

# Product Datasheet

## CD31/PECAM-1 Antibody (MEC13.3) - BSA Free NB600-1475

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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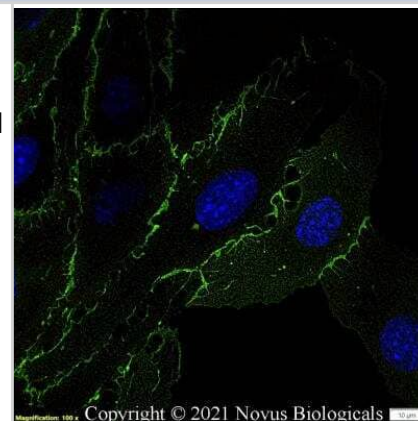
**NB600-1475****CD31/PECAM-1 Antibody (MEC13.3) - BSA Free**

<b>Product Information</b>	
<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	1.0 mg/ml
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Clonality</b>	Monoclonal
<b>Clone</b>	MEC13.3
<b>Preservative</b>	0.02% Sodium Azide
<b>Isotype</b>	IgG2a Kappa
<b>Purity</b>	Protein G purified
<b>Buffer</b>	PBS
<b>Target Molecular Weight</b>	82.5 kDa
<b>Product Description</b>	
<b>Host</b>	Rat
<b>Gene ID</b>	5175
<b>Gene Symbol</b>	PECAM1
<b>Species</b>	Human, Mouse
<b>Reactivity Notes</b>	Mouse (PMID: 7956830) and Human (PMID: 30626719) reactivity reported in scientific literature.
<b>Immunogen</b>	This CD31/PECAM-1 Antibody (MEC13.3) was developed against mouse endothelial cell line T-end.
<b>Product Application Details</b>	
<b>Applications</b>	Flow Cytometry, Flow (Cell Surface), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, In vitro assay, In vivo assay, Immunoprecipitation, CyTOF-ready
<b>Recommended Dilutions</b>	Flow Cytometry 2.5 ug/ml, Immunohistochemistry 1:100-1:500, Immunocytochemistry/ Immunofluorescence 1:500 - 1:1000, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Frozen 1:100 , In vitro assay reported in scientific literature (PMID 24647208), In vivo assay reported in scientific literature, Flow (Cell Surface) 2.5 ug/ml, CyTOF-ready
<b>Application Notes</b>	This CD31/PECAM1 Antibody (MEC13.3) is useful for in vitro and in vivo blocking of CD31-mediated cell-cell interactions. Please Note: NB600-1475 works well on acetone-fixed frozen sections as well as zinc-fixed paraffin-embedded sections. However, inconsistent results were observed for formalin-fixed paraffin-embedded sections. This antibody is CyTOF ready.

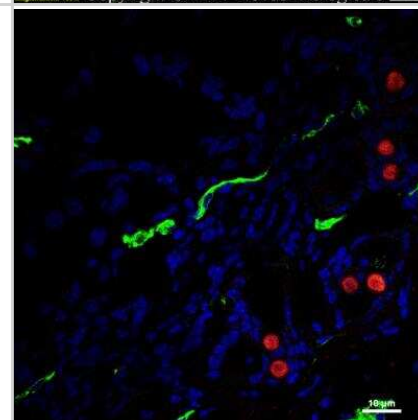


## Images

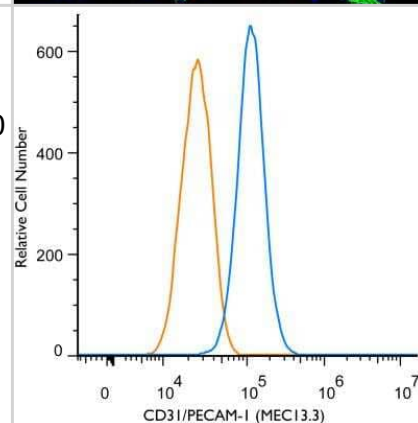
**Immunocytochemistry/Immunofluorescence:** CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475] - Mouse MS1 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD31/PECAM-1 Antibody [MEC13.3] (NB600-1475) at 1ug/ml 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.



