

Product Datasheet

CD68/SR-D1 Antibody (KP1) - BSA Free NB100-683-0.1mg

Unit Size: 0.1 mg

Store at 4C. Do not freeze.

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NB100-683-0.1mg

CD68/SR-D1 Antibody (KP1) - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Monoclonal
Clone	KP1
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS

Product Description	
Description	Novus Biologicals Mouse CD68/SR-D1 Antibody (KP1) - BSA Free (NB100-683) is a monoclonal antibody validated for use in IHC, WB, Flow, Dual RNAscope ISH-IHC, ICC/IF and IP. Anti-CD68/SR-D1 Antibody: Cited in 68 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	968
Gene Symbol	CD68
Species	Human, Mouse, Rat
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 25058444)
Marker	Macrophage Marker
Specificity/Sensitivity	This CD68/SR-D1 Antibody (KP1) is specific to macrophages in a wide variety of human tissues. It reacts with myeloid precursors and peripheral blood granulocytes. It also stains a cell population known as Plasmacytoid T cells.
Immunogen	This CD68/SR-D1 Antibody (KP1) was developed against subcellular fraction of human alveolar macrophages.

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Electron Microscopy, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation, Dual RNAscope ISH-IHC
Recommended Dilutions	Western Blot 2 ug/ml, Flow Cytometry reported in scientific literature (PMID 18405323), Immunohistochemistry 1:100-1:200, Immunocytochemistry/ Immunofluorescence 1-5 ug/ml, Immunoprecipitation, Immunohistochemistry-Paraffin 1:100-1:200, Immunohistochemistry-Frozen 1:100-1:200, Electron Microscopy reported in scientific literature (PMID 8962141), Dual RNAscope ISH-IHC
Application Notes	IHC: For an ABC system, dilute 1:20 and incubate for 30-60 minutes at RT. IHC-P tissue sections require high temperature antigen unmasking with 10 mM citrate buffer, pH 6.0 prior to immunostaining.

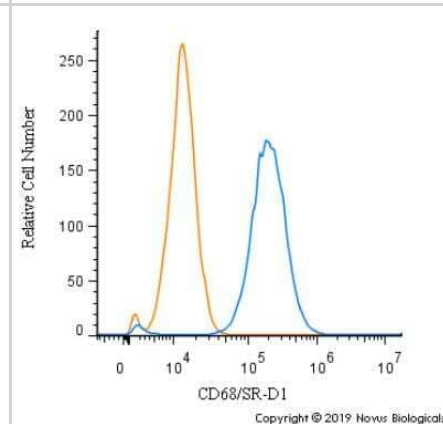


Images

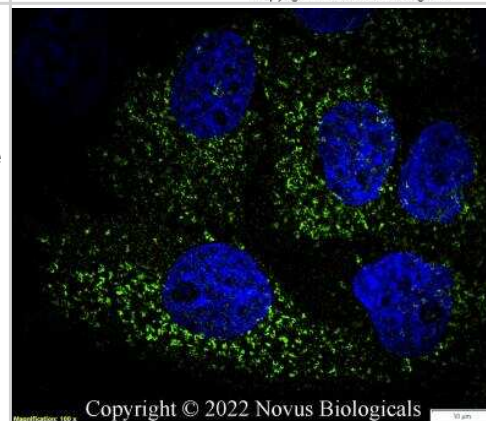
Immunohistochemistry: CD68/SR-D1 Antibody (KP1) [NB100-683] - Representative images (40x) of infiltrated macrophages stained with anti-CD 68 in the gingiva. The black arrowheads indicate the infiltrated macrophages. The right graphs show the quantitative data of CD68-positive cells in HPF (magnification of 200x). Data are expressed as the mean +/- SEM of three slides. * $p < 0.05$ compared with control cells. Image collected and cropped by CiteAb from the following publication ([//doi.org/10.1371/journal.pone.0102450](https://doi.org/10.1371/journal.pone.0102450)) licensed under a CC-BY license.



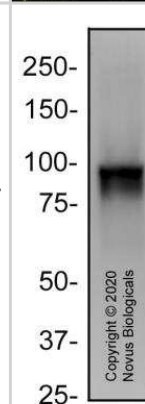
Flow Cytometry: CD68/SR-D1 Antibody (KP1) [NB100-683] - An intracellular stain was performed on THP-1 cells with CD68/SR-D1 Antibody NB100-683 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1:50 for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody.



