

Anti-CD146/Mcam Antibody Picoband™

Catalog Number: A01683

About Mcam

In humans, the CD146 protein is encoded by the MCAM gene. It is mapped to 9; 9 A5.1. CD146 (cluster of differentiation 146) also known as the melanoma cell adhesion molecule (MCAM) or cell surface glycoprotein MUC18, is a 113kDa cell adhesion molecule currently used as a marker for endothelial cell lineage. MCAM functions as a receptor for laminin alpha 4, a matrix molecule that is broadly expressed within the vascular wall. Accordingly, MCAM is highly expressed by cells that are components of the blood vessel wall, including vascular endothelial cells, smooth muscle cells and pericytes. Its function is still poorly understood, but evidence points to it being part of the endothelial junction associated with the actin cytoskeleton. A member of the Immunoglobulin superfamily, it consists of five Ig domains, a transmembrane domain, and a cytoplasmic region. It is expressed on chicken embryonic spleen and thymus, activated human T cells, endothelial progenitors such as angioblasts and mesenchymal stem cells, and strongly expressed on blood vessel endothelium and smooth muscle.

Overview

Product Name	Anti-CD146/Mcam Antibody Picoband™
Reactive Species	Mouse, Rat
Description	Boster Bio Anti-CD146/Mcam Antibody Picoband™ catalog # A01683. Tested in ELISA, Flow Cytometry, IF, IHC, WB applications. This antibody reacts with Mouse, Rat.
Application	ELISA, Flow Cytometry, IF, IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	Q8R2Y2

Technical Details

Immunogen	E.coli-derived mouse CD146/Mcam recombinant protein (Position: E27-E621).
Predicted Reactive Species	Human
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG

Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.1-0.25ug/ml, Mouse, Rat</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Mouse, Rat</p> <p>Immunofluorescence, 2ug/ml, Mouse</p> <p>Flow Cytometry, 1-3ug/1x10⁶ cells, Mouse, Rat</p> <p>Direct ELISA, 0.1-0.5ug/ml, Mouse</p>

Anti-CD146/Mcam Antibody Picoband™ (A01683) Images

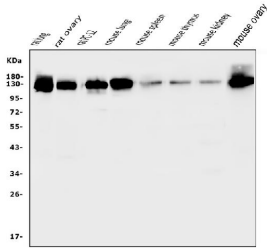


Figure 1. Western blot analysis of MCAM using anti-MCAM antibody (A01683).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: rat lung tissue lysates,

Lane 2: rat ovary tissue lysates,

Lane 3: rat PC-12 whole cell lysates,

Lane 4: mouse lung tissue lysates,

Lane 5: mouse spleen tissue lysates,

Lane 6: mouse thymus tissue lysates,

Lane 7: mouse kidney tissue lysates,

Lane 8: mouse ovary tissue lysates,

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes.

Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-MCAM antigen affinity purified polyclonal antibody (Catalog # A01683) at 0.25 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for MCAM at approximately 120KD. The expected band size for MCAM is at 120KD.

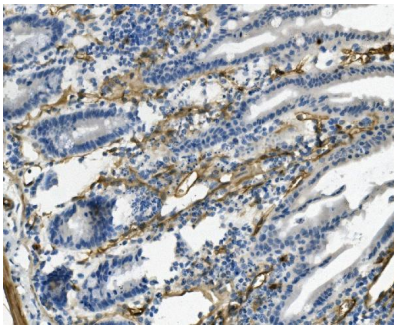


Figure 2. IHC analysis of MCAM using anti-MCAM antibody (A01683).

MCAM was detected in paraffin-embedded section of mouse intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-MCAM Antibody (A01683) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

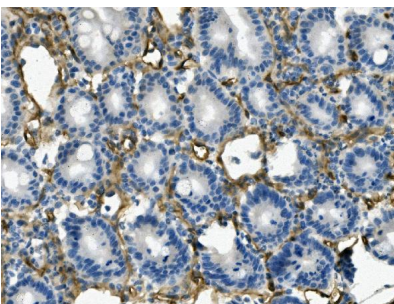


Figure 3. IHC analysis of MCAM using anti-MCAM antibody (A01683).

MCAM was detected in paraffin-embedded section of mouse intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-MCAM Antibody (A01683) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

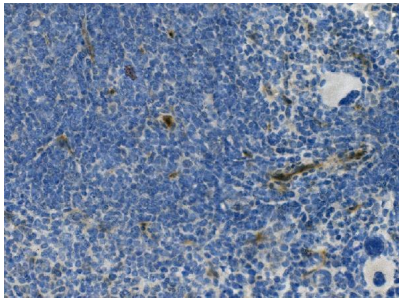


Figure 4. IHC analysis of MCAM using anti-MCAM antibody (A01683).
MCAM was detected in paraffin-embedded section of rat spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-MCAM Antibody (A01683) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

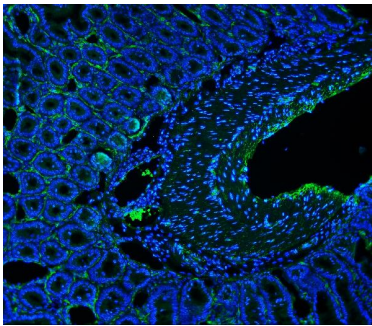


Figure 5. IF analysis of MCAM using anti-MCAM antibody (A01683).
MCAM was detected in paraffin-embedded section of mouse intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/mL rabbit anti-MCAM Antibody (A01683) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

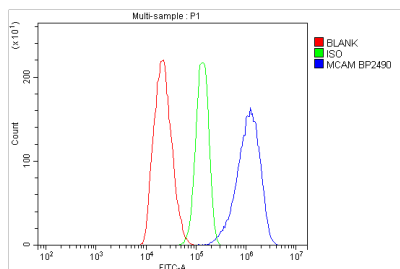


Figure 6. Flow Cytometry analysis of C6 cells using anti-MCAM antibody (A01683).
Overlay histogram showing C6 cells stained with A01683 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-MCAM Antibody (A01683, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

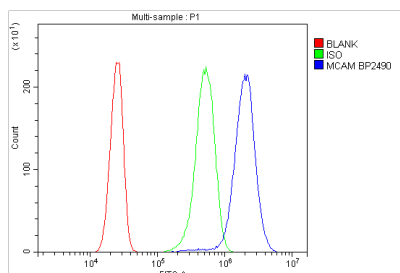


Figure 7. Flow Cytometry analysis of Neuro-2a cells using anti-MCAM antibody (A01683).
Overlay histogram showing Neuro-2a cells stained with A01683 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-MCAM Antibody (A01683, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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