abcam

Product datasheet

Anti-CD34 antibody [EP373Y] - BSA and Azide free ab198395

Recombinant RabMAb



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Overview

Product name Anti-CD34 antibody [EP373Y] - BSA and Azide free

Description Rabbit monoclonal [EP373Y] to CD34 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: IHC-Fr, Flow Cyt (Intra), IHC-P, ICC/IF, IP

Unsuitable for: WB

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Sheep, Dog, African bush elephant

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control Flow Cyt (intra): TF-1 cells. IHC-P: Human kidney tissue; Mouse normal brain, prostate and kidney

tissues; Rat kidney tissue. ICC/IF: HUVEC cells; Human embryonic stem cell-derived endothelial

cells. IHC-Fr: Mouse and rat lung tissue. Rat kidney. IP: TF-1 cell lysate.

General notes ab198395 is the carrier-free version of ab81289.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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.

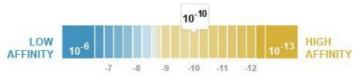
Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**[®] **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Dissociation constant (K_D) $K_D = 1.15 \times 10^{-10} M$



Learn more about K_D

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EP373Y

Isotype IgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab198395 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	★★★★ ☆ <u>(1)</u>	Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
IHC-P	★★★★★ (2)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Application notes Is unsuitable for WB.

Target

Function Possible adhesion molecule with a role in early hematopoiesis by mediating the attachment of

stem cells to the bone marrow extracellular matrix or directly to stromal cells. Could act as a scaffold for the attachment of lineage specific glycans, allowing stem cells to bind to lectins expressed by stromal cells or other marrow components. Presents carbohydrate ligands to

selectins.

Tissue specificity Selectively expressed on hematopoietic progenitor cells and the small vessel endothelium of a

variety of tissues.

Sequence similarities Belongs to the CD34 family.

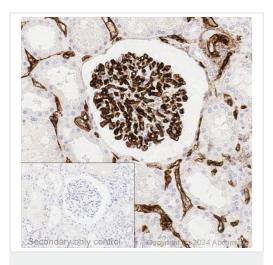
Developmental stage On early hematopoietic progenitor cells.

Post-translational Highly glycosylated.

modifications Phosphorylated on serine residues by PKC.

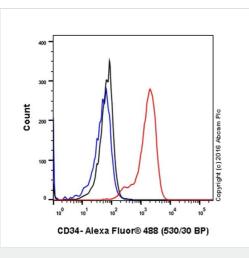
Cellular localization Membrane.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

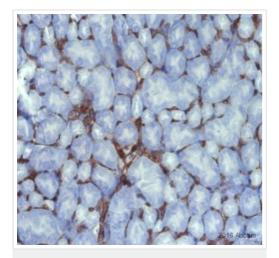
Immunohistochemical analysis of formalin fixed paraffin embedded human kidney labelling CD34 with ab198395 at a concentration of 0.5µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32 min with ULTRA cell conditioning solution (CC1 pH 8.5). ab198395 anti CD34 antibody was incubated at 37°C for 16min. Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

Intracellular Flow Cytometry analysis of TF-1 (human erythroleukemia) cells labeling CD34 with purified <u>ab81289</u> at 1/50 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit lgG (Alexa Fluor[®] 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal lgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab81289).

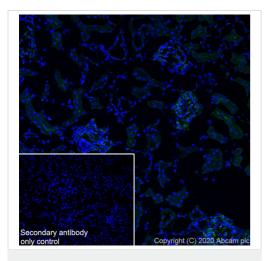


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD34 antibody [EP373Y]

- BSA and Azide free (ab198395)

This image is courtesy of an Abreview submitted by Rudolf Jung.

Paraformaldehyde-fixed, paraffin-embedded mouse kidney tissue stained for CD34 using <u>ab81289</u> at 1/200 dilution in immunohistochemical analysis.

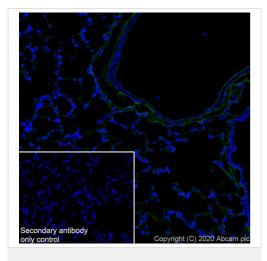


Immunohistochemistry (Frozen sections) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

This data was developed using <u>ab81289</u>, the same antibody clone in a different buffer formulation.