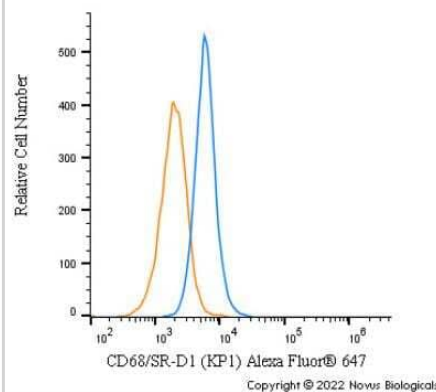
Immunocytochemistry/Immunofluorescence: CD68/SR-D1 Antibody (KP1) [NB100-683] - A431 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD68/SR-D1 Antibody [KP1] (NB100-683) at 1 ug/ml overnight at 4C and detected with an anti-mouse 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.



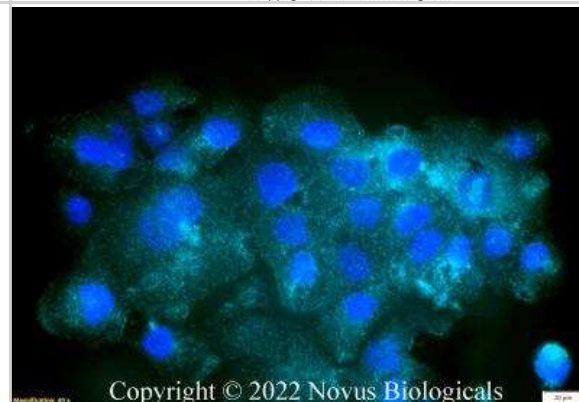
Western Blot: CD68/SR-D1 Antibody (KP1) [NB100-683] - Total protein from mouse Raw cells was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-CD68 (NB100-683) in blocking buffer and detected with an anti-mouse HRP secondary antibody using NovaLume chemiluminescence detection reagent (NPB2-61915).



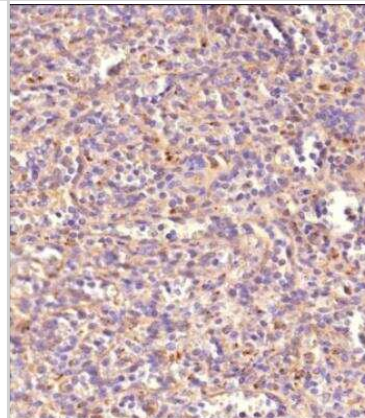
Flow Cytometry: CD68/SR-D1 Antibody (KP1) [NB100-683] - An intracellular stain was performed on A431 cells with CD68/SR-D1 [KP1] Antibody NB100-683AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



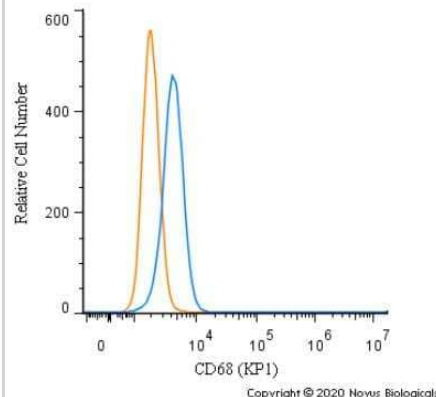
Immunocytochemistry/Immunofluorescence: CD68/SR-D1 Antibody (KP1) [NB100-683] - A431 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD68/SR-D1 Antibody [KP1] conjugated to Alexa Fluor 647 (NB100-683AF647) at 5 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



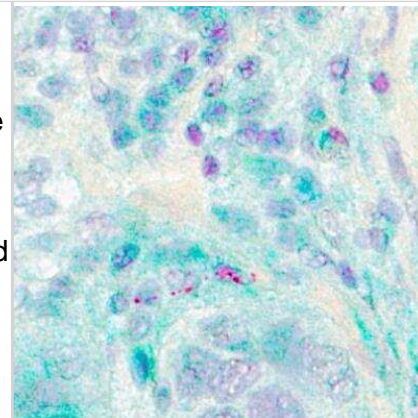
Immunohistochemistry-Paraffin: CD68/SR-D1 Antibody (KP1) [NB100-683] - Analysis of a FFPE tissue section of human spleen using 1:200 dilution of CD68 antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



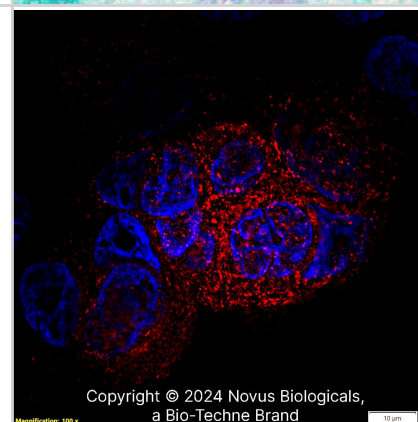
Flow Cytometry: CD68/SR-D1 Antibody (KP1) [NB100-683] - An intracellular stain was performed on THP-1 cells with CD68 Antibody [KP1] NB100-683 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).



