

Product datasheet

Anti-CD146 antibody [EPR3208] ab75769

KO VALIDATED Recombinant RabMAB

★★★★☆ 19 Abreviews 57 References 17 Images

Overview

Product name	Anti-CD146 antibody [EPR3208]
Description	Rabbit monoclonal [EPR3208] to CD146
Host species	Rabbit
Tested applications	Suitable for: ICC/IF, IHC-Fr, WB, IHC-P, Flow Cyt
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) corresponding to Human CD146 aa 600 to the C-terminus. Database link: P43121
Positive control	WB: A375, HUVEC and B16-F0 cell lysate; Human fetal artery lysate and Rat placenta ICC/IF: Murine bone marrow cell lysates. IHC-Fr: Mouse spleen tissue. IHC-P: Melanoma, breast carcinoma vessel, urinary bladder transitional carcinoma vessel, glioma vessel, normal tonsil and normal spleen tissue. FC: A375 and HUVEC cells
General notes	Our RabMAB [®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab[®] patents . We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team. This product is a recombinant rabbit monoclonal antibody .

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR3208
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab75769** in the following tested applications.

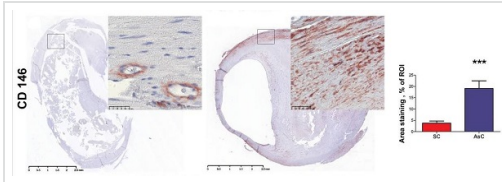
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★☆	1/100 - 1/250.
IHC-Fr	★★★★☆	1/250.
WB	★★★★☆	1/1000. Predicted molecular weight: 72 kDa. Can be blocked with CD146 peptide (ab219161) . For unpurified use at 1/10000 - 1/50000.
IHC-P	★★★★☆	1/250 - 1/500. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol. See IHC antigen retrieval protocols .
Flow Cyt		1/20 - 1/80. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

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.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD146 antibody

[EPR3208] (ab75769)

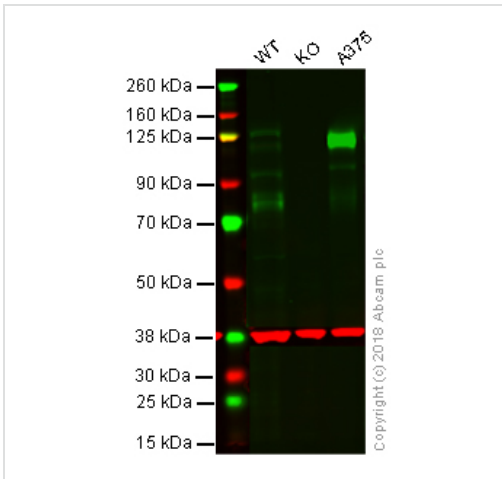
Davaine, J.M. et al PLoS One. 2014 Sep 26;9(9):e107642. doi: 10.1371/journal.pone.0107642. eCollection 2014

Immunohistochemistry experiments were used to compare symptomatic carotid plaques (SC) and asymptomatic carotid plaques (AsC)

Asymptomatic lesions presented higher CD146⁺ pericyte infiltration, $p < 0.001$. Representative images are on the left with corresponding quantification on the right.

ab75769 used at 1/200 dilution.

(After Figure 2 of Davaine et al)



Western blot - Anti-CD146 antibody [EPR3208] (ab75769)

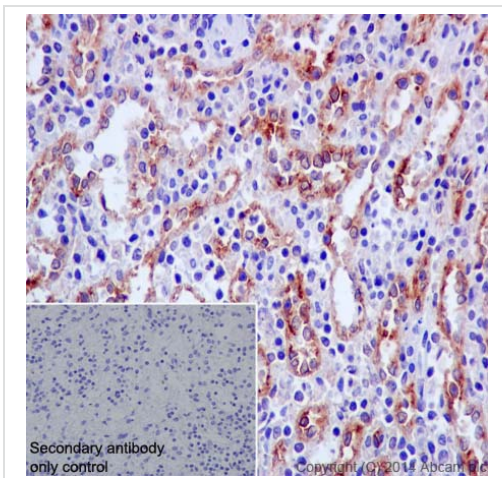
Lane 1: Wild-type HAP1 whole cell lysate (40 μ g)

Lane 2: CD146 knockout HAP1 whole cell lysate (40 μ g)

Lane 3: A375 whole cell lysate (20 μ g)

Lanes 1 - 4: Merged signal (red and green). Green - ab75769 observed at 120-72 kDa. Red - loading control, ab9484, observed at 37 kDa.

