Overview

Product name  Anti-CD146 antibody [P1H12]  ab24577
Description   Mouse monoclonal [P1H12] to CD146
Host species  Mouse
Tested applications  Suitable for: ICC/IF, IHC-P, WB, ELISA, IHC-Fr, IP, Flow Cyt
Species reactivity  Reacts with: Human
   Predicted to work with: Mouse, Dog
Immunogen  Tissue/ cell preparation: human umbilical vein endothelial cells (HUVECs).
Positive control  In Western Blot, ab24577 gave a positive signal in HUVEC whole cell lysate and human artery membrane lysate. In IHC, this antibody gave a positive signal in formalin-fixed paraffin-embedded human aorta tissue sections.
General notes  This antibody clone is manufactured by Abcam.
   If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

Properties

Form  Liquid
Storage instructions  Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer  pH: 7.40
   Preservative: 0.02% Sodium azide
   Constituent: PBS
Purity  Protein G purified
Clonality  Monoclonal
Clone number  P1H12
Isotype  IgG1

Applications

Our Abpromise guarantee covers the use of ab24577 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
**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 surface receptor that triggers tyrosine phosphorylation of FYN and PTK2, and a transient increase in the intracellular calcium concentration.

**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.

**Sequence similarities**

Contains 3 Ig-like C2-type (immunoglobulin-like) domains.
Contains 2 Ig-like V-type (immunoglobulin-like) domains.

**Cellular localization**

Membrane.

**Application** | **Abreviews** | **Notes**
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ICC/IF | | Use at an assay dependent concentration.
IHC-P | | Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB | | Use a concentration of 5 µg/ml. Detects a band of approximately 110 kDa (predicted molecular weight: 72 kDa).
ELISA | | Use a concentration of 1 - 10 µg/ml.
IHC-Fr | | 1/1000. See Abreview.
IP | | 1/150.
Flow Cyt | | 1/1000.  
ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

**Target**

**Images**
Flow cytometry analysis of hBMSCs cultured in complete a-MEM supplemented with 1 ng/mL TGF-β1 for 7 days, staining CD146 with ab24577.

Cells were trypsinized, counted and resuspended in 2% BSA at a concentration of 2500 cells/µL. Cells were first incubated for 45 min at 4°C, protected from light, with primary antibody antibody (1/100). Cells were then incubated for 45 minutes, protected from light, at room temperature with AlexaFluor®488-conjugated goat anti-mouse secondary antibody (1/500).

ab24577 staining CD146 in murine bone marrow leukocytes by Immunocytochemistry/ Immunofluorescence. The cells were fixed in methanol and then blocked using 5% serum for 2 hours at 25°C. Samples were then incubated with the primary antibody at 1/400 for 12 hours at 4°C. The secondary antibody used was a goat anti-mouse IgG conjugated to Alexa Fluor® 594 (red) used at a 1/500 dilution.

IHC image of CD146 staining in human aorta formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab24577, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
**Western blot - Anti-CD146 antibody [P1H12] (ab24577)**

- **All lanes**: Anti-CD146 antibody [P1H12] (ab24577) at 5 µg/ml
- **Lane 1**: HUVEC (Human Umbilical Vein Endothelial Cell) Whole Cell Lysate
- **Lane 2**: Human blood vessel: artery normal tissue lysate - membrane extract (ab28989)

Lysates/proteins at 25 µg per lane.

**Secondary**

- **All lanes**: Goat polyclonal Secondary Antibody to Mouse IgG - H&L (HRP), pre-adsorbed at 1/5000 dilution

Performed under reducing conditions.

**Predicted band size**: 72 kDa

**Observed band size**: 110 kDa

**why is the actual band size different from the predicted?**

**Additional bands at**: 55 kDa. We are unsure as to the identity of these extra bands.

**Exposure time**: 20 minutes

This blot was produced using a 10% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% Milk before being incubated with ab24577 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution.

**Immunohistochemistry (Frozen sections) - Anti-CD146 antibody [P1H12] (ab24577)**

- ab24577 at 1/1000 dilution staining mouse brain tissue sections by Immunohistochemistry (Frozen sections). Mice were processed by transcardial perfusion first with saline, then 4% PF. After overnight incubation in 4% PF, brains were transferred to sucrose. Upon saturation, brains were frozen, sectioned with a cryostat, and then the sections were immediately mounted on slides. The tissue was incubated with ab24577 for 2 hours and then an Alexa Fluor® 594 goat anti-mouse IgG was used as the secondary (red). DAPI staining is shown in blue. Images were taken with a confocal microscope in comparable cortex regions of the lesion or contralateral side in the same section. The lesion image shown is
from this model of ischemia-hypoxia, with 1 hour of recovery time after injury, when endothelial cell activation is quite robust.

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