

# Product Datasheet

## CD11b Antibody - BSA Free

### NB110-89474

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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**NB110-89474**

CD11b Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	127.2 kDa

Product Description	
Description	Novus Biologicals Rabbit CD11b Antibody - BSA Free (NB110-89474) is a polyclonal antibody validated for use in IHC, WB, Flow, Dual RNAscope ISH-IHC, ICC/IF and Simple Western. Anti-CD11b Antibody: Cited in 123 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	3684
Gene Symbol	ITGAM
Species	Human, Mouse, Rat, Bovine, C. elegans, Chinese Hamster, Monkey, Rhesus Macaque
Reactivity Notes	Use in Mouse reported in scientific literature (PMID:35398596). Monkey reactivity reported in scientific literature (PMID: 26443820). Rhesus Macaque reactivity reported in scientific literature (PMID: 29760177). Use in C. elegans reported in scientific literature (PMID:32058942).
Marker	Microglia Marker, Myeloid Marker
Immunogen	Rabbit Polyclonal CD11b Antibody was made to a synthetic peptide within residues 250-350 of the mouse CD11b protein. [Swiss-Prot# P05555]

Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, Flow Cytometry, Flow (Cell Surface), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, In-situ Hybridization, Dual RNAscope ISH-IHC, Single Cell Western
Recommended Dilutions	Western Blot 2 ug/mL. Use reported in scientific literature (PMID 31082627), Simple Western 1:50, Flow Cytometry reported in scientific literature (PMID 21422470), Immunohistochemistry 1:400, Immunocytochemistry/ Immunofluorescence 1:200. Use reported in multiple pieces of scientific literature (PMID 23980916), Immunohistochemistry-Paraffin 1:400. Use reported in scientific literature (PMID 31022918), Immunohistochemistry-Frozen reported in scientific literature (PMID 23980916), In-situ Hybridization reported in scientific literature (PMID 27133471), Flow (Cell Surface) 1:10 - 1:1000, Single Cell Western 1:10, Dual RNAscope ISH-IHC

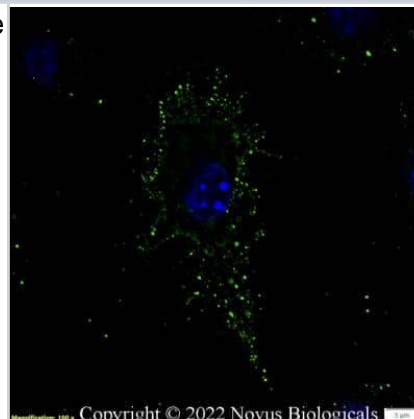


**Application Notes**

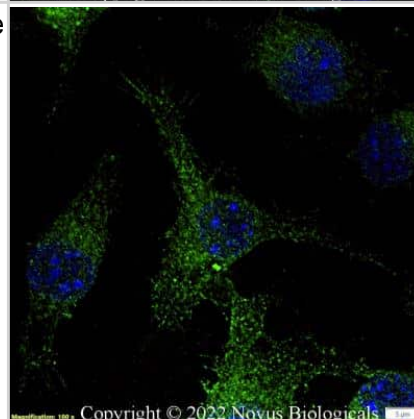
In WB a specific band is observed ~160 kDa and an apparant non-specific band is observed ~56 kDa. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. In ICC/IF, membrane staining was observed in Raw 264.7 cells. This antibody does not appear to work in human samples with WB. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

**Images**

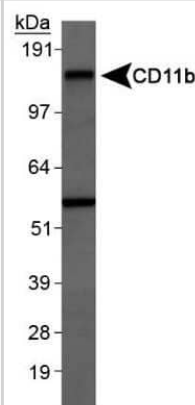
Immunocytochemistry/Immunofluorescence: CD11b Antibody - BSA Free [NB110-89474] - Raw264.7 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD11b Antibody (NB110-89474) at 1ug/ml overnight at 4C and detected with an anti-rabbit 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.



