abcam

Product datasheet

Anti-CD146 antibody [EPR3208] ab75769

敲除验证 重组 RabMAb

41 Abreviews 130 References 18 图像

概述

产品名称	Anti-CD146抗体[EPR3208]
描述	兔单克隆抗体[EPR3208] to CD146
宿主	Rabbit
经测试应用	**适用于:** ICC/IF, Flow Cyt (Intra), WB, IHC-P **不适用于:** IHC-Fr
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HeLa, 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. Flow Cyt (intra): A375 and HUVEC cells
常规说明	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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆

克隆编号	EPR3208
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab75769于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF	★★★★☆ (9)	1/100 - 1/250.
Flow Cyt (Intra)		1/20 - 1/80. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (4)	1/1000. Predicted molecular weight: 72 kDa. **For unpurified use at 1/10000 - 1/50000.**
IHC-P	★★★★☆ (13)	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**.

应用说明 Is unsuitable for IHC-Fr.

靶标

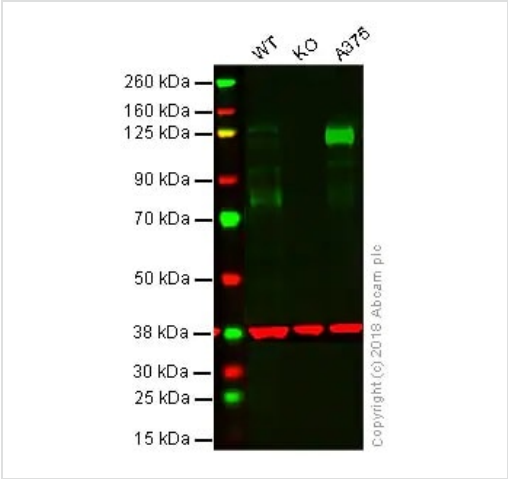
功能 Plays a role in cell adhesion, and in cohesion of the endothelial monolayer at intercellular junctions in vascular tissue. Its expression may allow melanoma cells to interact with cellular elements of the vascular system, thereby enhancing hematogeneous tumor spread. Could be an adhesion molecule 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.

组织特异性 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.

序列相似性 Contains 3 Ig-like C2-type (immunoglobulin-like) domains.
Contains 2 Ig-like V-type (immunoglobulin-like) domains.

细胞定位 Membrane.

图片

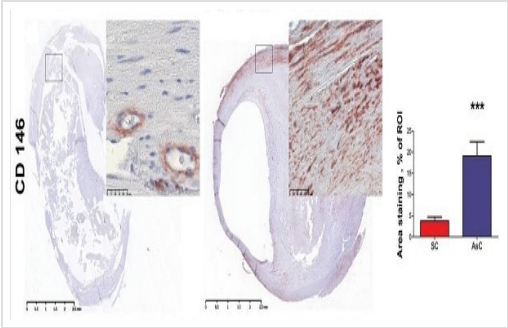


Western blot - Anti-CD146 antibody [EPR3208] (ab75769)

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

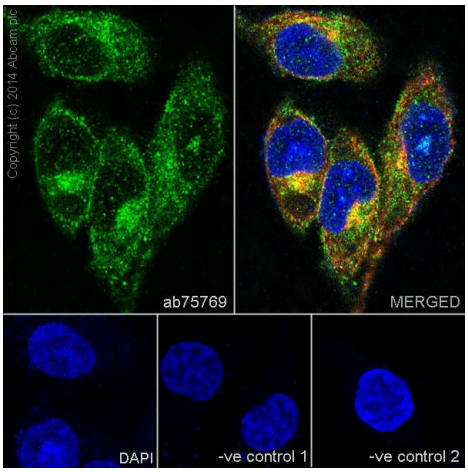
Davaine, J.M et al PLoS One. 2014 Sep 26;9(9):e107642. doi: 10.1371/journal.pone.0107642. eCollection 2014 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

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)

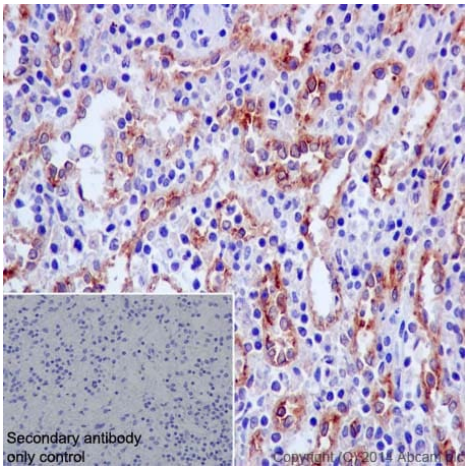


Immunocytochemistry/ Immunofluorescence - Anti-CD146 antibody [EPR3208] (ab75769)

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.

