abcam

Product datasheet

Anti-CD31 antibody [EPR3094] ab76533

Recombinant RabMAb

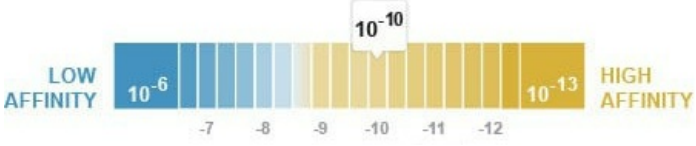
12 Abreviews 68 References 13 Images

Overview

Product name	Anti-CD31 antibody [EPR3094]
Description	Rabbit monoclonal [EPR3094] to CD31
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P **Unsuitable for:** Flow Cyt or IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: THP-1 and Jurkat cell lysates IHC-P: Human kidney tissue and human muscle tissue ICC/IF: THP-1 cell lysate.
General notes	This antibody shows no cross-reactivity with rat and mouse samples in WB. However it can give some non specific staining on mouse smooth muscle tissues. Plesae contact our Scientific support for more information. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Dissociation constant (K_D)	$K_D = 1.79 \times 10^{-10}$ M



Learn more about K_D

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	EPR3094
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab76533 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		1/500. It is recommended to incubate cells with 0.1% Triton-X for 5 min to detect nuclear antigen. Use 0.3M glycine to quench autofluorescence caused by aldehydes. Positive Control: HUVEC cells
WB	★★★★★ (1)	1/5000 - 1/20000. Predicted molecular weight: 83 kDa. Treat samples with PNGase F or phosphatase to confirm the specificity of bands if necessary. The observed band size of CD31 may not the same as predicted MWs in WB due to the different forms and modifications of CD31. Hu Isoform 1-6: 79-83 kDa (predicted)
IHC-P	★★★★☆ (7)	1/100 - 1/500. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol. We suggest to program the pressure cooker to run for 30 seconds at 125° C, followed by 10 seconds at 90° C. Then let the slide cool down to room temperature (10 - 20 minutes). The ideal fixation time will depend on the size of the tissue block

Application notes Is unsuitable for Flow Cyt or IP.

Target

Function Induces susceptibility to atherosclerosis (By similarity). Cell adhesion molecule which is required for leukocyte transendothelial migration (TEM) under most inflammatory conditions. Tyr-690 plays a critical role in TEM and is required for efficient trafficking of PECAM1 to and from the lateral border recycling compartment (LBRC) and is also essential for the LBRC membrane to be targeted around migrating leukocytes. Prevents phagocyte ingestion of closely apposed viable cells by transmitting 'detachment' signals, and changes function on apoptosis, promoting tethering

of dying cells to phagocytes (the encounter of a viable cell with a phagocyte via the homophilic interaction of PECAM1 on both cell surfaces leads to the viable cell's active repulsion from the phagocyte. During apoptosis, the inside-out signaling of PECAM1 is somehow disabled so that the apoptotic cell does not actively reject the phagocyte anymore. The lack of this repulsion signal together with the interaction of the eat-me signals and their respective receptors causes the attachment of the apoptotic cell to the phagocyte, thus triggering the process of engulfment). Isoform Delta15 is unable to protect against apoptosis. Modulates BDKRB2 activation. Regulates bradykinin- and hyperosmotic shock-induced ERK1/2 activation in human umbilical cord vein cells (HUVEC).

Tissue specificity

Expressed on platelets and leukocytes and is primarily concentrated at the borders between endothelial cells. Isoform Long predominates in all tissues examined. Isoform Delta12 is detected only in trachea. Isoform Delta14-15 is only detected in lung. Isoform Delta14 is detected in all tissues examined with the strongest expression in heart. Isoform Delta15 is expressed in brain, testis, ovary, cell surface of platelets, human umbilical vein endothelial cells (HUVECs), Jurkat T-cell leukemia, human erythroleukemia (HEL) and U937 histiocytic lymphoma cell lines (at protein level).

Sequence similarities

Contains 6 Ig-like C2-type (immunoglobulin-like) domains.

Domain

The Ig-like C2-type domains 2 and 3 contribute to formation of the complex with BDKRB2 and in regulation of its activity.