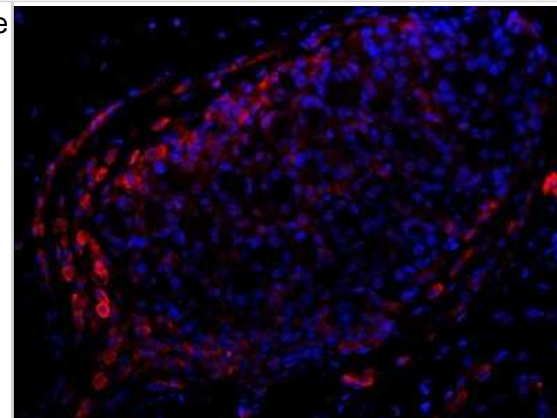
Immunocytochemistry/Immunofluorescence: CD11b Antibody - BSA Free [NB110-89474] - Raw264.7 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD11b Antibody conjugated to Alexa Fluor 488 (NB110-89474AF488) 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.



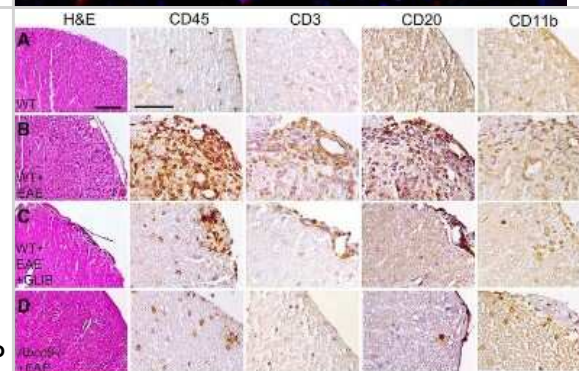
Western Blot: CD11b Antibody - BSA Free [NB110-89474] - Western Blot detection of CD11b in RAW 264.7 whole cell lysates using [NB110-89474].



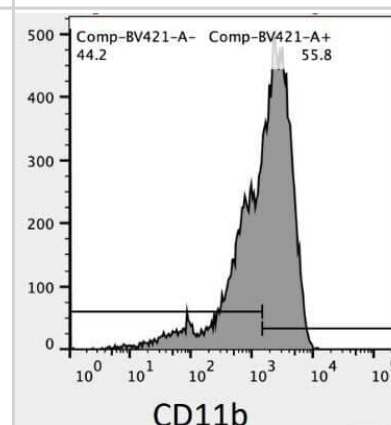
**Immunocytochemistry/Immunofluorescence: CD11b Antibody - BSA Free [NB110-89474] - Staining of mouse pancreas using [NB110-89474] at 1:400 dilution. Nuclei counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue). ICC/IF image submitted by a verified customer review.**



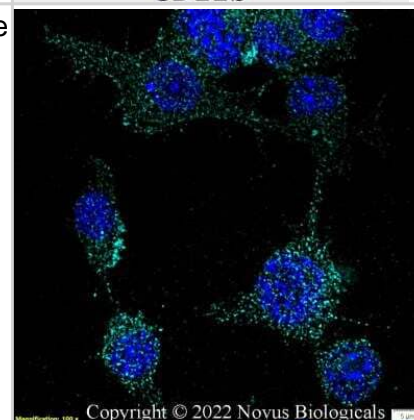
**Immunohistochemistry: CD11b Antibody - BSA Free [NB110-89474] - Glibenclamide and Abcc8-/- suppress immune cell infiltration in EAE.** White matter of lumbar spinal cord sections from WT control (a), untreated pid-30 WT/EAE (b), glibenclamide-treated pid-30 WT/EAE (c), and pid-30 Abcc8-/-/EAE (d) mice, stained with H&E or immunolabeled for CD45 (leucocyte), CD3 (T cells), CD20 (B cells), or CD11b (macrophage/microglia), as indicated; original magnification, x200 (H&E) or x400 (all immunolabelings). Left panel: percent of quadrants with inflammatory cells on H&E; four mice/group. Four right panels: Quantification of CD-45-, CD3-, CD20-, and CD11b-expressing cells in white matter; four mice/group;  $##P < 0.01$  with respect to WT control;  $**P < 0.01$ , and  $***P < 0.001$  with respect to WT/EAE; scale bars, 100  $\mu$ m. Image collected and cropped by CiteAb from the following publication (<https://www.jneuroinflammation.com/content/12/1/210>) licensed under a CC-BY license.



**Flow (Cell Surface): CD11b Antibody - BSA Free [NB110-89474] - Surface staining of CD11b in CT26 colorectal carcinoma tumor model.** Using Alexa Fluor 405 conjugated version of the antibody (NB110-89474AF405). Image from verified customer review.

