10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

Anti-CD146/MCAM Antibody Picoband™ (monoclonal, 4C12)

Catalog # ABO14945

Specification

Anti-CD146/MCAM Antibody Picoband™ (monoclonal, 4C12) - Product Information

Application	**WB, IHC, IHC-F, FC**
Primary Accession	P43121
Host	**Mouse**
Isotype	**Mouse IgG2a**
Reactivity	**Human**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-CD146/MCAM Antibody Picoband™ (monoclonal, 4C12) . Tested in Flow Cytometry, IHC, IHC-F, WB applications. This antibody reacts with Human.

Reconstitution
Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Anti-CD146/MCAM Antibody Picoband™ (monoclonal, 4C12) - Additional Information

Gene ID 4162

Other Names
Cell surface glycoprotein MUC18, Cell surface glycoprotein P1H12, Melanoma cell adhesion molecule, Melanoma-associated antigen A32, Melanoma-associated antigen MUC18, S-endo 1 endothelial-associated antigen, CD146, MCAM, MUC18

Calculated MW
120 kDa KDa

Application Details
Western blot, 0.1-0.5 µg/ml, Human
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml, Human
 Immunohistochemistry (Frozen Section), 0.5-1 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Subcellular Localization
Membrane. Single-pass type I membrane protein.

Tissue Specificity
Detected in endothelial cells in vascular tissue throughout the body. May appear at the surface of neural crest cells during their embryonic migration. Appears to be limited to vascular smooth muscle in normal adult tissues. Associated with tumor progression and the development of metastasis in human malignant melanoma. Expressed most strongly on metastatic lesions and advanced primary tumors and is only rarely detected in benign melanocytic nevi and thin primary melanomas with a low probability of metastasis.

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na₂HPO₄,

0.05mg NaN₃.

Immunogen
E.coli-derived human CD146 recombinant protein (Position: H59-A401). Human CD146 shares 73% amino acid (aa) sequence identity with both mouse and rat CD146.

Purification
Immunogen affinity purified.

Cross Reactivity
No cross-reactivity with other proteins.

Storage	**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.**

Anti-CD146/MCAM Antibody Picoband™ (monoclonal, 4C12) - Protein Information

Name MCAM

Synonyms MUC18

Function
Plays a role in cell adhesion, and in cohesion of the endothelial monolayer at intercellular junctions in vascular tissue. Its expression may allow melanoma cells to interact with cellular elements of the vascular system, thereby enhancing hematogeneous tumor spread. Could be an adhesion molecule active in neural crest cells during embryonic development. Acts as a surface receptor that triggers tyrosine phosphorylation of FYN and PTK2/FAK1, and a transient increase in the intracellular calcium concentration.

Cellular Location
Membrane; Single-pass type I membrane protein.

Tissue Location
Detected in endothelial cells in vascular tissue throughout the body. May appear at the surface of neural crest cells during their embryonic migration. Appears to be limited to vascular smooth muscle in normal adult tissues. Associated with tumor progression and the development of metastasis in human malignant melanoma. Expressed most strongly on metastatic lesions and advanced primary tumors and is only rarely detected in benign melanocytic nevi and thin primary melanomas with a low probability of metastasis

Anti-CD146/MCAM Antibody Picoband™ (monoclonal, 4C12) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety

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- Cell Culture

Anti-CD146/MCAM Antibody Picoband™ (monoclonal, 4C12) - Images

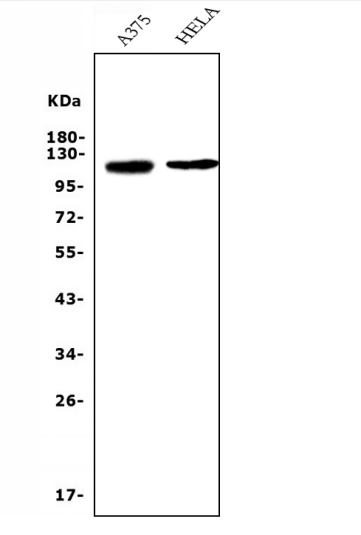

Figure 1. Western blot analysis of CD146/MCAM using anti-CD146/MCAM antibody (M01683-3).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human A375 whole cell lysates;
Lane 2: human Hela whole cell 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 mouse anti-CD146/MCAM antigen affinity purified monoclonal antibody (Catalog # M01683-3) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for CD146/MCAM at approximately 120KD. The expected band size for CD146/MCAM is at 72KD.