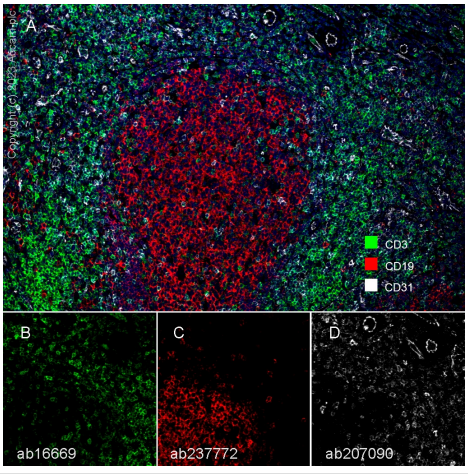
Post-translational modifications

Phosphorylated on Ser and Tyr residues after cellular activation. In endothelial cells Fyn mediates mechanical-force (stretch or pull) induced tyrosine phosphorylation.

Cellular localization

Membrane. Cell junction. Localizes to the lateral border recycling compartment (LBRC) and recycles from the LBRC to the junction in resting endothelial cells and Cell junction. Localizes to the lateral border recycling compartment (LBRC) and recycles from the LBRC to the junction in resting endothelial cells.

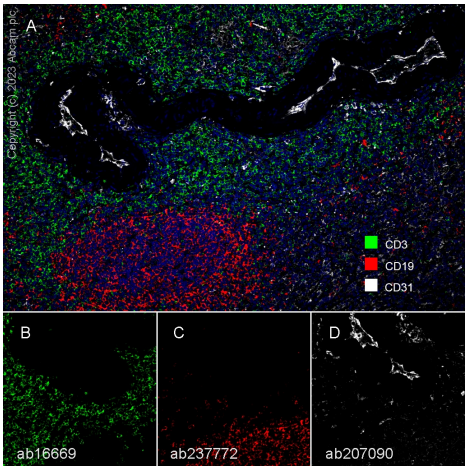
Images



Multiplex immunohistochemistry - Anti-CD31 antibody [EPR3094] (ab76533)

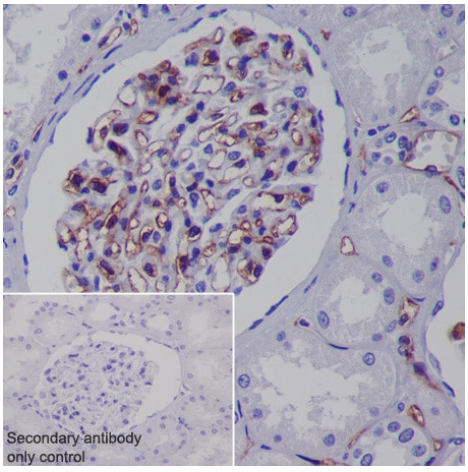
Panel A: merged staining of anti-CD31 (gray; Opal™690), anti-CD3 (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human tonsil. Secondary antibody was Opal Polymer HRP Ms + Rb, nuclear counterstain was DAPI.
Panel B: anti-CD3 stained on T cells with **ab16669** at 1/500 dilution
Panel C: anti-CD19 stained on B cells with **ab237772** at 1/5000 dilution
Panel D: anti-CD31 stained on endothelial cells and immune cell subsets with **ab207090** at 1/500 dilution
The section was incubated in three rounds of staining: in the order of **ab207090** and **ab16669** for 30 mins, then **ab237772** for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope

retrieval solution2) for 20 mins



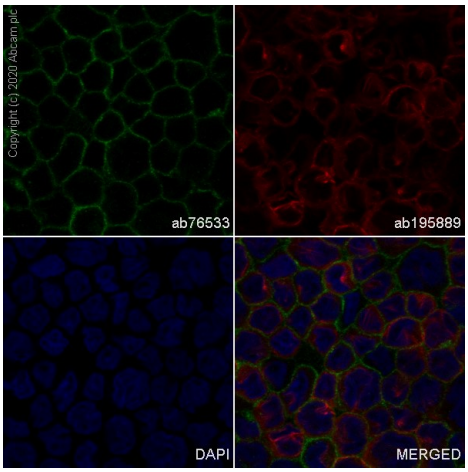
Multiplex immunohistochemistry - Anti-CD31 antibody [EPR3094] (ab76533)

Panel A: merged staining of anti-CD31 (gray; Opal™690), anti-CD3 (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human spleen. Secondary antibody was Opal Polymer HRP Ms + Rb, nuclear counterstain was DAPI.
Panel B: anti-CD3 stained on T cells with **ab16669** at 1/500 dilution
Panel C: anti-CD19 stained on B cells with **ab237772** at 1/5000 dilution
Panel D: anti-CD31 stained on endothelial cells with **ab207090** at 1/500 dilution
The section was incubated in three rounds of staining: in the order of **ab207090** and **ab16669** for 30 mins, then **ab237772** for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD31 antibody [EPR3094] (ab76533)