Secondary antibody control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) at 1000 dilution.<\p>

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



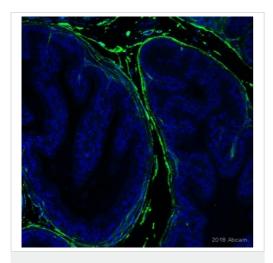
Immunohistochemistry (Frozen sections) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

This data was developed using <u>ab81289</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat lung tissue labeling CD34 with **ab81289** at 1/50 (11.04 µg/mL) dilution followed by **ab150077** Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) at 1/1000 dilution (Green). Positive staining on rat lung. is observed. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) at 1000 dilution.<\p>

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

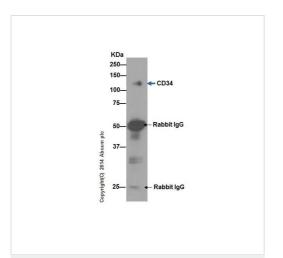


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

This image is courtesy of an anonymous Abreview.

Paraformaldehyde-fixed, paraffin-embedded mouse prostate tissue stained for CD34 using <u>ab81289</u> at 1/150 dilution in immunohistochemical analysis.

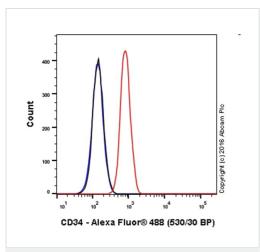
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab81289).



Immunoprecipitation - Anti-CD34 antibody [EP373Y]

- BSA and Azide free (ab198395)

CD34 was immunoprecipitated from TF-1 (Human bone marrow erythroleukemia cells) cells using purified <u>ab81289</u> at 1/30 dilution. Western blot was performed from the immunoprecipitate using <u>ab81289</u>. Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated was used as secondary antibody at 1/1000 dilution. Blocking and dilution buffer and concentration: 5% NFDM/TBST



Flow Cytometry (Intracellular) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

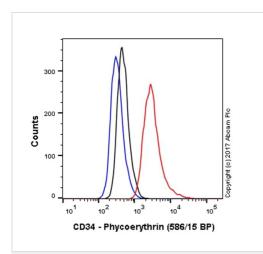
Clone EP373Y (ab198395) has been successfully conjugated by Abcam. This image was generated using Anti-CD34 antibody [EP373Y] (Alexa Fluor[®] 488). Please refer to **ab195013** for protocol details.

Overlay histogram showing HUVEC cells stained with <u>ab195013</u> (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab195013, 1/500 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal)
Alexa Fluor[®] 488 (<u>ab199091</u>) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

This antibody gave a positive signal in HUVEC cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



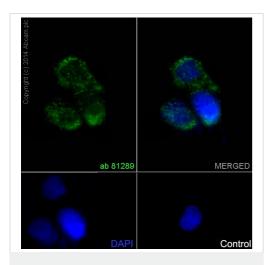
Flow Cytometry (Intracellular) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

Clone EP373Y (ab198395) has been successfully conjugated by Abcam. This image was generated using Anti-CD34 antibody [EP373Y] (PE). Please refer to ab223930 for protocol details.

Overlay histogram showing HUVEC cells stained with <u>ab223930</u> (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab223930, 1/500 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Phycoerythrin (<u>ab209478</u>) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter.

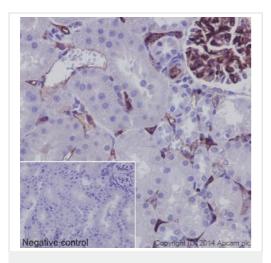


Immunocytochemistry/ Immunofluorescence - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

Immunocytochemistry/Immunofluorescence analysis of HUVEC (Human umbilical vein endothelial cell line) cells labelling CD34 with purified ab81289 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. An Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (ab150077) (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counter stain.