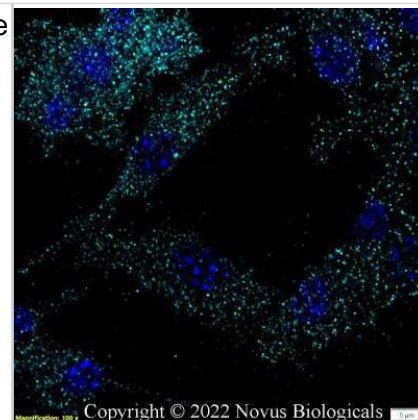


**Immunocytochemistry/Immunofluorescence: CD11b Antibody - BSA Free [NB110-89474] - Raw264.7 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD11b Antibody conjugated to Alexa Fluor 647 (NB110-89474AF647) at 5  $\mu$ g/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.**

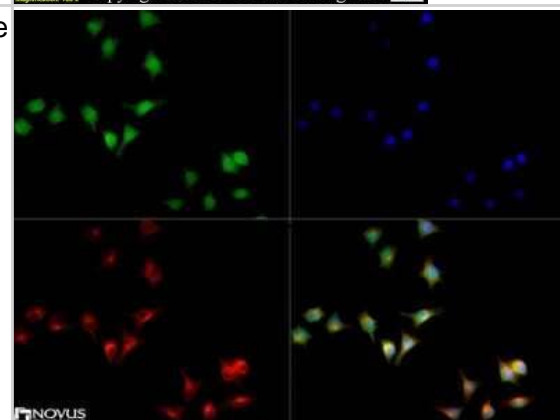




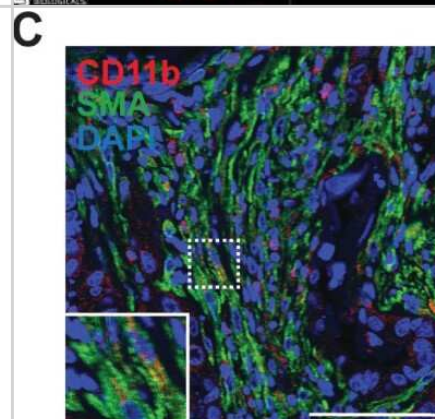
Immunocytochemistry/Immunofluorescence: CD11b Antibody - BSA Free [NB110-89474] - Raw264.7 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD11b Antibody conjugated to DyLight 650 (NB110-8947C) at 5 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



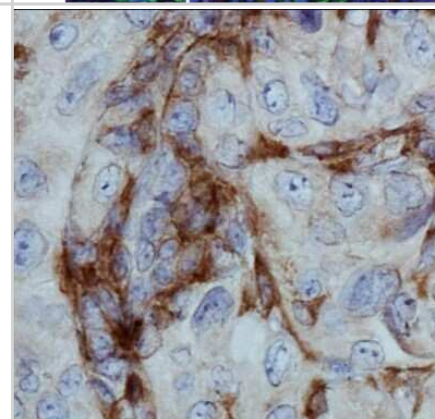
Immunocytochemistry/Immunofluorescence: CD11b Antibody - BSA Free [NB110-89474] - CD11b Antibody [NB110-89474] - CD11b antibody [NB110-89474] was tested in Raw264.7 cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue) and DyLight 550 (red).



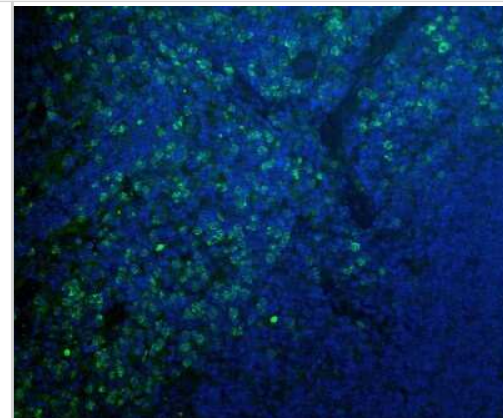
Immunohistochemistry: CD11b Antibody - BSA Free [NB110-89474] - Population of CD11b + myeloid progenitor cells differentiate into SMA + stromal cells within tumors and in vitro. Representative image of CD11b + SMA + double positive cells within tumor stroma. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32731354/>) licensed under a CC-BY license.



