

Product Datasheet

CD31/PECAM-1 Antibody (MEC 7.46) - BSA Free NB100-1642

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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NB100-1642

CD31/PECAM-1 Antibody (MEC 7.46) - BSA Free

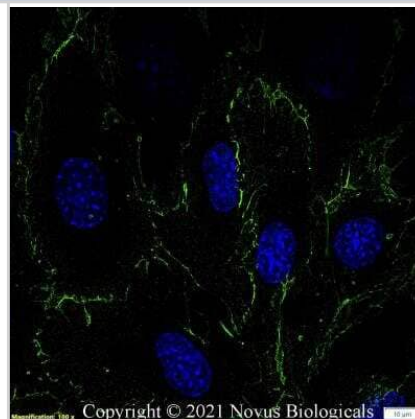
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	MEC 7.46
Preservative	0.05% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	82.5 kDa

Product Description	
Host	Rat
Gene ID	5175
Gene Symbol	PECAM1
Species	Mouse
Immunogen	This CD31/PECAM-1 Antibody (MEC 7.46) was developed against mouse endothelial cell line T-end.

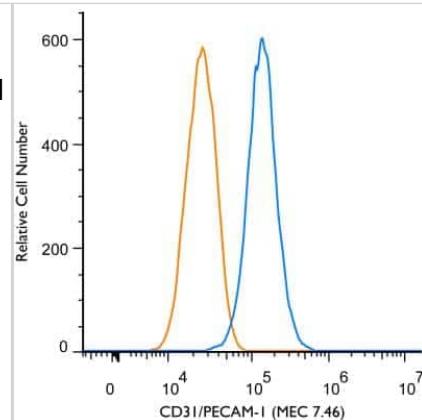
Product Application Details	
Applications	Flow Cytometry, Flow (Cell Surface), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vivo assay, Immunoprecipitation
Recommended Dilutions	Flow Cytometry 1:10-1:1000, Immunohistochemistry 1:100-1:500, Immunocytochemistry/ Immunofluorescence 1:100-1:500, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:10-1:500. Use reported in scientific literature (PMID 25525387), Immunohistochemistry-Frozen 1:100-1:500, In vivo assay reported in scientific literature (PMID 10544206), Flow (Cell Surface)

Images

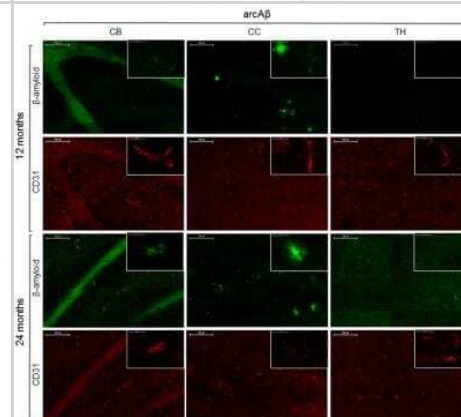
Immunocytochemistry/Immunofluorescence: CD31/PECAM-1 Antibody (MEC 7.46) [NB100-1642] - Mouse MS1 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD31/PECAM-1 Antibody [MEC 7.46] (NB100-1642) at 1ug/ml overnight at 4C and detected with an anti-rat DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



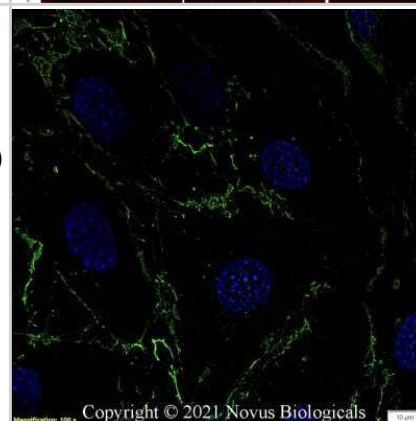
Flow (Cell Surface): CD31/PECAM-1 Antibody (MEC 7.46) [NB100-1642] - A surface stain was performed on MS-1 Cells with CD31/PECAM-1 Antibody (MEC 7.46) NB100-1642(blue) and a matched isotype control (orange). Cells were incubated in an antibody dilution of 2.5 ug/mL for 20 minutes at room temperature, followed by rat F(ab)2 IgG (H+L) APC-conjugated secondary antibody (F0113, R&D Systems).