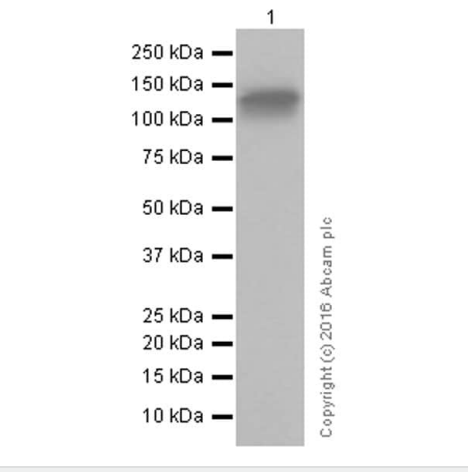
ab76533 staining CD31 in human kidney tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti-rabbit IgG H&L (HRP) **ab97051** was used as the secondary antibody at a dilution of 1/500.



Immunocytochemistry/ Immunofluorescence - Anti-CD31 antibody [EPR3094] (ab76533)

Immunocytochemistry analysis of THP-1 (Human monocytic leukemia monocyte) cell line labeling CD31 with ab76533 at 1/250 (4.9 µg/mL). **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used as a counterstain at 1/200 dilution. DAPI (blue) was used as nuclear counterstain.

Confocal image showing membranous staining in THP-1 cells.



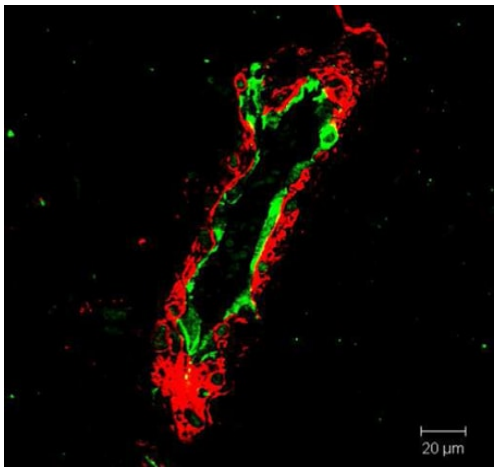
Western blot - Anti-CD31 antibody [EPR3094] (ab76533)

Anti-CD31 antibody [EPR3094] (ab76533) at 1/10000 dilution + THP-1 (Human monocytic leukemia cell line) whole cell lysate at 20 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 83 kDa
Additional bands at: 125 kDa. We are unsure as to the identity of these extra bands.

Blocking and diluting buffer: 5% NFDM /TBST

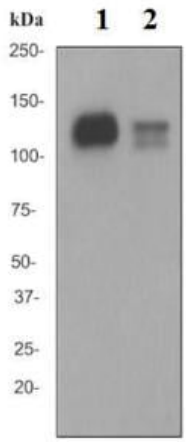


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD31 antibody [EPR3094] (ab76533)

Image from Hofmann NA et al., PLoS One. 2012;7(9):e44468. doi: 10.1371/journal.pone.0044468. Epub 2012 Sep 7. Fig 3.; doi:10.1371/journal.pone.0044468; September 7, 2012, PLoS ONE 7(9): e44468.

Immunohistochemical analysis of endothelial colony forming progenitor cell plugs, staining CD31 (green) with ab76533.

Following antigen retrieval and blocking, sections were incubated with primary antibody (1/1000) overnight at 4°C. A Cy5®-conjugated anti-rabbit IgG (2 µg/ml) was used as the secondary antibody.



Western blot - Anti-CD31 antibody [EPR3094] (ab76533)

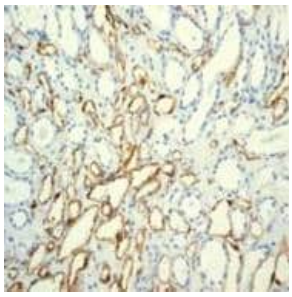
All lanes : Anti-CD31 antibody [EPR3094] (ab76533) at 1/20000 dilution

Lane 1 : THP-1 cell lysate
Lane 2 : Jurkat cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

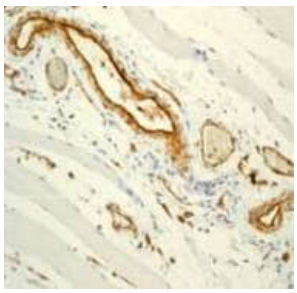
Predicted band size: 83 kDa
Observed band size: 125 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD31 antibody [EPR3094] (ab76533)

Immunohistochemical analysis of paraffin embedded human kidney tissue using ab76533 at a 1/250 dilution. Note positive staining of endothelial cells.