Immunohistochemistry: CD11b Antibody - BSA Free [NB110-89474] - Immunohistochemical analysis of CD11b in human renal cancer with [NB110-89474] using 3,3'-Diaminobenzidine (DAB) with hematoxylin counterstain.



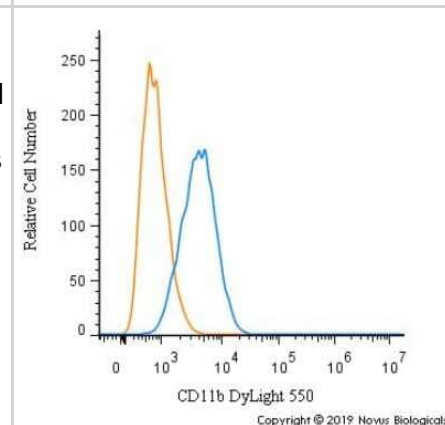
**Immunohistochemistry-Frozen: CD11b Antibody - BSA Free [NB110-89474]** - Immunohistochemical analysis of frozen sections of mouse spleen using [NB110-89474]. IHC-Fr image submitted by a verified customer review.



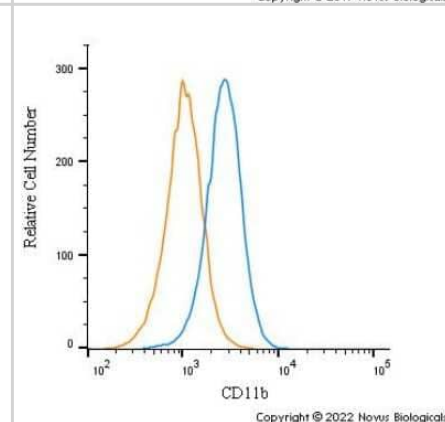
**Immunohistochemistry: CD11b Antibody - BSA Free [NB110-89474]** - Representative photomicrographs of cross sections of uteri collected from wild type (WT) mice 24 h after intrauterine infusion with 100  $\mu$ l of vehicle or 100  $\mu$ g ultrapure LPS from *E. coli* O111:B4, and Tlr4<sup>-/-</sup> mice similarly infused intrauterine with vehicle or LPS. Cross sections were stained with haematoxylin and eosin, and immunostained using a CD11b antibody for granulocytes (red) and DAPI for cell nuclei (white), and images collected at 10X magnification sections (C, F, I, L; bars = 200  $\mu$ m). At least 4 sections per animal and at least 4 fields per section were examined. Only WT mice infused with LPS show evidence of inflammation and accumulation of granulocytes in the endometrium. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0012906>) licensed under a CC-BY license.



**Flow (Cell Surface): CD11b Antibody - BSA Free [NB110-89474]** - CD11b Antibody [NB110-89474] - A surface stain was performed on Raw264.7 cells with DyLight 550-conjugated [NB110-89474R] (blue) and a matched isotype control (orange). Cells were incubated in an antibody dilution of 10  $\mu$ g/mL for 20 minutes at room temperature. Both antibodies were conjugated to DyLight 550.



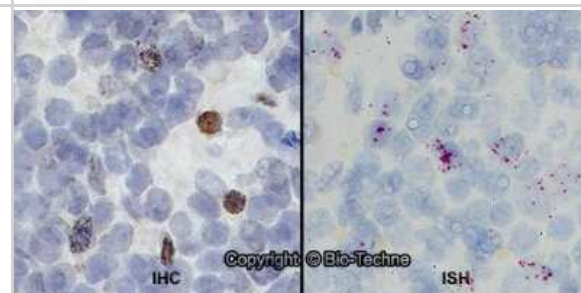
**Flow (Cell Surface): CD11b Antibody - BSA Free [NB110-89474]** - An intracellular stain was performed on THP-1 cells with CD11b NB110-89474 (blue) and a matched isotype control NBP2-24891 (orange). Cells were incubated in an antibody dilution of 2.5  $\mu$ g/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, DyLight 550 (SA5-10033, Thermo Fisher).



