## Overview

<table>
<thead>
<tr>
<th><strong>Product name</strong></th>
<th>Anti-CD146 antibody [EPR3208]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Rabbit monoclonal [EPR3208] to CD146</td>
</tr>
<tr>
<td><strong>Host species</strong></td>
<td>Rabbit</td>
</tr>
<tr>
<td><strong>Tested applications</strong></td>
<td>Suitable for: ICC/IF, IHC-Fr, WB, IHC-P, Flow Cyt</td>
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<tr>
<td><strong>Species reactivity</strong></td>
<td>Reacts with: Mouse, Rat, Human</td>
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<tr>
<td><strong>Immunogen</strong></td>
<td>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</td>
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<tr>
<td><strong>Positive control</strong></td>
<td>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</td>
</tr>
<tr>
<td><strong>General notes</strong></td>
<td>This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information see here. 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.</td>
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</table>

## Properties

<table>
<thead>
<tr>
<th><strong>Form</strong></th>
<th>Liquid</th>
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<tbody>
<tr>
<td><strong>Storage instructions</strong></td>
<td>Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td></td>
<td>1/100 - 1/250.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td></td>
<td>1/250.</td>
</tr>
<tr>
<td>WB</td>
<td></td>
<td>1/1000. Predicted molecular weight: 72 kDa. For unpurified use at 1/10000 - 1/50000.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/20 - 1/80. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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</table>

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.
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)

Lane 1: Wild-type HAP1 whole cell lysate (40 µg)
Lane 2: CD146 knockout HAP1 whole cell lysate (40 µg)
Lane 3: A375 whole cell lysate (20 µg)

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) 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 analysis of A375 (human malignant melanoma) cells labeling CD146 with unpurified ab75769 at 1/20 dilution (10µg/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 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<sup>TM</sup> 488-conjugated goat anti-rabbit IgG (1/500).

**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.

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) analysis of melanoma tissue labelling CD146 with unpurified ab75769 at 1/250. A HRP/AP polymerized secondary antibody was used.

Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

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 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.
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) analysis of breast carcinoma vessels tissue labelling CD146 with unpurified ab75769.
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.
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) analysis of urinary bladder transitional carcinoma vessels tissue labelling CD146 with unpurified ab75769.

Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of glioma vessels tissue labelling CD146 with unpurified ab75769.

Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

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

Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

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

Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

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