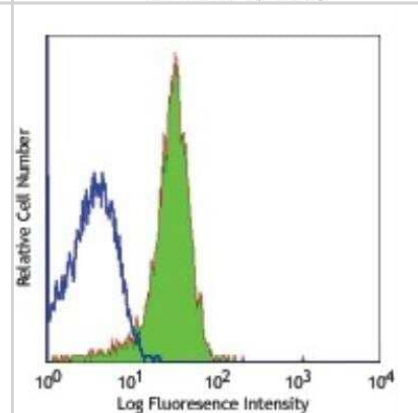
**Immunohistochemistry-Frozen:** CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475] - 4T1 tumor sections were stained for CD31 (green) and CD105 (red).



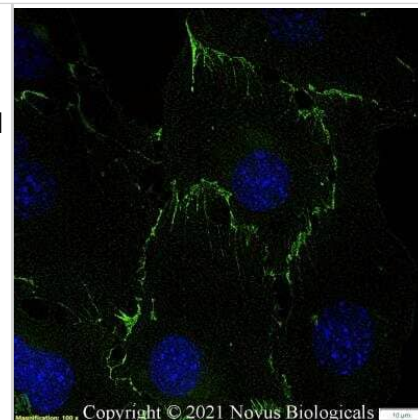
**Flow (Cell Surface):** CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475] - A surface stain was performed on WEHI-3 Cells with CD31/PECAM-1 Antibody (MEC13.3) (NB600-1475, blue) and a matched isotype control (orange). Cells were incubated in an antibody dilution of 2.5 ug/mL for 20 minutes at room temperature, followed by rat F(ab)2 IgG (H+L) APC-conjugated secondary antibody (F0113, R&D Systems).



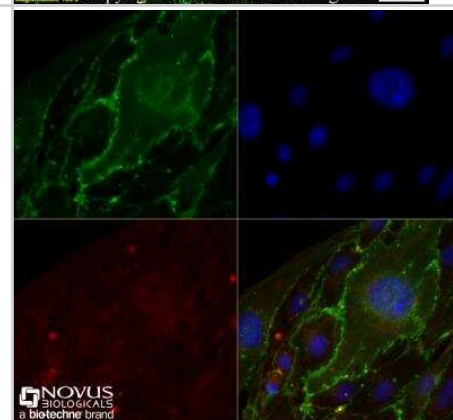
**Flow Cytometry:** CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475] - C57BL/6 mouse splenocytes stained with purified CD31/PECAM-1 Antibody (MEC13.3), followed by anti-rat IgG FITC



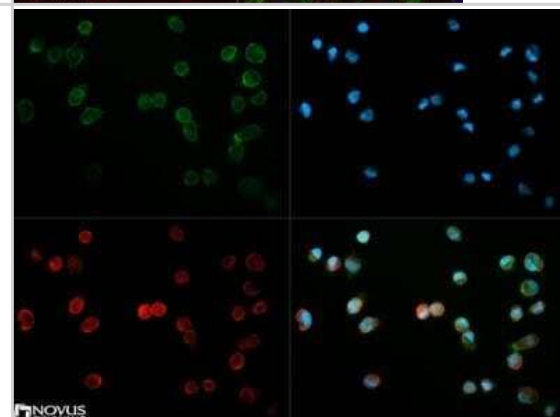
Immunocytochemistry/Immunofluorescence: CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475] - Mouse MS1 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD31/PECAM-1 Antibody [MEC13.3] conjugated to DyLight 488 (NB600-1475G) 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.



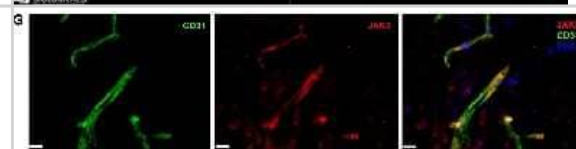
Immunocytochemistry/Immunofluorescence: CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475] - MS1 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with at 5.0 ug/ml overnight at 4C and detected with an anti-mouse 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.