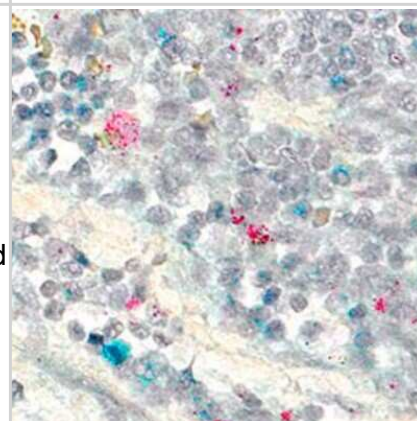
Simple Western: CD11b Antibody - BSA Free [NB110-89474] - Specific band for Cd11b in 1.0 mg/mL of Dentate Gyrus from Rat Brain. [NB110-89474] was used at a dilution of 1:50. This experiment was performed under reducing conditions using the 12-230 kDa separation system. Simple Western image submitted by a verified customer review.



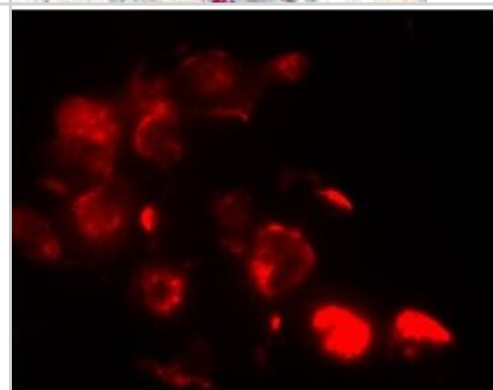
Dual RNAscope ISH-IHC: CD11b Antibody - BSA Free [NB110-89474] - Formalin-fixed paraffin-embedded tissue sections of human lymph node were probed for CD11b mRNA (ACD RNAscope Probe, catalog #555098; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using Rabbit Polyclonal (Novus Biologicals catalog #NB110-89474) at 1:50 dilution with 1 hour incubation at room temperature followed by incubation with anti-rabbit IgG VisUCyte HRP Polymer Antibody (Catalog # VC003) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes.



Dual RNAscope ISH-IHC: CD11b Antibody - BSA Free [NB110-89474] - CD14 mRNA (red) and CD11b protein (green) were detected in formalin-fixed paraffin-embedded tissue sections of human malignant lymph node. ACD's Integrated Co-Detection Workflow was performed using ACD RNAscope Probe Hs-CD14 and CD11b antibody at 1:200 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 RNA scope (TM) 2.5 LS Green Accessory Pack. Tissue was counterstained with 50% hematoxylin (blue).



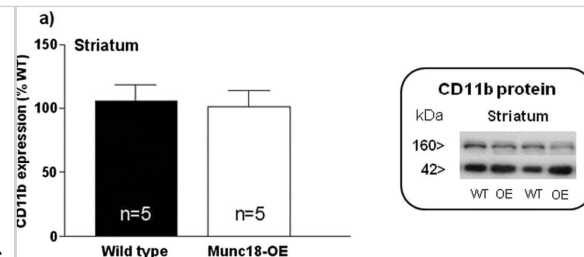
CD11b staining in IMhu. Cells were stained with antibodies against the CD11b surface antigen. (A) Panel A shows positive staining for CD11b and (B) panel B shows nuclear DAPI staining. Magnitude 40×.



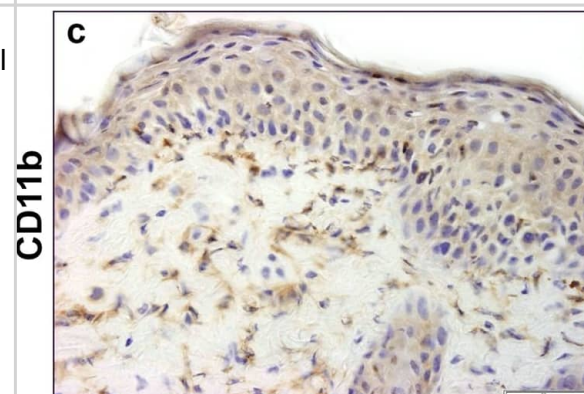
(A)



