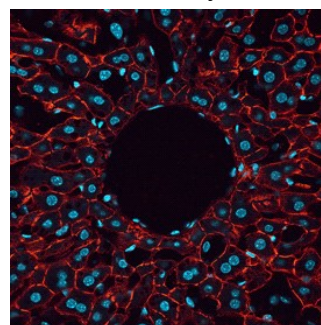
**Detection of ALCAM/CD166 in Mouse Splenocytes by Flow Cytometry.** Mouse splenocytes either (A) activated or (B) resting were stained with Goat Anti-Mouse/Rat/Canine ALCAM/CD166 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1172) followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary 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



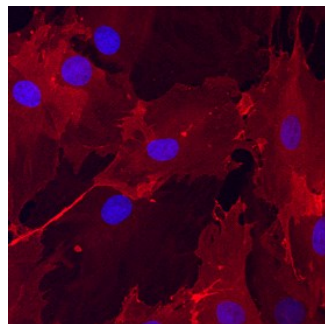
**Detection of ALCAM/CD166 in Canine PBMCs by Flow Cytometry.** Canine peripheral blood mononuclear cells (PBMCs) treated with PMA and Calcium Ionomycin for 24 hours were stained with Goat Anti-Mouse/Rat/Canine ALCAM/CD166 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1172, filled histogram)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

### Immunohistochemistry



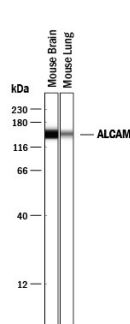
**ALCAM/CD166 in Mouse Liver.** ALCAM/CD166 was detected in perfusion fixed frozen sections of mouse liver using Goat Anti-Mouse/Rat/Canine ALCAM/CD166 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1172) at 1.7 µg/mL overnight at 4 °C. Tissue was stained using the NorthernLights™ 557-conjugated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

## 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

|                                |   |
|--------------------------------|---|
| <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.<br>*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C  |
| <b>Stability &amp; Storage</b> | <p><b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b></p> <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

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.