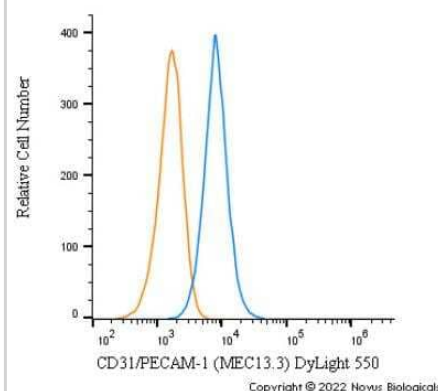
Immunocytochemistry/Immunofluorescence: CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475] - was tested in Wehi-3 cells with DyLight 488 (green). Nuclei and beta-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



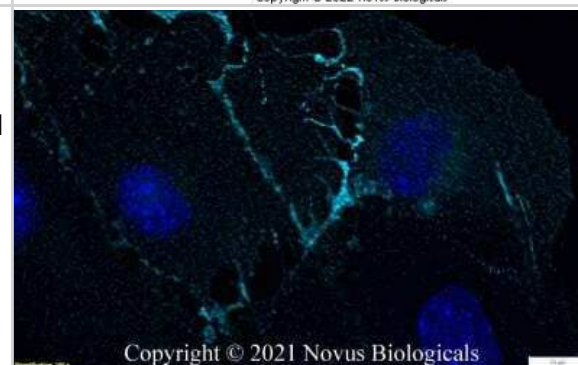
Immunohistochemistry-Frozen: CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475] - JAK3 colocalized with endothelial cells labeled with CD31. Image collected and cropped by CiteAb from the following publication (<https://journal.frontiersin.org/article/10.3389/fneur.2017.00363/full>), licensed under a CC-BY license.



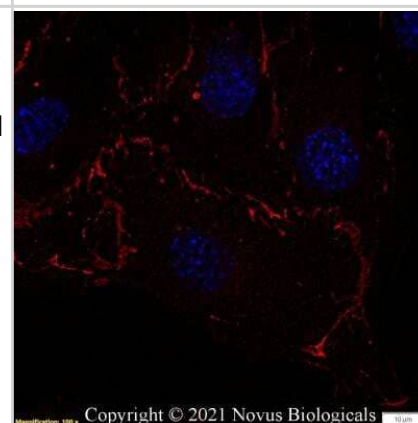
**Flow Cytometry: CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475] - A surface stain was performed on MS1 cells with CD31/PECAM-1 [MEC13.3] Antibody NB600-1475R (blue) and a matched isotype control (orange). Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 550.**



**Immunocytochemistry/Immunofluorescence: CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475] - 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 [MEC13.3] conjugated to Alexa Fluor 647 (NB600-1475AF647) 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 (MEC13.3) [NB600-1475] - 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 [MEC13.3] conjugated to DyLight 550 (NB600-1475R) 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.**

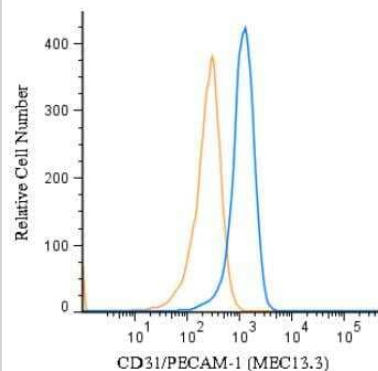


**Immunohistochemistry-Frozen: CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475] - Staining of mouse CD31 in non-small cell lung cancer tumor sections from a human NSCLC tumor-bearing mouse. This image was submitted via customer Review.**



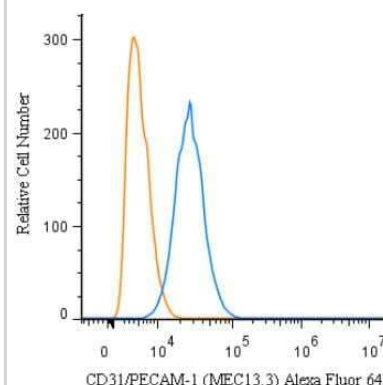


**Flow (Cell Surface): CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475]** - A surface stain was performed on WEHI-3 Cells with CD31 (MEC13.3) antibody NB600-1475 (blue) and a matched isotype control (orange). Cells were incubated in an antibody dilution of 2.5 ug/mL for 20 minutes at room temperature, followed by rat F(ab)<sub>2</sub> IgG (H+L) APC-conjugated secondary antibody (F0113, R&D Systems).



