

# Product Datasheet

## CD11b/c Antibody - BSA Free NB110-40766

Unit Size: 0.1 ml

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

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**NB110-40766**

CD11b/c Antibody - BSA Free

Product Information	
<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	1.0 mg/ml
<b>Storage</b>	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
<b>Clonality</b>	Polyclonal
<b>Preservative</b>	0.02% Sodium Azide
<b>Isotype</b>	IgG
<b>Purity</b>	Immunogen affinity purified
<b>Buffer</b>	PBS + 30% Glycerol
<b>Target Molecular Weight</b>	160 kDa

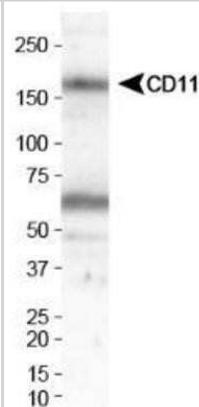
Product Description	
<b>Description</b>	Novus Biologicals Rabbit CD11b/c Antibody - BSA Free (NB110-40766) is a polyclonal antibody validated for use in IHC, WB, Flow and ICC/IF. Anti-CD11b/c Antibody: Cited in 33 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
<b>Host</b>	Rabbit
<b>Gene ID</b>	3684
<b>Gene Symbol</b>	ITGAM
<b>Species</b>	Human, Mouse, Rat
<b>Reactivity Notes</b>	Rat reactivity reported in scientific literature (PMID: 25150592)
<b>Marker</b>	Microglia Marker, Macrophage Marker
<b>Immunogen</b>	A synthetic peptide made to an internal region (within residues 500-600) of the mouse CD11 (b/c) protein. [Swiss-Prot: P05555]

Product Application Details	
<b>Applications</b>	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen
<b>Recommended Dilutions</b>	Western Blot 2-4 ug/ml, Flow Cytometry 1:50 - 1:200, Immunohistochemistry 5-10 ug/ml, Immunocytochemistry/ Immunofluorescence 1:50-1:200, Immunohistochemistry-Paraffin 5-10 ug/ml, Immunohistochemistry-Frozen reported in scientific literature (PMID 29467366)
<b>Application Notes</b>	In Western blot, a specific band is observed at ~ 160 kDa and an apparent non-specific band is observed at ~ 60 kDa. Prior to immunostaining paraffin tissues, antigen retrieval with citrate buffer (pH 6.0) is recommended. In ICC/IF, membrane staining was observed in Raw 264.7 cells.

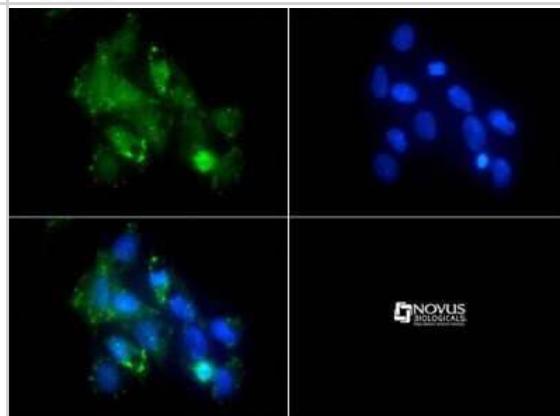


## Images

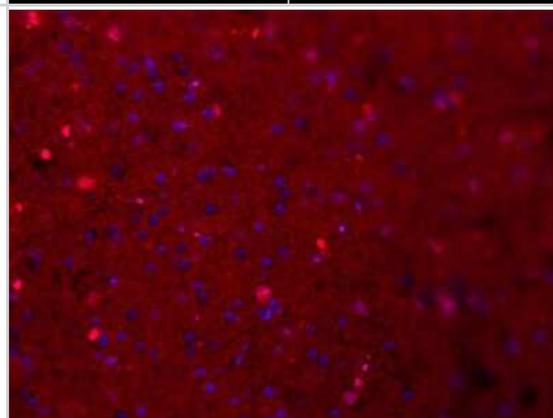
Western Blot: CD11b/c Antibody - BSA Free [NB110-40766] - Analysis of CD11b/c in Raw 264.7 whole cell lysate.