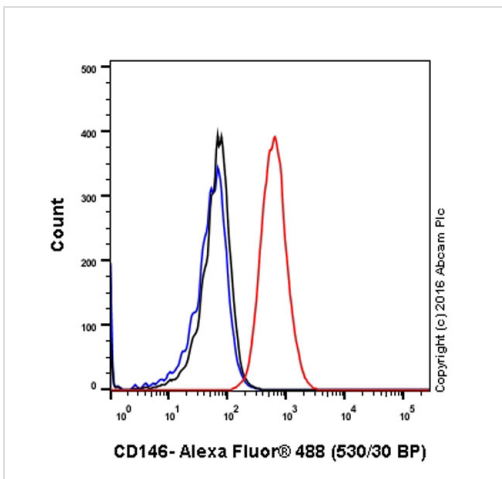
ab75769 was shown to specifically react with CD146 in wild-type HAP1 cells as signal was lost in CD146 knockout cells. Wild-type and CD146 knockout samples were subjected to SDS-PAGE. ab75769 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/10000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD146 antibody

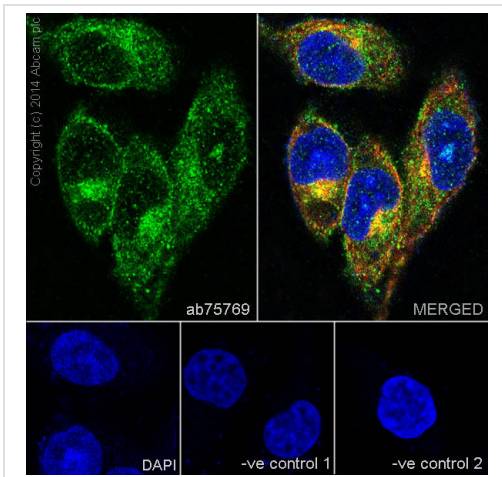
[EPR3208] (ab75769)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human spleen tissue labelling CD146 with purified ab75769 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a goat anti-rabbit IgG H&L (HRP) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Flow Cytometry - Anti-CD146 antibody [EPR3208] (ab75769)

Flow Cytometry analysis of A375 (human malignant melanoma) cells labeling CD146 with unpurified ab75769 at 1/20 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor®488) at 1/2000 dilution was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.

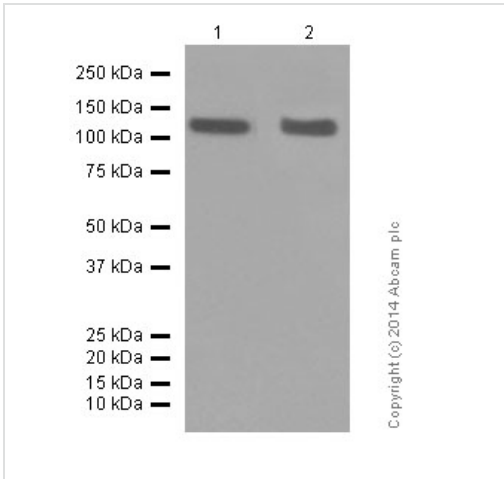


Immunocytochemistry/ Immunofluorescence - Anti-CD146 antibody [EPR3208] (ab75769)

Immunocytochemistry/Immunofluorescence analysis of A375 (human malignant melanoma) cells labelling CD146 with purified ab75769 at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

Control 1: primary antibody (1/100) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).



Western blot - Anti-CD146 antibody [EPR3208]
(ab75769)

All lanes : Anti-CD146 antibody [EPR3208] (ab75769) at 1/10000 dilution (purified)

Lane 1 : A375 cell lysate

Lane 2 : Human fetal artery lysate

Lysates/proteins at 20 µg per lane.