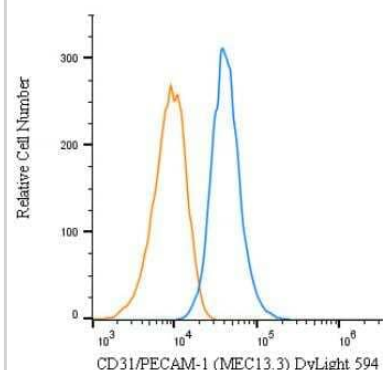
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**Flow Cytometry: CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475]** - A surface stain was performed on MS-1 cells with CD31/PECAM-1 Antibody (MEC13.3) (NB600-1475AF647, blue) and a matched isotype control (orange). Cells were incubated in an antibody dilution of 5 ug/mL for 20 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



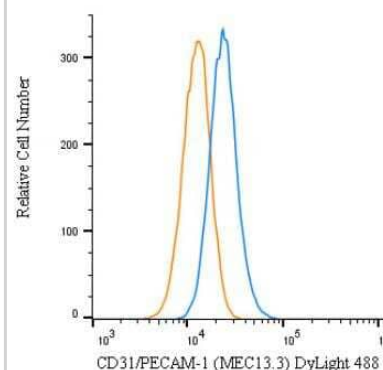
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**Flow Cytometry: CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475]** - A surface stain was performed on MS1 cells with CD31/PECAM-1 [MEC13.3] Antibody NB600-1475DL594 (blue) and a matched isotype control (orange). Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 594.



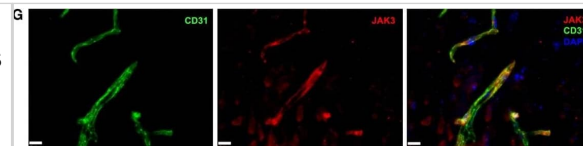
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**Flow Cytometry: CD31/PECAM-1 Antibody (MEC13.3) [NB600-1475]** - A surface stain was performed on MS1 cells with CD31/PECAM-1 [MEC13.3] Antibody NB600-1475G (blue) and a matched isotype control (orange). Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 488.



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Immunocytochemistry/ Immunofluorescence: CD31/PECAM-1 Antibody (MEC13.3) - BSA Free [NB600-1475] - (A) Representative Western blots depicting phosphorylated JAK3 (pJAK3), total JAK3, & corresponding  $\beta$ -actin levels in the ipsilateral (CXI) versus the contralateral (CXC) cortex at 24 h in sham & mice subjected to permanent middle cerebral artery occlusion (pMCAO). (B,C) Quantified pJAK3 & JAK3 values normalized to  $\beta$ -actin in the cortex of sham & mice subjected to stroke. Both pJAK3 & JAK3 are significantly increased in the ipsilateral cortex of stroked animals (\* $P < 0.05$  & \*\* $P < 0.01$  with respect to sham ipsilateral/contralateral & stroke contralateral; sham,  $n = 5$ ; stroke,  $n = 13$ ). Data were analyzed using a two-way ANOVA (ipsilateral/contralateral or sham/stroke) with Bonferroni post-test. (D) Diagram showing peri-infarct brain region from which images were taken ( $n = 3$ ). Scale bar is  $10\ \mu\text{m}$ , & magnification is  $60\times$ . (E) JAK3 colocalized with microglia/macrophages labeled with Iba-1. (F) JAK3 colocalized with neurons labeled with NeuN. (G) JAK3 colocalized with endothelial cells labeled with CD31. (H) JAK3 is not colocalized with astrocytes labeled with GFAP. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28790974>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Yang C, Gao Q, Xu N et al. Human Dental Pulp Stem Cells Are Subjected to Metabolic Reprogramming and Repressed Proliferation and Migration by the Sympathetic Nervous System via  $\alpha$ 1B-Adrenergic Receptor Journal of endodontics 2023-09-27 [PMID: 37769871]