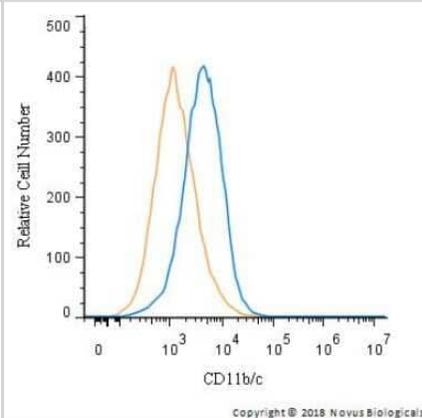
Immunocytochemistry/Immunofluorescence: CD11b/c Antibody - BSA Free [NB110-40766] - CD11 antibody was tested in Raw264.7 cells with FITC (green). Nuclei were counterstained with DAPI (blue).



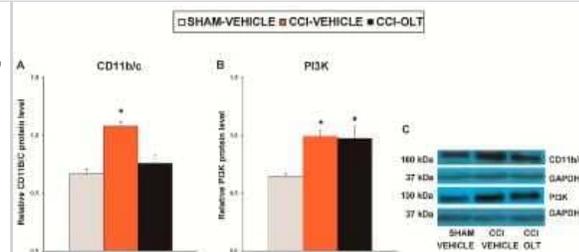
Immunohistochemistry-Frozen: CD11b/c Antibody - BSA Free [NB110-40766] - 10 um Cryosection of murine acute brain slices stained with CD11b/c antibody (1:300 = 3.3 ug/ml) and Alexa Fluor 555 donkey anti rabbit IgG. Antibody shows specific staining, but the background is quite heavy. Image from verified customer review.



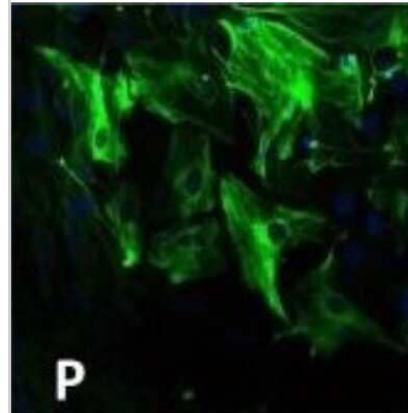
Flow Cytometry: CD11b/c Antibody - BSA Free [NB110-40766] - A surface stain was performed on RAW 264.7 with NB110-40766 and a matched isotype control. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H +L) Cross-Adsorbed Secondary Antibody, DyLight 550.



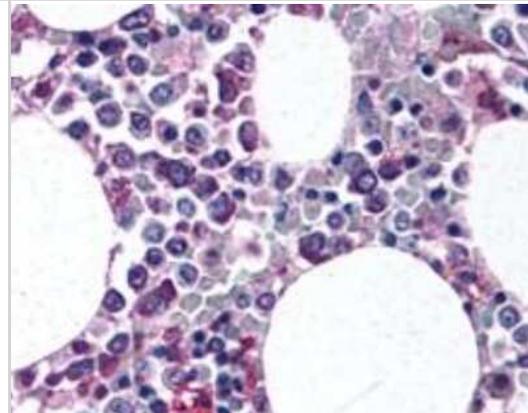
Western Blot: CD11b/c Antibody - BSA Free [NB110-40766] - Effects of oltipraz on the expression of CD11b/c, phosphoinositide 3-kinase (PI3K), phosphorylated protein kinase B (p-Akt), and phosphorylated inhibitor of kB alpha (p-IkB alpha) in the spinal cord of the CCI-injured mice. The relative protein levels of (A) CD11b/c and (B) PI3K on the ipsilateral side of the spinal cord in the CCI-injured mice treated with oltipraz (OLT) or vehicle are represented. (C) Representative examples of blots for CD11b/c (160 kDa), PI3K (130 kDa), and GAPDH (37 kDa). Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31234342/>) licensed under a CC-BY license.



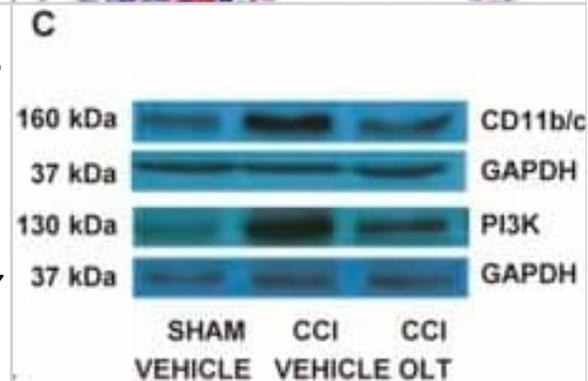
Immunocytochemistry/Immunofluorescence: CD11b/c Antibody - BSA Free [NB110-40766] - Phase contrast microscopy images of HFA and brain tumor cells and immunofluorescence images of cultured primary brain tumor cells, AA and HFA. HFA were derived from three different fetal brains. HFA from an 18-week-old fetus (passaged once) - GFAP (DAKO) merged with DAPI and CD68 (zoomed: 2.1x magnification); GFAP (green and red) was used as an astrocyte marker and CD68 (red) and CD11b (green) were used as markers for microglia. Nuclei indicated by DAPI (blue) in all images. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0112945>), licensed under a CC-BY license.



