abcam

Product datasheet

Anti-CD31 antibody [JC/70A] ab9498

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Overview

Product name	Anti-CD31 antibody [JC/70A]
Description	Mouse monoclonal [JC/70A] to CD31
Host species	Mouse
Tested applications	**Suitable for:** IHC-Fr, WB, ICC, IHC-P, Flow Cyt
Species reactivity	**Reacts with:** Human **Predicted to work with:** Cynomolgus monkey
Immunogen	Tissue, cells or virus. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HUVEC and HeLa whole cell lysate. Human spleen and kidney tissue lysate. IHC-P: Human tonsil tissue. ICC: HUVEC cells. Flow Cyt: PBMC.
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.02% Sodium azide Constituent: PBS
Purity	Protein G purified
Clonality	Monoclonal
Clone number	JC/70A
Myeloma	unknown

Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab9498 in the following tested applications.

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

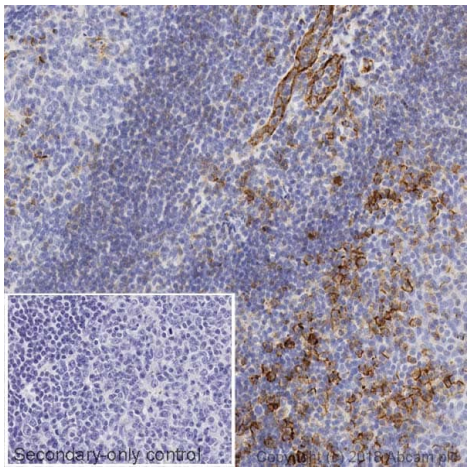
Application	Abreviews	Notes
IHC-Fr	★★★★★ (2)	Use a concentration of 1 µg/ml.
WB	★★★★★ (1)	1/1000. Detects a band of approximately 130 kDa (predicted molecular weight: 82 kDa). Treat samples with PNGase F or phosphatase to confirm the specificity of bands if necessary. The observed band size of CD31 may not the same as predicted MWs in WB due to the different forms and modifications of CD31.
ICC	★★★★★ (4)	Use a concentration of 1 µg/ml. 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
IHC-P	★★★★☆ (26)	Use a concentration of 0.5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. The ideal fixation time will depend on the size of the tissue block and the type of tissue, but fixation between 18–24h is suitable for most samples. Positive Control: Hu tonsil tissue
Flow Cyt	★★★☆☆ (1)	1/20.

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

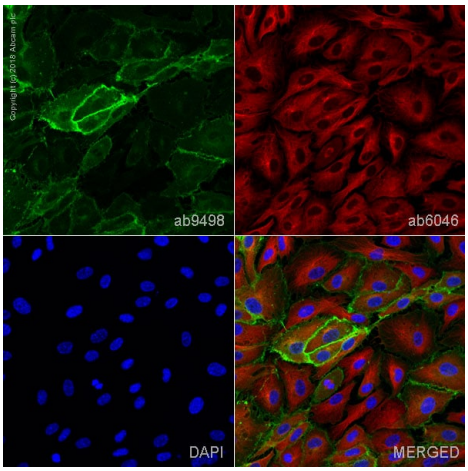


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD31 antibody [JC/70A] (ab9498)

IHC image of CD31 staining in a section of formalin-fixed paraffin-embedded normal human tonsil* 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 20mins. The section was then incubated with ab9498, 0.5ug/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. The inset secondary-only control image is taken from an identical assay without primary antibody.

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.

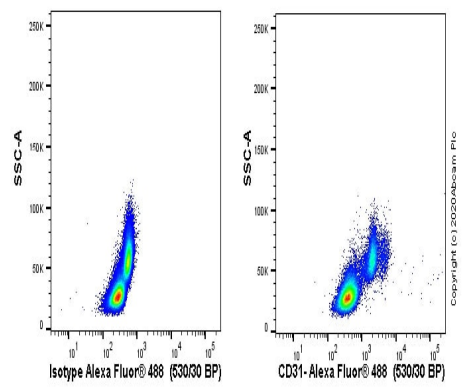
**Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre*