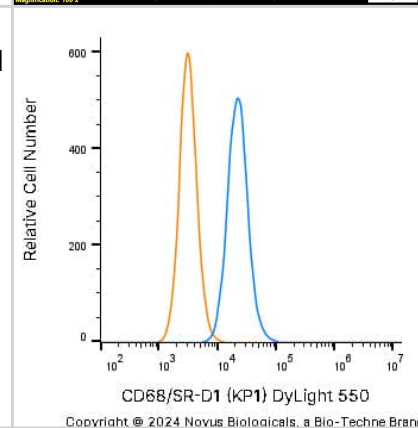
Dual RNAscope ISH-IHC: CD68/SR-D1 Antibody (KP1) [NB100-683] - NOS2 mRNA (red) and CD68 protein (green) were detected in formalin-fixed paraffin-embedded tissue sections of human breast cancer. ACD's Integrated Co-Detection Workflow was performed using ACD RNAscope Probe Hs-NOS2 and CD68/SR-D1 antibody (KP1) at 1:100 dilution. Tissue was stained on Leica Bond RX using RNAscope (TM) 2.5 LS Reagent Kit-RED, BOND Polymer Refine Detection (DAB) and Hematoxylin, BOND Polymer Refine Red Detection and Hematoxylin and RNAscope (TM) 2.5 LS Green Accessory Pack. Tissue was counterstained with 50% hematoxylin (blue).



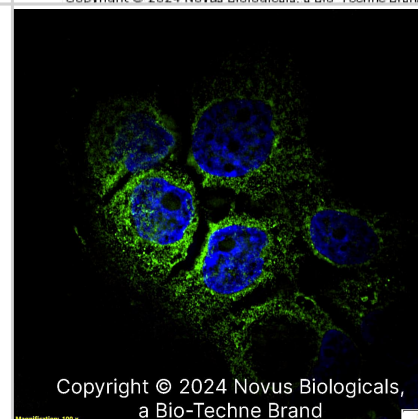
CD68/SR-D1 (KP1) was detected in immersion fixed A431 human skin carcinoma cell line using Mouse anti-CD68/SR-D1 (KP1) Protein-G purified Monoclonal Antibody conjugated to DyLight 550 (Catalog # NB100-683R) (red) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



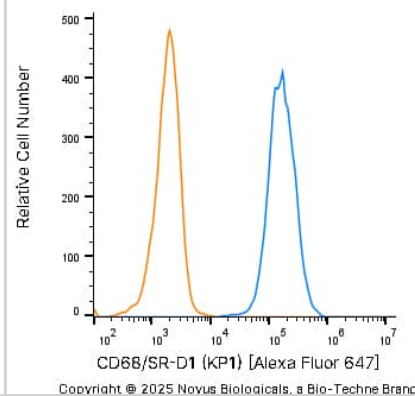
An intracellular stain was performed on A431 human skin carcinoma cell line using Mouse anti- CD68/SR-D1 (KP1) Protein-G purified Monoclonal Antibody conjugated to DyLight 550 (Catalog # NB100-683R, blue histogram) or matched control antibody (orange histogram) at 2.5 µg/mL for 30 minutes at RT.



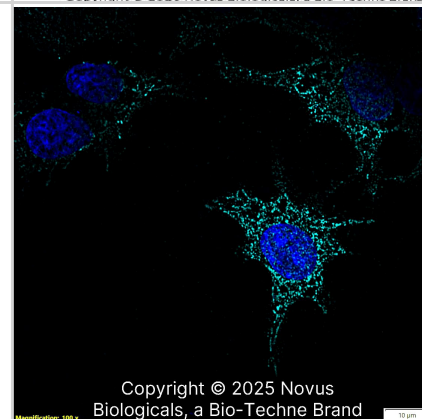
CD68/SR-D1 (KP1) was detected in immersion fixed A431 human skin carcinoma cell line using Mouse anti-CD68/SR-D1 (KP1) Protein-G purified Monoclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NB100-683AF488) (green) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



An intracellular stain was performed on THP-1 human acute monocytic leukemia cell line with Mouse anti-CD68/SR-D1 (KP1) Protein-G purified Monoclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NB100-683AF647, blue histogram) or matched control antibody (orange histogram) at 2.5 µg/mL for 30 minutes at RT.