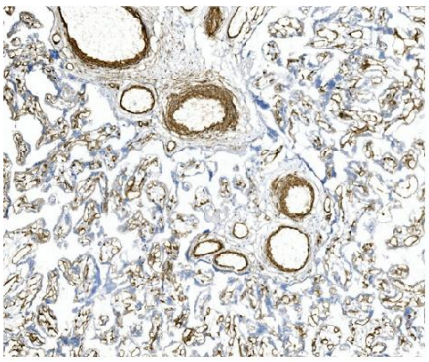

Figure 2. IHC analysis of CD146/MCAM using anti-CD146/MCAM antibody (M01683-3).
CD146/MCAM was detected in paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD146/MCAM Antibody (M01683-3) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue

section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

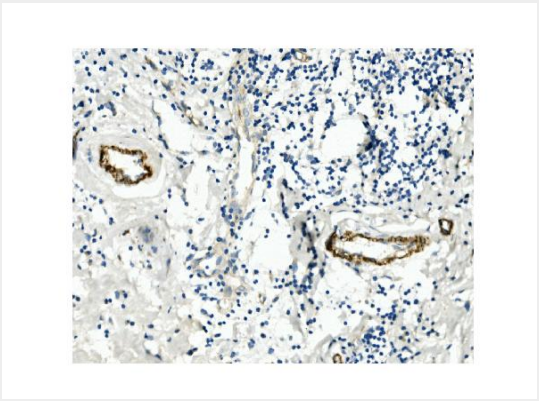

Figure 3. IHC analysis of CD146/MCAM using anti-CD146/MCAM antibody (M01683-3).
CD146/MCAM was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD146/MCAM Antibody (M01683-3) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

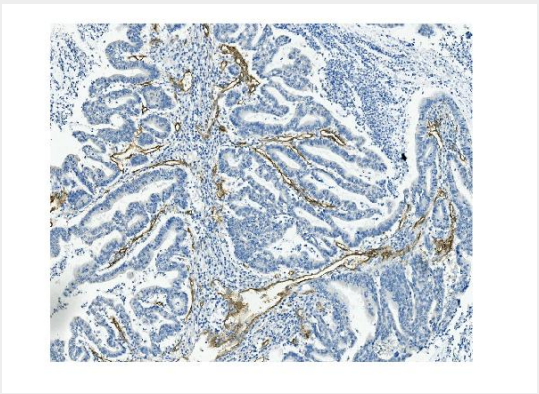

Figure 4. IHC analysis of CD146/MCAM using anti-CD146/MCAM antibody (M01683-3).
CD146/MCAM was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD146/MCAM Antibody (M01683-3) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

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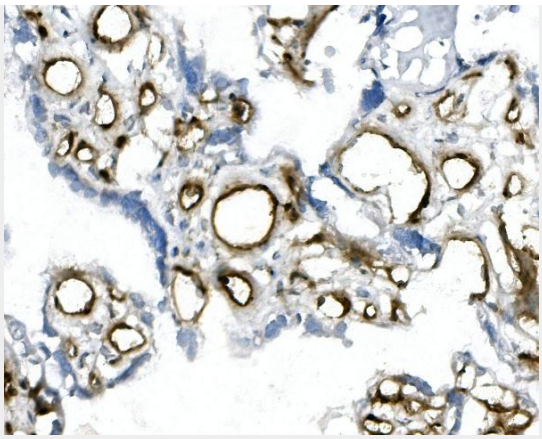

Figure 5. IHC analysis of CD146/MCAM using anti-CD146/MCAM antibody (M01683-3).
CD146/MCAM was detected in frozen section of human placenta tissue. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD146/MCAM Antibody (M01683-3) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

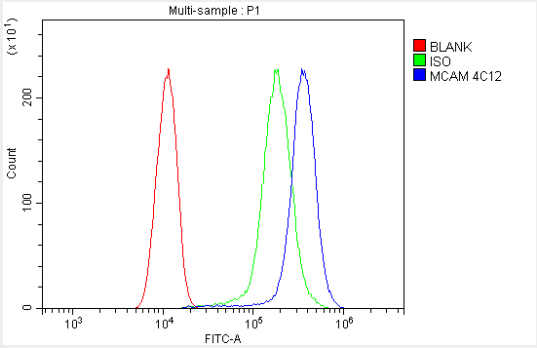

Figure 6. Flow Cytometry analysis of SiHa cells using anti-CD146/MCAM antibody (M01683-3).
Overlay histogram showing SiHa cells stained with M01683-3 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-CD146/MCAM Antibody (M01683-3, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Anti-CD146/MCAM Antibody Picoband™ (monoclonal, 4C12) - Background

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, a member of the immunoglobulin superfamily, is homologous to several cell adhesion molecules and is associated with tumor progression and the development of metastasis in human malignant melanoma. By radiation hybrid analysis, this gene is mapped to chromosome 11q23.3. MCAM has been demonstrated to appear on a small subset of T and B lymphocytes in the peripheral blood of healthy individuals. MCAM has been seen as a marker for mesenchymal stem cells isolated from multiple adult and fetal organs, and its expression may be linked to multipotency mesenchymal stem cells with greater differentiation potential express higher levels of MCAM on the cell surface.