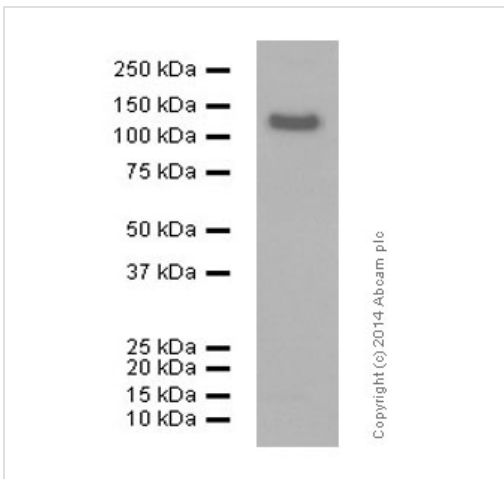
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 72 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-CD146 antibody [EPR3208]
(ab75769)

Anti-CD146 antibody [EPR3208] (ab75769) at 1/10000 dilution (purified) + HUVEC cell lysate at 20 µg

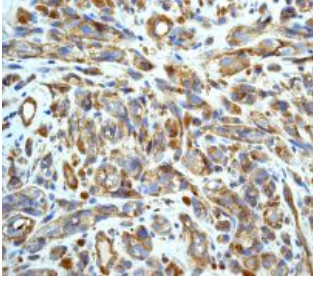
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 72 kDa

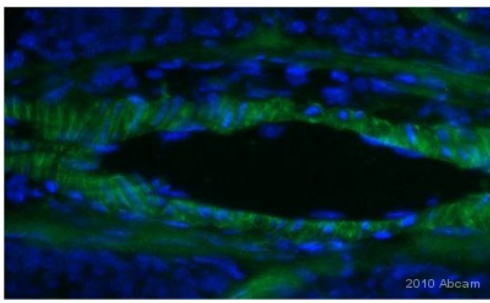
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



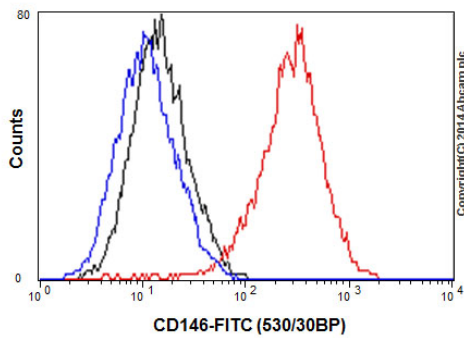
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD146 antibody [EPR3208] (ab75769)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of melanoma tissue labelling CD146 with unpurified ab75769 at 1/250. A HRP/AP polymerized secondary antibody was used.



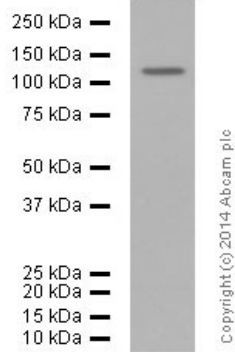
Immunohistochemistry (Frozen sections) - Anti-CD146 antibody [EPR3208] (ab75769)
This image is courtesy of an anonymous Abreview

Immunohistochemistry (Frozen sections) analysis of mouse spleen tissue labelling CD146 with unpurified ab75769 at 1/250. Tissue samples were fixed with acetone and blocked with 5% serum for 2 hours at 25°C. The sample was incubated with primary antibody at 4°C for 12 hours. An Alexa Fluor®488-conjugated Goat polyclonal to rabbit IgG (1/250) was used as secondary antibody. Nuclear staining was with DAPI (blue).