Immunocytochemistry - Anti-CD31 antibody [JC/70A] (ab9498)

ab9498 staining CD31 in HUVEC cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1%PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab9498 at 1µg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control, at 1/1000 dilution. Cells were then incubated with ab150117, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (shown in green) and ab150084, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolor red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

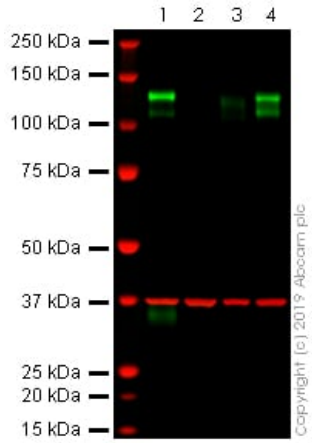


Flow Cytometry - Anti-CD31 antibody [JC/70A] (ab9498)

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with ab9498 (right) or mouse IgG1κ; (ab170190) isotype (left). PBMCs were incubated for 30 min on ice in 1x PBS containing 10 µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (ab9498) or mouse IgG1κ; (ab170190) isotype ($1x10^6$ in 100 µL at 0.04 µg/ml) for 30 min on ice.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor ® 488, pre-adsorbed) (ab150117) was used at 1:2000 dilution for 30 min on ice.

Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. Events were gated on alive cells.



Western blot - Anti-CD31 antibody [JC/70A] (ab9498)

All lanes : Anti-CD31 antibody [JC/70A] (ab9498) at 1 µg/ml

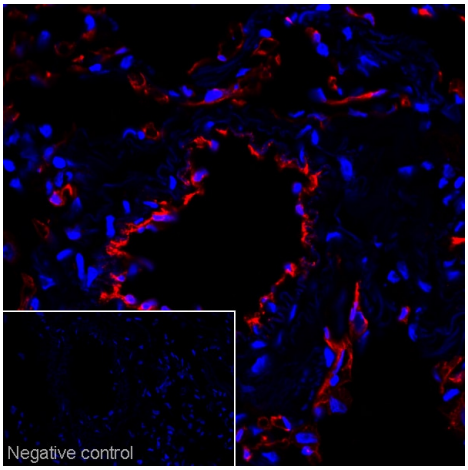
Lane 1 : HUVEC whole cell lysate
Lane 2 : HeLa whole cell lysate
Lane 3 : Human kidney tissue lysate
Lane 4 : Human spleen tissue lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 82 kDa
Observed band size: 130 kDa

This blot was produced using a 4-12% Bis-tris under the MOPS buffer system. The gel was run at 200V for 55 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was blocked for an hour using 3% milk before ab9498 and ab181602 (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at a 1ug/ml concentration and 1/20000 dilution respectively. Antibody binding was detected using Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



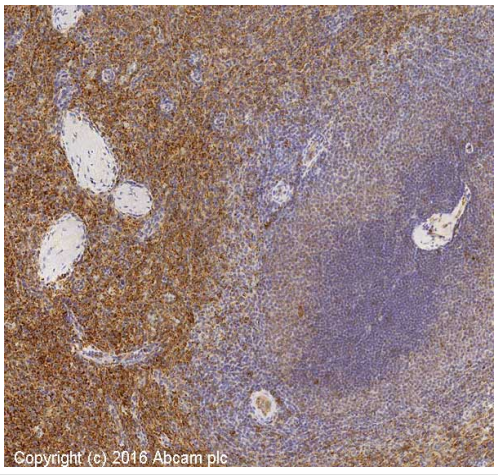
Immunohistochemistry (Frozen sections) - Anti-CD31 antibody [JC/70A] (ab9498)

IHC image of ab9498 staining in 10% formaldehyde fixed frozen tissue section of human lung.

Non-specific protein-protein interactions were blocked using TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 3% (w/v) BSA for 1 hour at room temperature. The section was then incubated with ab9498 (1µg/ml concentration) in TBS containing 0.025% (v/v) Triton X-100 and 3% (w/v) BSA overnight at +4°C. The section was then incubated with ab150119 (Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 647)) and DAPI for 1 hour at room temperature.

The DAPI only control (no antibody) inset shows no autofluorescence, demonstrating that any Alexa Fluor® 647 signal is derived directly from bound ab9498.

For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antibody concentrations and incubation times.