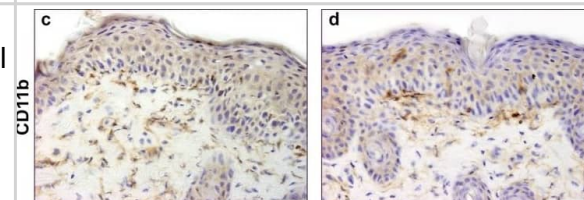
**Western Blot: CD11b Antibody - BSA Free [NB110-89474] -** Immunodensities of (a) CD11b, (b) GFAP & (c) NF- $\kappa$ B (p65) proteins with representative immunoblots in striatum from Munc18-OE (n = 5) & wild-type (n = 5) mice. Bar graphs are ratios of optical densities of our proteins of interest to  $\beta$ -actin (42 kDa band), expressed as immunoreactivity in percentage of mean value of the WT group (100%). No differences were detected between groups for any of the analyzed proteins. Right panels are representative immunoblots for target proteins &  $\beta$ -actin which included Munc18-OE (OE) & wild-type (WT) mice samples. The molecular masses were estimated from referenced standards. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/25069615>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



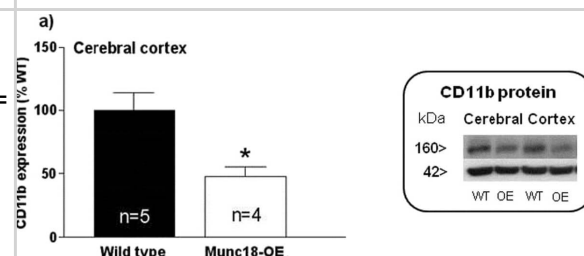
**Immunohistochemistry: CD11b Antibody - BSA Free [NB110-89474] -** Comparison of Ki-67 or CD11b immunoreactivity of Aldara-treated dorsal skin samples of C57BL/6 mice using OP or MP. Nuclear proliferation marker Ki-67 immunostaining on OP (a) & MP (b) dorsal skin tissue samples at 400x magnification. Dermal dendritic cells were labelled with anti-CD11b antibody on OP (c) & MP samples (d) at 400x magnification. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30842501>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



**Immunohistochemistry: CD11b Antibody - BSA Free [NB110-89474] -** Comparison of Ki-67 or CD11b immunoreactivity of Aldara-treated dorsal skin samples of C57BL/6 mice using OP or MP. Nuclear proliferation marker Ki-67 immunostaining on OP (a) & MP (b) dorsal skin tissue samples at 400x magnification. Dermal dendritic cells were labelled with anti-CD11b antibody on OP (c) & MP samples (d) at 400x magnification. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30842501>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

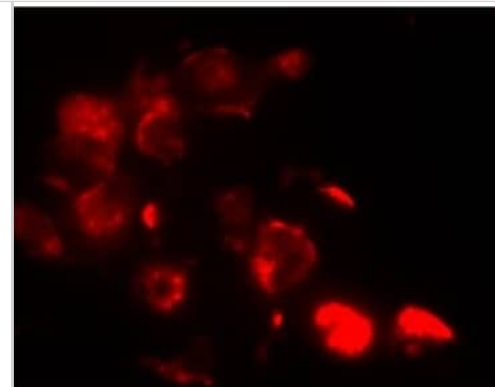


**Western Blot: CD11b Antibody - BSA Free [NB110-89474] -** Immunodensities of (a) CD11b, (b) GFAP & (c) NF- $\kappa$ B (p65) proteins with representative immunoblots in cerebral cortex from Munc18-OE (n = 5) & wild-type (n = 5) mice. Bar graphs are ratios of optical densities of our proteins of interest to  $\beta$ -actin (42 kDa band), expressed as immunoreactivity in percentage of the mean value of the WT group (100%). CD11b was significantly decreased in Munc18-OE mice compared to the WT (t = 3.01; P < 0.05). No differences between groups were observed in the levels of GFAP or NF- $\kappa$ B. Right panels are representative immunoblots for target proteins &  $\beta$ -actin which included Munc18-OE (OE) & wild-type (WT) mice samples. The molecular masses were estimated from referenced standards. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/25069615>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





Immunocytochemistry/ Immunofluorescence: CD11b Antibody - BSA Free [NB110-89474] - CD11b staining in IMhu. Cells were stained with antibodies against the CD11b surface antigen. (A) Panel A shows positive staining for CD11b & (B) panel B shows nuclear DAPI staining. Magnitude 40x. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31096716>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