Flow Cytometry - Anti-CD146 antibody [EPR3208] (ab75769)

Flow Cytometry analysis of HUVEC cells labelling CD146 with purified ab75769 at 1/50 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Western blot - Anti-CD146 antibody [EPR3208]
(ab75769)

Anti-CD146 antibody [EPR3208] (ab75769) at 1/10000 dilution
(purified) + B16-F0 cell lysate at 20 µg/ml

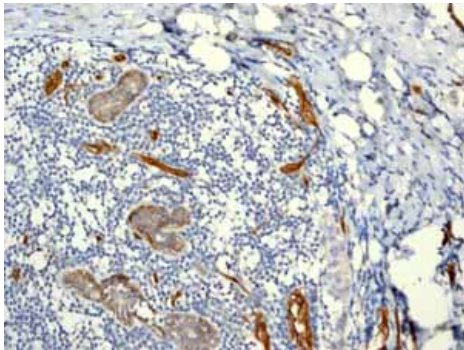
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution
(Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 72 kDa

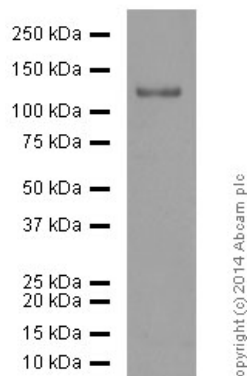
Blocking buffer and concentration: 5% NFDm/TBST.

Diluting buffer and concentration: 5% NFDm /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-
embedded sections) - Anti-CD146 antibody
[EPR3208] (ab75769)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded
sections) analysis of breast carcinoma vessels tissue labelling
CD146 with unpurified ab75769.



Western blot - Anti-CD146 antibody [EPR3208] (ab75769)

Anti-CD146 antibody [EPR3208] (ab75769) at 1/10000 dilution (purified) + Rat placenta lysate at 20 µg

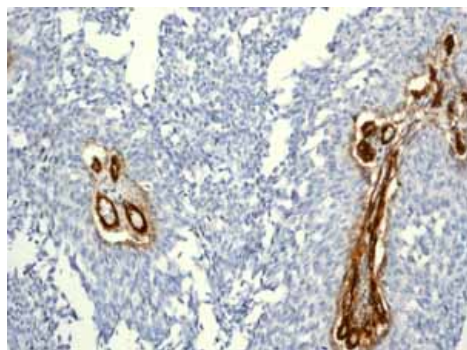
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 72 kDa

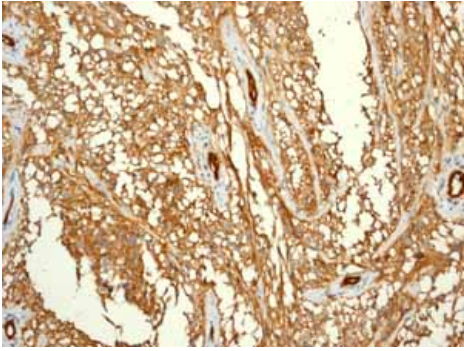
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



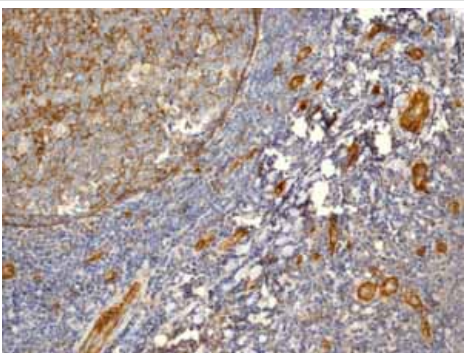
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD146 antibody [EPR3208] (ab75769)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of urinary bladder transitional carcinoma vessels tissue labelling CD146 with unpurified ab75769.



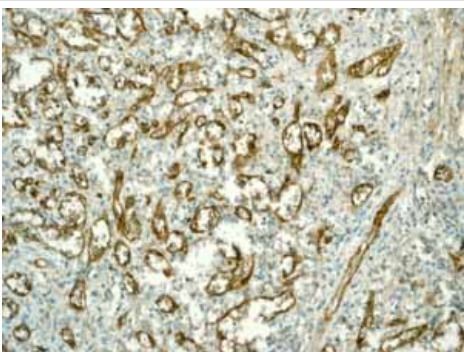
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of glioma vessels tissue labelling CD146 with unpurified ab75769.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD146 antibody [EPR3208] (ab75769)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of normal tonsil tissue labelling CD146 with unpurified ab75769.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD146 antibody [EPR3208] (ab75769)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of normal spleen tissue labelling CD146 with unpurified ab75769.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD146 antibody [EPR3208] (ab75769)

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