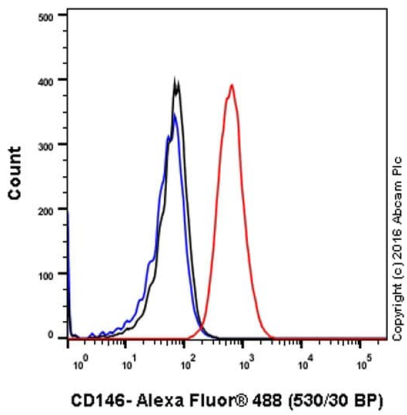
Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

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



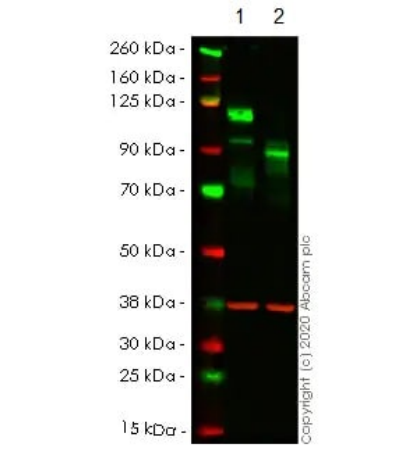
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD146 antibody [EPR3208] (ab75769)

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 (Intracellular) - Anti-CD146 antibody [EPR3208] (ab75769)

Intracellular 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 Fluorr®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.



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

All lanes : Anti-CD146 antibody [EPR3208] (ab75769) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : MCAM CRISPR/Cas9 edited HeLa cell lysate

Lysates/proteins at 20 µg per lane.

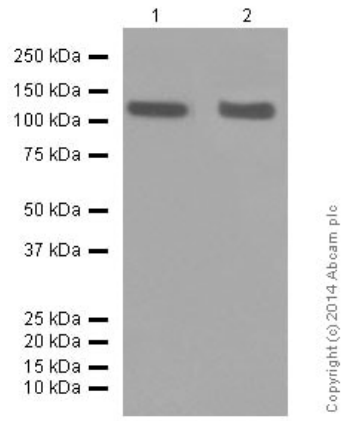
Performed under reducing conditions.

Predicted band size: 72 kDa
Observed band size: 120 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab75769 observed at 120 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab75769 was shown to react with CD146 in wild-type HeLa cells in western blot. The band observed in CRISPR/Cas9 edited cell line **ab261790** (CRISPR/Cas9 edited cell lysate **ab256985**) lane below 120kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and MCAM CRISPR/Cas9 edited HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab75769 and Anti-GAPDH

antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



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

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

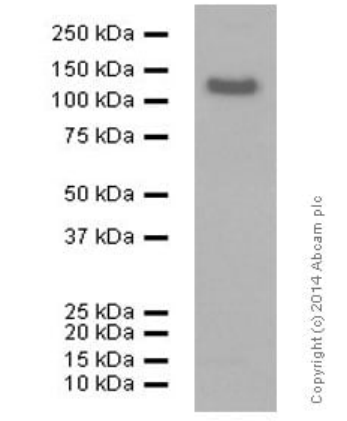
Lane 1 : A375 cell lysate
Lane 2 : Human fetal artery lysate

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.



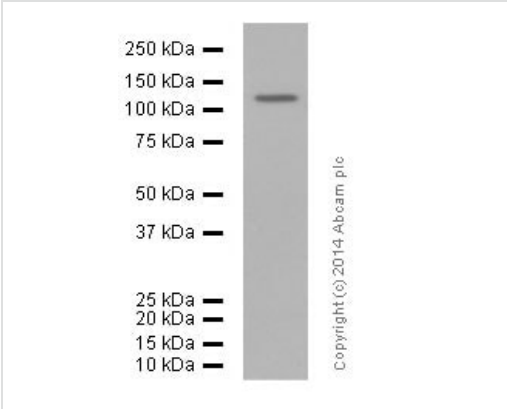
Western blot - Anti-CD146 antibody [EPR3208] (ab75769)

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.



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

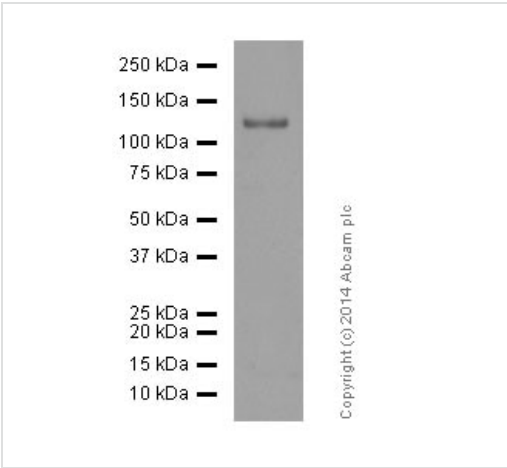
Anti-CD146 antibody [EPR3208] (ab75769) at 1/10000 dilution (purified) + B16-F0 cell lysate at 20 µg/ml

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.