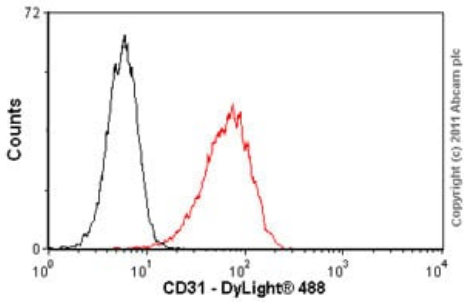


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD31 antibody [JC/70A] (ab9498)

IHC image of CD31 staining in human spleen 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 ab9498, 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.

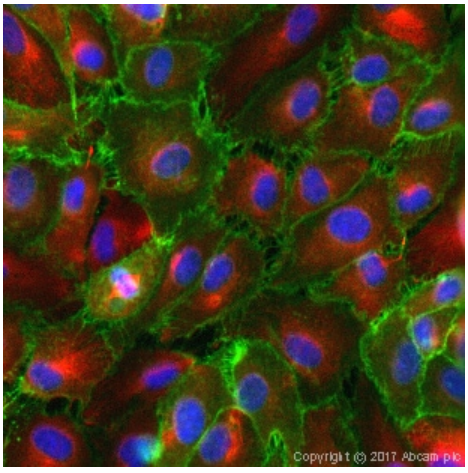
**Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre*



Flow Cytometry - Anti-CD31 antibody [JC/70A] (ab9498)

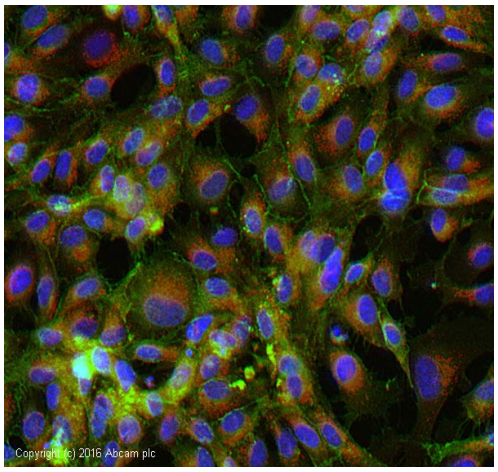
Overlay histogram showing Jurkat cells stained with ab9498 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab9498, 1/20 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Jurkat cells fixed with methanol (5 min) used under the same conditions.

Please note that Abcam do not have data for use of this antibody on non-fixed cells. We welcome any customer feedback.



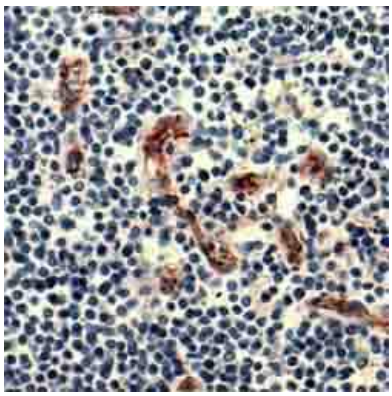
Immunocytochemistry - Anti-CD31 antibody [JC/70A] (ab9498)

ab9498 stained in HUVEC cells. Cells were fixed with 100% methanol (5 min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab9498 at 5 µg/ml and ab6046 (Rabbit polyclonal to beta Tubulin) at 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150080 (pseudo-colored red) and ab150117 (colored green) used at 1 ug/ml for 1hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43µM for 1hour at room temperature



Immunocytochemistry - Anti-CD31 antibody [JC/70A] (ab9498)

ab9498 stained HUVEC cells. The cells were 100% methanol fixed for 5 minutes at -20°C and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1hour at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab9498 at 5µg/ml) overnight at +4°C. The secondary antibody (pseudo-colored green) was Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (ab150117) used at a 1/1000 dilution for 1hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD31 antibody [JC/70A] (ab9498)

This image is courtesy of an anonymous Abreview

ab9498 at 1/100 staining human lymph node tissue sections by IHC-P. The tissue was paraformaldehyde fixed and a heat based antigen retrieval step was performed. The tissue was then blocked with serum and incubated with ab9498 overnight. An HRP conjugated goat antibody was used as the secondary.

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