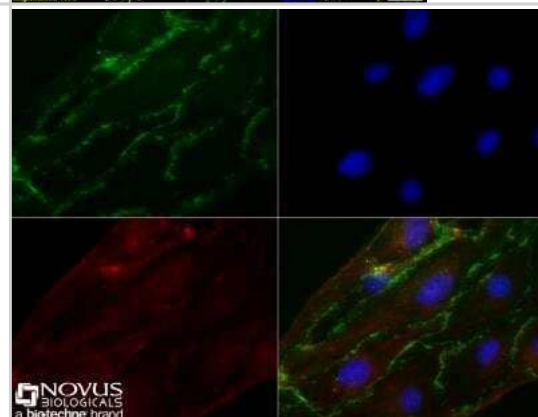
Immunohistochemistry: CD31/PECAM-1 Antibody (MEC 7.46) [NB100-1642] - Immunohistochemistry for vessel staining (CD31) and beta-amyloid comparing 13- and 24-month old arcAbeta mice. Overviews (scale bars = 200 um) of the corpus callosum (CC), thalamus (TH), and cerebellum (CB). Insets show the regions with higher magnification (scale bar = 50 um). Image collected and cropped by CiteAb from the following publication (<https://journal.frontiersin.org/Article/10.3389/fnagi.2015.00241/abstract>), licensed under a CC-BY license.



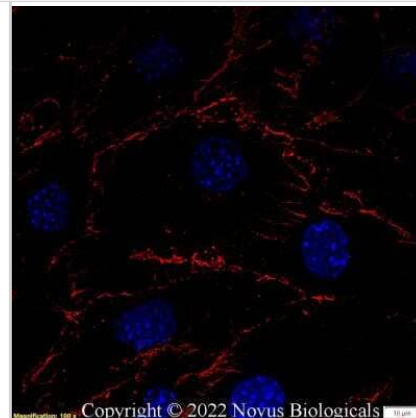
Immunocytochemistry/Immunofluorescence: CD31/PECAM-1 Antibody (MEC 7.46) [NB100-1642] - Mouse MS1 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD31/PECAM-1 Antibody [MEC 7.46] conjugated to Alexa Fluor 488 (NB100-1642AF488) at 5 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



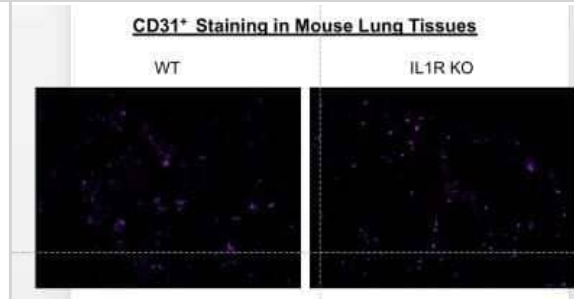
Immunocytochemistry/Immunofluorescence: CD31/PECAM-1 Antibody (MEC 7.46) [NB100-1642] - MS1 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with at 5.0 ug/ml overnight at 4C and detected with an anti-mouse DyLight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



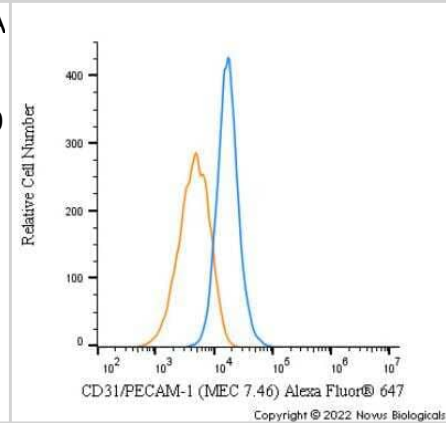
Immunocytochemistry/Immunofluorescence: CD31/PECAM-1 Antibody (MEC 7.46) [NB100-1642] - Mouse MS1 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD31/PECAM-1 Antibody [MEC 7.46] conjugated to DyLight 550 (NB100-1642R) at 5 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