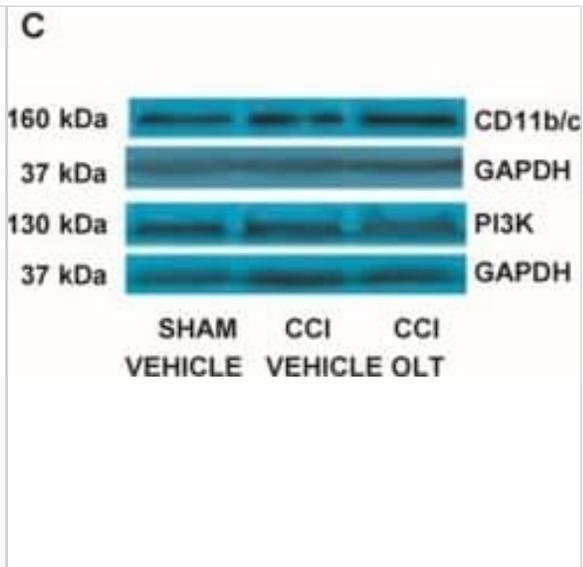
Immunohistochemistry: CD11b/c Antibody - BSA Free [NB110-40766] - Staining of human bone marrow, myeloid precursors.



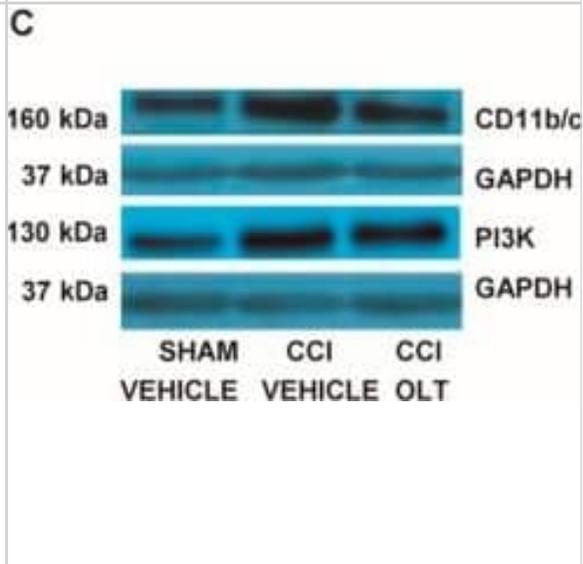
Western Blot: CD11b/c Antibody - BSA Free [NB110-40766] - Effects of oltipraz on the expression of CD11b/c, phosphoinositide 3-kinase (PI3K), phosphorylated protein kinase B (p-Akt), & phosphorylated inhibitor of kB alpha (p-IkB alpha) in the spinal cord of the CCI-injured mice. The relative protein levels of (A) CD11b/c, (B) PI3K, (D) p-Akt, & (E) p-IkB alpha on the ipsilateral side of the spinal cord in the CCI-injured mice treated with oltipraz (OLT) or vehicle are represented. The sham-operated mice (SHAM) treated with vehicle were used as controls. (C) Representative examples of blots for CD11b/c (160 kDa), PI3K (130 kDa), & GAPDH (37 kDa), & (F) for p-Akt (60 kDa), Akt (60 kDa), p-IkB alpha (40 kDa) & I kB alpha (40 kDa). CD11b/c & PI3K are expressed relative to GAPDH levels whereas phosphorylated proteins are expressed relative to their corresponding total proteins. In all panels, \* denotes significant differences vs. sham-operated mice treated with vehicle ( $p < 0.05$ ; one-way ANOVA followed by the SNK test). Results are presented as the mean  $\pm$  SEM;  $n = 5$  samples per experimental group. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31234342/>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