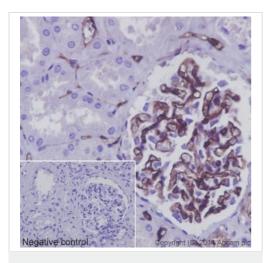
Control: Secondary antibody only, <u>ab150120</u>, an Alexa Fluor[®] 488-conjugated goat anti-mouse IgG (1/500).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab81289).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

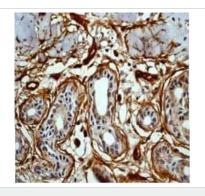
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat kidney tissue labelling CD34 with purified **ab81289** at 1/2500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. Goat Anti-Rabbit lgG H&L (HRP) (**ab97051**) was used as the secondary antibody at 1/500. Negative control using PBS instead of primary antibody. Counter stained with Hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue labelling CD34 with purified ab81289 at 1/2500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. Goat Anti-Rabbit IgG H&L (HRP) (ab97051) was used as the secondary antibody at 1/500. Negative control using PBS instead of primary antibody. Counter stained with Hematoxylin.

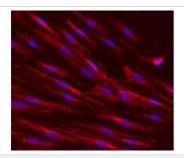
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab81289).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human angiosarcoma labeling CD34 with unpurified <u>ab81289</u> at 1/100-1/250.

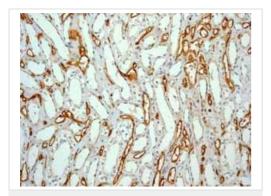
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab81289).



Immunocytochemistry/ Immunofluorescence - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

Image from F?Ides G et al. PLoS One. 2010 May 5;5(5):e10501 Fig 3.

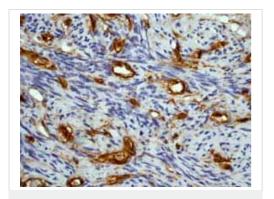
Immunocytochemistry/Immunofluorescence analysis of human embryonic stem cell-derived endothelial cells labeling CD34 with unpurified <u>ab81289</u> at 1/200. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.2% aTriton X-100. An Alexa Fluor[®] 647-conjugated secondary antibody was used at a 1/400 dilution. DAPI (blue) was used as the nuclear counter stain.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Normal kidney vessels tissue labeling CD34 with unpurified <u>ab81289</u> at 1/100-1/250.

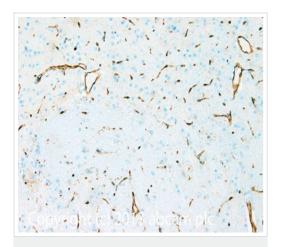
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81289**).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Normal uterus vessels tissue labeling CD34 with unpurified **ab81289** at 1/100-1/250.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab81289).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

IHC image of CD34 staining in Mouse normal brain formalin fixed paraffin embedded tissue section, performed on a Leica BondTM system using the standard protocol B. 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 unpurified ab81289, 1/250 dilution, for 15 mins at room temperature. A Goat anti-Rabbit biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC 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

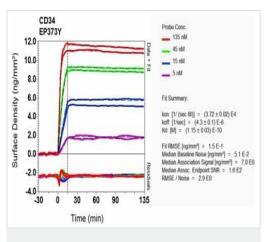
incubation times.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab81289).

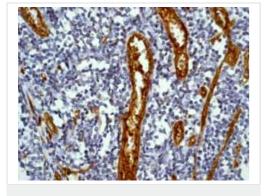
Equilibrium disassociation constant (K_D) Learn more about K_D

Click here to learn more about KD

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab81289).



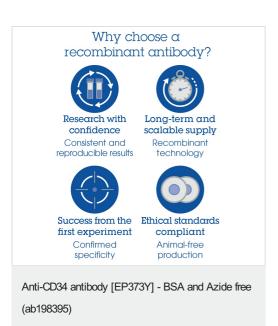
Ol-RD Scanning - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD34 antibody [EP373Y] - BSA and Azide free (ab198395)

This IHC data was generated using the same anti-CD34 antibody clone, EP373Y, in a different buffer formulation (cat# <u>ab81289</u>).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Normal tonsil vessels tissue labeling CD34 with unpurified <u>ab81289</u> at 1/100-1/250.



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