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

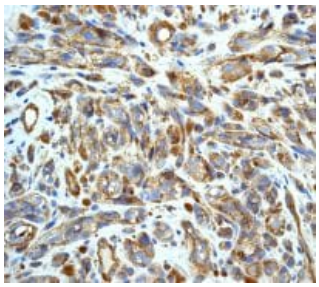
Anti-CD146 antibody [EPR3208] (ab75769) at 1/10000 dilution (purified) + Rat placenta lysate at 20 µg

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

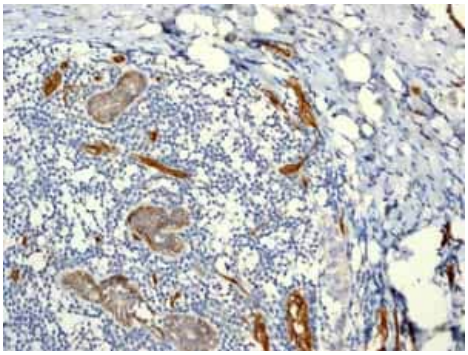
Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.



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

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

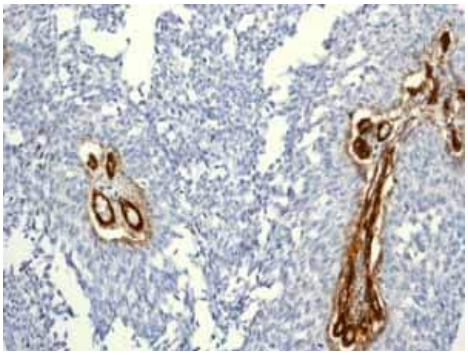
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



Immunohistochemistry (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.

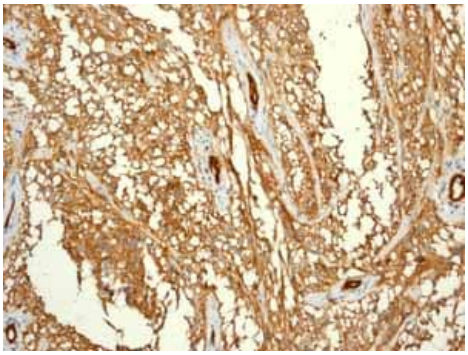
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



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.

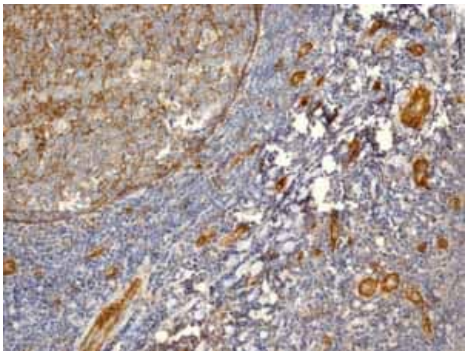
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



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

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

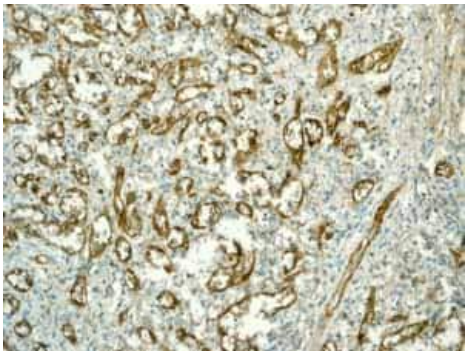
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



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

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

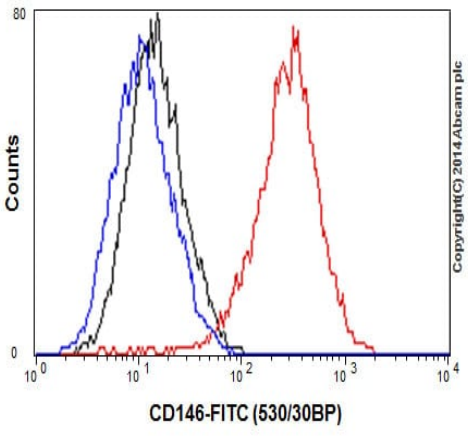
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



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

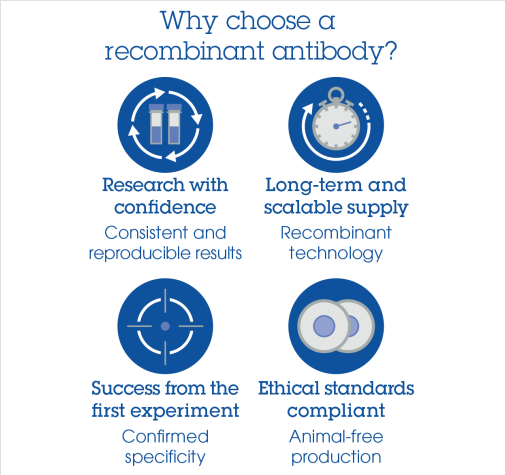
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of normal spleen tissue labelling CD146 with unpurified ab75769.

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



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

Intracellular Flow Cytometry analysis ofHUVEC 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)

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