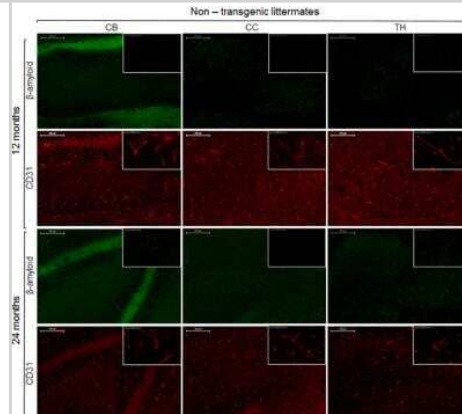
Immunohistochemistry-Frozen: CD31/PECAM-1 Antibody (MEC 7.46) [NB100-1642] - Frozen mouse lung tissues of WT B6 and IL1R^{-/-} (knockout) mice were blocked with 1% BSA in PBS and were double stained with Alexa647-conjugated CD31/PECAM-1 antibody (MEC 7.46) and PE-conjugated F4/80 for 2 hours at the room temperature. Image using the Alexa Fluor 647 format of this antibody.



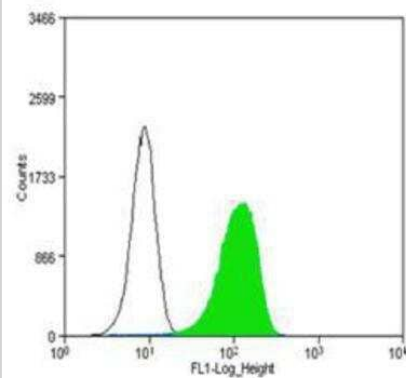
Flow Cytometry: CD31/PECAM-1 Antibody (MEC 7.46) [NB100-1642] - A surface stain was performed on MS1 cells with CD31/PECAM-1 [MEC 7.46] Antibody NB100-1642AF647 (blue) and a matched isotype control (orange). Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



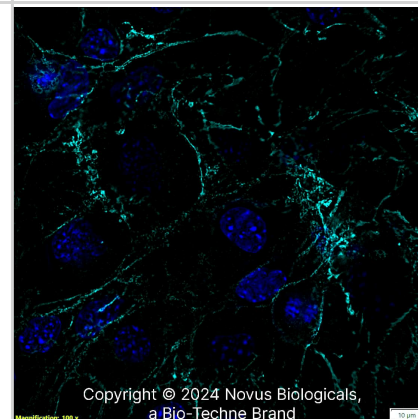
Immunohistochemistry: CD31/PECAM-1 Antibody (MEC 7.46) [NB100-1642] - Immunohistochemistry for vessel staining (CD31/PECAM-1) and beta-amyloid comparing 13- and 24-month old non-transgenic littermates. Overviews (scale bars = 200 um) of the corpus callosum (CC), thalamus (TH), and cerebellum (CB). Insets show the regions with higher magnification (scale bar = 50 um). Image collected and cropped by CiteAb from the following publication (<https://journal.frontiersin.org/Article/10.3389/fnagi.2015.00241/abstract>), licensed under a CC-BY license.



Flow Cytometry: CD31/PECAM-1 Antibody (MEC 7.46) [NB100-1642] - CD31/PECAM-1 Antibody (MEC 7.46) was tested at 1:250 in WEHI-3 cells with DyLight 488 (green) alongside a matched isotype control (black).



CD31/PECAM-1 (MEC 7.46) was detected in immersion fixed MS1 mouse pancreas/Islet of Langerhans endothelial cell line using Rat anti-CD31/PECAM-1 (MEC 7.46) Protein-G purified Monoclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NB100-1642AF647) (light blue) at 2 $\mu\text{g}/\text{mL}$ overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



Publications

Tallman MM Adult and pediatric brain tumors targeted with the small molecule drug CBL0137 Thesis 2023-01-01 (IHC)

Gau D, Daoud A, Allen A et al. Vascular endothelial profilin-1 drives a pro-tumorigenic tumor microenvironment and tumor progression in renal cancer *The Journal of biological chemistry* 2023-07-12 [PMID: 37451478] (IHC, Mouse)

Acharya BR, Fang JS, Jeffery ED et al. Connexin 37 sequestering of activated-ERK in the cytoplasm promotes p27-mediated endothelial cell cycle arrest *Life science alliance* 2023-08-01 [PMID: 37197981]