Honig MG, Dorian CC, Worthen JD et al. Progressive long-term spatial memory loss following repeat concussive and subconcussive brain injury in mice, associated with dorsal hippocampal neuron loss, microglial phenotype shift, and vascular abnormalities European Journal of Neuroscience 2021-09-01 [PMID: 32090401]

Zhang Y, Wang D, Zhao Z et al. Nephronectin promotes cardiac repair post myocardial infarction via activating EGFR/JAK2/STAT3 pathway International Journal of Medical Sciences 2022-05-13 [PMID: 35693734] (Immunohistochemistry-Frozen, Immunocytochemistry/ Immunofluorescence)

Ugur M, Labios RJ, Fenton C et al. Lymph node medulla regulates the spatiotemporal unfolding of resident dendritic cell networks Immunity 2023-07-07 [PMID: 37463581] (ICC/IF)

Details:

Alexa Fluor 700 conjugation used

Pang L, Dunterman M, Xuan W et al. Circadian regulator CLOCK promotes tumor angiogenesis in glioblastoma Cell reports 2023-02-14 [PMID: 36795563] (IHC, Human)

Wei W, Liu Q, Jiang D et al. Tissue Factor-Targeted ImmunoPET Imaging and Radioimmunotherapy of Anaplastic Thyroid Cancer Adv Sci (Weinh) 2020-07-17 [PMID: 32670751]

Deng W, Guo S, van Veluw SJ et al. Effects of cerebral amyloid angiopathy on the brain vasculome Aging cell 2022-07-18 [PMID: 35851991] (IF/IHC, Mouse)

Chen y, Wang H, yang Q et al. Single-cell RNA landscape of the osteoimmunology microenvironment in periodontitis Theranostics 2022-01-01 [PMID: 35154475] (IHC-P, Human)

Ensan, S, Li, A Et al. Self-renewing resident arterial macrophages arise from embryonic CX3CR1(+) precursors and circulating monocytes immediately after birth. Nat Immunol 2016-02-01 [PMID: 26642357] (FLOW, Human)

Yang C, Lavayen BP, Liu L Et al. Neurovascular protection by adropin in experimental ischemic stroke through an endothelial nitric oxide synthase-dependent mechanism Redox biology 2021-11-22 [PMID: 34826783] (IF/IHC, Mouse)

Park M, Kim J, Kim T Et al. REDD1 is a determinant of low-dose metronomic doxorubicin-elicited endothelial cell dysfunction through downregulation of VEGFR-2/3 expression Experimental & molecular medicine 2021-10-25 [PMID: 34697389] (IF/IHC, Mouse)

Mao XW, Nishiyama NC, Byrum SD et al. Spaceflight induces oxidative damage to blood-brain barrier integrity in a mouse model FASEB J. 2020-09-26 [PMID: 32981077] (IF/IHC, Mouse)

More publications at <http://www.novusbio.com/NB600-1475>





## Procedures

### Immunocytochemistry/ Immunofluorescence Protocol for CD31/PECAM-1 Antibody (NB600-1475)

#### 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.

### Flow (Cell Surface) Protocol for CD31/PECAM-1 Antibody (NB600-1475)

#### Protocol for Flow Cytometry Cell Surface Staining

##### Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between  $2 \times 10^5$  and  $1 \times 10^6$  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 uL 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  $1 \times 10^6$  cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL 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 ug 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 ug per sample, as experimentally determined) to 100 uL 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 uL 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 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.





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General: novus@novusbio.com

### **Products Related to NB600-1475**

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HAF005	Goat anti-Rat IgG Secondary Antibody [HRP]
NBP1-75398	Goat anti-Rat IgG (H+L) Secondary Antibody (Pre-adsorbed)
NBP1-43321-0.5mg	Rat IgG2a Kappa Light Chain Isotype Control (R2a)
NB600-1475AF647	CD31/PECAM-1 Antibody (MEC13.3) [Alexa Fluor® 647]

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### **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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