(A)

## Publications

Noval MG, Spector SN, Bartnicki E et al. MAVS signaling is required for preventing persistent chikungunya heart infection and chronic vascular tissue inflammation Nature Communications 2023-08-03 [PMID: 37537212] (Immunohistochemistry, Human)

Singh AK, Wang R, Lombardo KA et al. Intravenous BCG vaccination reduces SARS-CoV-2 severity and promotes extensive reprogramming of lung immune cells iScience 2023-10-20 [PMID: 37674985] (Immunohistochemistry, Human)

Reinfeld BI, Madden MZ, Wolf MM et al. Cell-programmed nutrient partitioning in the tumour microenvironment Nature 2021-05-13 [PMID: 33828302] (Immunohistochemistry, Human)

Snyder B, Duong P, Trieu J, Cunningham RL. Androgens modulate chronic intermittent hypoxia effects on brain and behavior. Horm Behav. 2018-10-06 [PMID: 30268884] (Immunohistochemistry, Human)

Kienzl M, Hasenoehrl C, Maitz K, Sarsembayeva A, Taschler U, Valadez-Cosmes P, Kindler O, Ristic D, Raftopoulou S, Santiso A, Barnthaler T, Brcic L, Hahnefeld L, Gurke R, Thomas D, Geisslinger G, Kargl J, Schicho R Monoacylglycerol lipase deficiency in the tumor microenvironment slows tumor growth in non-small cell lung cancer. Oncoimmunology, 2021-09-11;10(1):1965319. 2021-09-11 [PMID: 34527428] (Immunohistochemistry, Human)

Hillers-Ziemer LE, Williams AE, Janquart A et al. Obesity-Activated Lung Stromal Cells Promote Myeloid Lineage Cell Accumulation and Breast Cancer Metastasis Cancers (Basel) 2021-02-28 [PMID: 33670906] (Immunohistochemistry, Human)

Wang D, Wang K, Liu Z et al. Valproic acid-labeled chitosan nanoparticles promote recovery of neuronal injury after spinal cord injury Aging (Albany NY) 2020-05-28 [PMID: 32463791] (Immunohistochemistry, Human)

Lim YJ, Koh J, Choi M et al. Prognostic stratification based on the levels of tumor-infiltrating myeloid-derived suppressor cells and PD-1/PD-L1 axis in locally advanced rectal cancer Frontiers in Oncology 2022-10-25 [PMID: 36387259] (Immunohistochemistry, Human)

Tripathi A, Thangaraj A, Chivero ET et al. N-Acetylcysteine Reverses Antiretroviral-Mediated Microglial Activation by Attenuating Autophagy-Lysosomal Dysfunction Frontiers in Neurology 2020-09-04 [PMID: 33013619] (Immunohistochemistry, Human)

Ochoa MC, Sanchez-Gregorio S, de Andrea CE et al. Synergistic effects of combined immunotherapy strategies in a model of multifocal hepatocellular carcinoma Cell reports. Medicine 2023-04-18 [PMID: 37040772]

Ahn JH, da Silva Pedrosa M, Lopez LR, Tibbs TN et Al. Intestinal E. coli-produced yersiniabactin promotes profibrotic macrophages in Crohn's disease Cell Host Microbe 2024-12-19 [PMID: 39701098]

Wang F, Peters R, Jia J, Mudd M et Al. ATG5 provides host protection acting as a switch in the atg8ylation cascade between autophagy and secretion Dev Cell 2023-04-13 [PMID: 37054706]

More publications at <http://www.novusbio.com/NB110-89474>

## Procedures

### Western Blot protocol for CD11b Antibody (NB110-89474)

#### Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer 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.

**\*\*Note:** Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

### Immunohistochemistry-Paraffin protocol for CD11b Antibody (NB110-89474)

#### 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.

##### Staining:

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



**Immunocytochemistry/Immunofluorescence protocol for CD11b Antibody (NB110-89474)****Immunocytochemistry Protocol**

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

\*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.





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### **Products Related to NB110-89474**

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NBP1-30158	Raw 264.7 Whole Cell - T0901317 treated Whole Cell Lysate
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NBP2-24891	Rabbit IgG Isotype Control

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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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