O'Brien A, Zhou T, White T et al. FGF1 Signaling Modulates Biliary Injury and Liver Fibrosis in the Mdr2^{-/-} Mouse Model of Primary Sclerosing Cholangitis *Hepatology communications* 2022-03-10 [PMID: 35271760]

Haxho F, Allison S, Alghamdi F et al. Oseltamivir phosphate monotherapy ablates tumor neovascularization, growth, and metastasis in mouse model of human triple-negative breast adenocarcinoma. *Breast Cancer Dove Med Press* 2015-12-19 [PMID: 25525387] (IHC-P, Mouse)

Meng R, Cai WK, Xu WM et al. Generation and identification of endothelial-specific Hrh2 knockout mice *Transgenic research* 2021-03-30 [PMID: 33786748] (IF/IHC, Mouse)

Bhatti FUR, Dadwal UC, Valuch CR et al The effects of high fat diet, bone healing, and BMP-2 treatment on endothelial cell growth and function *Bone* 2021-02-13 [PMID: 33581374] (ICC/IF, Mouse)

Details:

Citation using the DyLight 488 version of this antibody.

Serra A, Gallart-Palau X, Park J et al. Vascular Bed Molecular Profiling by Differential Systemic Decellularization In Vivo. *Arterioscler Thromb Vasc Biol* 2018-01-10 [PMID: 30354219] (ICC/IF, Mouse)

Aizawa E Characterization of in vitro systems to generate oocytes and substitutes of sperm from pluripotent stem cells Thesis 2019-01-01

Li Calzi S, Shaw LC, Moldovan L et al. Progenitor cell combination normalizes retinal vascular development in the oxygen-induced retinopathy (OIR) model *JCI Insight* 2019-11-02 [PMID: 31672944] (ICC/IF, Mouse)

Katori S, Noguchi-Katori Y, Itohara S, Iwasato T. Spinal RacGAP alpha-Chimaerin Is Required to Establish the Midline Barrier for Proper Corticospinal Axon Guidance *J. Neurosci.* 2017-08-09 [PMID: 28747385] (IF/IHC, Mouse)

Liu X, Hu J, Li Y et al. Mesenchymal stem cells expressing interleukin-18 inhibit breast cancer in a mouse model. *Oncol Lett* 2018-05-01 [PMID: 29725393]

More publications at <http://www.novusbio.com/NB100-1642>

Procedures

Flow (Cell Surface) Protocol for CD31/PECAM-1 Antibody (NB100-1642)

Protocol for Flow Cytometry Cell Surface Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2×10^5 and 1×10^6 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 μ L for counting, then transfer cell volume into a 15 mL conical tube and centrifuge for 4 minutes at 400 RCF.
 - a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1×10^6 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 μ L samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Cell surface staining

1. Recommended: Block non-specific interactions using 0.5-1 μ g of a species specific Fc-blocking reagent such as an anti-mouse CD16/CD32 antibody (NBP1-27946).
2. Add appropriate amount of each antibody (eg. 1 test or 1 μ g per sample, as experimentally determined) to 100 μ L of staining buffer (NBP2-26247) per sample (eg. use 1 mL of staining buffer for 10 samples).
3. Mix well and incubate at room temperature in dark for 20 minutes.
4. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
5. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 μ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
6. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
7. Incubate at room temperature in dark for 20 minutes.
8. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 μ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Resuspend in an appropriate volume of staining buffer (usually 500 μ L per sample) and proceed with analysis on your flow cytometer.

Immunocytochemistry/ Immunofluorescence Protocol for CD31/PECAM-1 Antibody (NB100-1642)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.

Immunohistochemistry-Paraffin Protocol for CD31/PECAM-1 Antibody (NB100-1642)

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.





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Products Related to NB100-1642

HAF005	Goat anti-Rat IgG Secondary Antibody [HRP]
F0105B	Goat anti-Rat IgG Secondary Antibody [Phycoerythrin]
DDXCR01	Rat IgG1 Isotype Control
NB100-1642AF647	CD31/PECAM-1 Antibody (MEC 7.46) [Alexa Fluor® 647]

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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