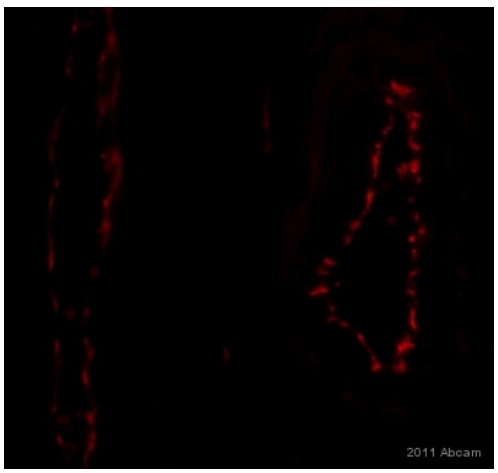
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD31 antibody [EPR3094] (ab76533)

Immunohistochemical analysis of paraffin embedded human muscle tissue using ab76533 at a 1/250 dilution. Note positive staining of endothelial cells.

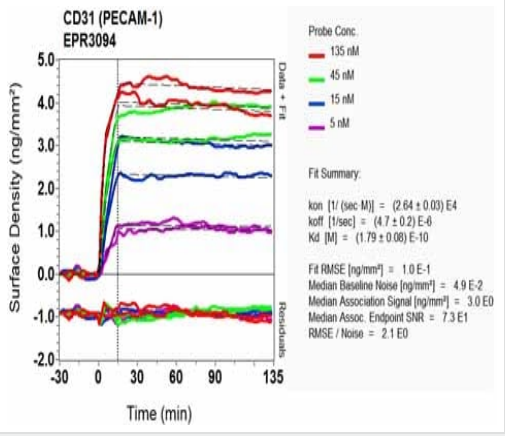
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD31 antibody [EPR3094] (ab76533)

Image courtesy of an anonymous Abreview.

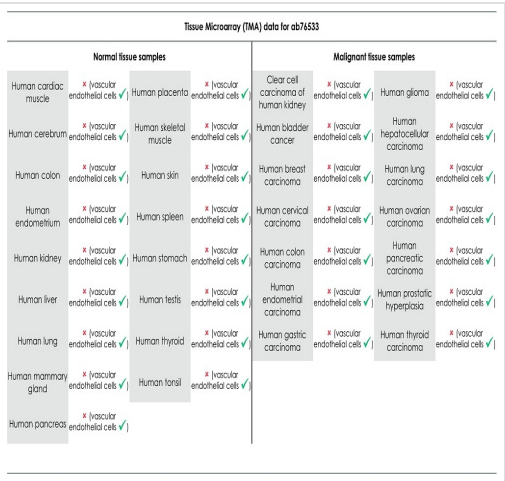
ab76533 staining CD31 in human muscle tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue was fixed with formaldehyde and a heat mediated antigen retrieval step was performed using citrate buffer pH 6.0. Samples were then blocked with 3% serum for 30 minutes at 20°C followed by incubation with the primary antibody at a 1/200 dilution for 12 hours at20°C. A Cy3®-conjugated goat anti-rabbit polyclonal was used as secondary antibody at a 1/200 dilution.



OI-RD Scanning - Anti-CD31 antibody [EPR3094] (ab76533)

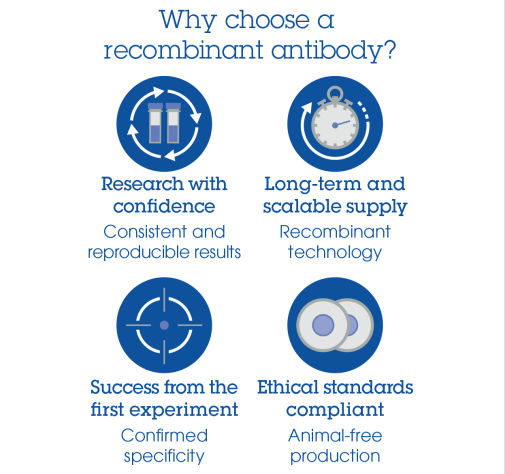
Equilibrium disassociation constant (K_D) measurement to determine antibody affinity to the target antigen.

Click here to learn more about K_D



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD31 antibody [EPR3094] (ab76533)

Tissue Microarrays stained for "Anti-CD31 antibody [EPR3094]" using "ab76533" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). The sections were incubated with ab76533 at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).



Anti-CD31 antibody [EPR3094] (ab76533)

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