CD68/SR-D1 Antibody (KP1) was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Mouse anti-CD68/SR-D1 Antibody (KP1) Protein G Purified Monoclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NB100-683AF647) (light blue) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



Publications

Chen LH, Yeh YM, Chen YF et al. Targeting interleukin-20 alleviates paclitaxel-induced peripheral neuropathy Pain 2020-06-01 [PMID: 32068666] (Human)

Liu W, Danckwardt-Lillieström N, Schrott-Fischer A et al. Distribution of Immune Cells Including Macrophages in the Human Cochlea Frontiers in Neurology 2021-11-22 [PMID: 34880828] (Human)

Lim CS, Veltri B, Kashon M et al. Multi-walled carbon nanotubes induce arachidonate 5-lipoxygenase expression and enhance the polarization and function of M1 macrophages in vitro Nanotoxicology 2023-04-28 [PMID: 37115655] (Human)

Jia X, Zhai T, Qu C et al. Metformin Reverses Hashimoto's Thyroiditis by Regulating Key Immune Events Frontiers in Cell and Developmental Biology 2021-05-28 [PMID: 34124070] (Human)

Zhang J, Yin Z, Yu L et al. Macrophage Rmp Ameliorates Myocardial Infarction by Modulating Macrophage Polarization in Mice Oxidative Medicine and Cellular Longevity 2022-09-01 [PMID: 36092156] (Human)

CY Lin, KY Huang, SH Kao, MS Lin, CC Lin, SC Yang, WC Chung, YH Chang, RJ Chein, PC Yang Small-molecule PIK-93 modulates the tumor microenvironment to improve immune checkpoint blockade response Science Advances, 2023-04-07;9(14):eade9944. 2023-04-07 [PMID: 37027467] (Human)

Maria A. Beamer, Cassandra Zamora, Andrea L. Nestor-Kalinoski, Veani Fernando, Vandana Sharma, Saori Furuta Novel 3D Flipwell system that models gut mucosal microenvironment for studying interactions between gut microbiota, epithelia and immunity Scientific Reports 2023-01-17 [PMID: 36650266]

Zhang T, Zhang Y, Yang Z et al. Echinococcus multilocularis protoscoleces enhance glycolysis to promote M2 Macrophages through PI3K/Akt/mTOR Signaling Pathway Pathogens and global health 2023-06-01 [PMID: 35876088] (IHC-P, Human)

Pan Y, Hu Q, Yang Y et al. Characterization of pain-related behaviors and gene expression profiling of peripheral sensory ganglia in a mouse model of acute ankle sprain Frontiers in Behavioral Neuroscience 2023-05-25 [PMID: 37304762] (IHC-Fr, Mouse)

Zhai M, Gong S, Luan P et al. Extracellular traps from activated vascular smooth muscle cells drive the progression of atherosclerosis Nature communications 2022-12-06 [PMID: 36473863] (WB, IHC-Fr, IHC-P, Mouse, Human)

Rivellese F, Surace AEA, Goldmann K Et al. Rituximab versus tocilizumab in rheumatoid arthritis: synovial biopsy-based biomarker analysis of the phase 4 R4RA randomized trial Nat Med 2022-05-19 [PMID: 35589854] (IHC-P, Human)

Details:

Citation using the Alexa Fluor 532 version of this antibody.

Real F, Zhu A, Huang B et al. S100A8-mediated metabolic adaptation controls HIV-1 persistence in macrophages in vivo Nature communications 2022-10-11 [PMID: 36220814] (IHC-P, Human)

More publications at <http://www.novusbio.com/NB100-683>

Procedures

Western Blot Protocol for CD68/SR-D1 Antibody (NB100-683)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.



Flow (Intracellular) Protocol for CD68/SR-D1 Antibody (NB100-683)

Protocol for Flow Cytometry Intracellular 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 50 mL conical tube and centrifuge for 8 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.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 μ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 μ L of a permeabilization buffer to every 1×10^6 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
 - a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
 - b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 μ L of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 μ g per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer + 0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
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. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. 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.
14. Resuspend in an appropriate volume of staining buffer (usually 500 μ L per sample) and proceed with analysis on your flow cytometer.



Immunohistochemistry-Paraffin Protocol for CD68/SR-D1 Antibody (NB100-683)

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 all the time).

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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General: novus@novusbio.com

Products Related to NB100-683-0.1mg

NBL1-08962	CD68/SR-D1 Overexpression Lysate
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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