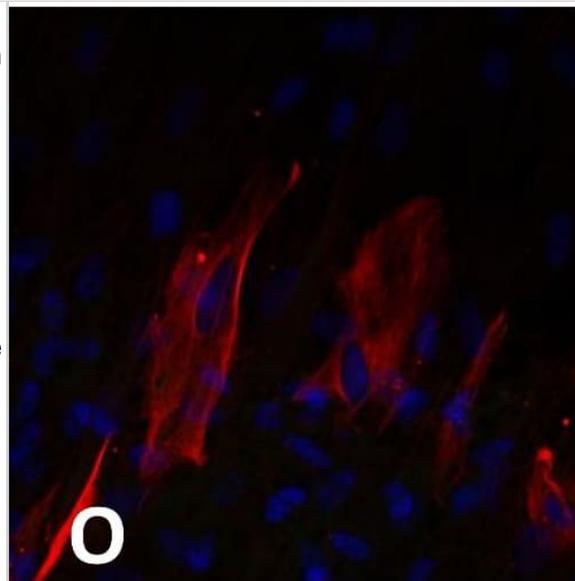
Western Blot: CD11b/c Antibody - BSA Free [NB110-40766] - Effects of oltipraz on the expression of CD11b/c, PI3K, p-Akt, & p-IkB $\alpha$  in the prefrontal cortex of the CCI-injured mice. The relative protein levels of (A) CD11b/c, (B) PI3K, (D) p-Akt, & (E) p-IkB $\alpha$  in the prefrontal cortex of the CCI-injured mice treated with oltipraz (OLT) or vehicle. The sham-operated mice (SHAM) treated with vehicle were used as controls. Representative examples of blots for (C) CD11b/c (160 kDa), PI3K (130 kDa) & GAPDH (37 kDa), & for (F) p-Akt (60 kDa), Akt (60 kDa), p-IkB $\alpha$  (40 kDa) & IKB $\alpha$  (40 kDa). CD11b/c & PI3K are expressed relative to GAPDH levels whereas phosphorylated proteins are expressed relative to their corresponding total proteins. In all panels, \* denotes significant differences vs. sham-operated mice treated with vehicle ( $p < 0.05$ ; one-way ANOVA followed by the SNK test). Results are presented as the mean  $\pm$  SEM;  $n = 4-5$  samples per experimental group. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31234342>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: CD11b/c Antibody - BSA Free [NB110-40766] - Effects of oltipraz on the expression of CD11b/c, PI3K, p-Akt, & p-IkB $\alpha$  in the hippocampus of the CCI-injured mice. The relative protein levels of (A) CD11b/c, (B) PI3K, (D) p-Akt, & (E) p-IkB $\alpha$  in the hippocampus of the CCI-injured mice treated with oltipraz (OLT) or vehicle. The sham-operated mice (SHAM) treated with vehicle were used as controls. Representative examples of blots for (C) CD11b/c (160 kDa), PI3K (130 kDa), & GAPDH (37 kDa); & (F) for p-Akt (60 kDa), Akt (60 kDa), p-IkB $\alpha$  (40 kDa), & IKB $\alpha$  (40 kDa). CD11b/c & PI3K are expressed relative to GAPDH levels whereas phosphorylated proteins are expressed relative to their corresponding total proteins. In all panels, \* denotes significant differences vs. sham-operated mice treated with vehicle ( $p < 0.05$ ; one-way ANOVA followed by the SNK test). Results are presented as the mean  $\pm$  SEM;  $n = 5$  samples per experimental group. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31234342>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: CD11b/c Antibody - BSA Free [NB110-40766] - Phase contrast microscopy images of HFA & brain tumor cells & immunofluorescence images of cultured primary brain tumor cells, AA & HFA. HFA were derived from three different foetal brains. (A) 18-week foetus (10× magnification); (B) 19-week foetus, first week in culture (10× magnification); (C) 19-week foetus, day 5 in culture (20× magnification); (D) Primary GBM after 2 trypsinisations (20x); (E) Recurrent GBM (20x); (F) Secondary GBM after 3 trypsinisations (10x); (G) OII 14 days in culture (10x); (H) All 13 days in culture (10x); (I) Negative control for NGS merged with DAPI; (J) Negative control for secondary antibodies merged with DAPI; (K) GBM cells derived from one patient – GFAP (DAKO) merged with DAPI & CD68 (zoomed: 2.4× magnification); (L) primary brain tumor cells from one patient (male, 40 years old) (passaged three times) - GFAP (Novocastra) merged with DAPI; (K & L) GFAP (green) was used as a GBM marker & CD68 (red) was used as a marker for microglia. (M) AA from one Female, 61 years old; (passaged twice) - GFAP (Novocastra) merged with DAPI; (N) AA from one male 40 years old; (passaged once) - GFAP (Sigma) merged with DAPI & CD68 (Abcam); (M & N) GFAP (green) was used as an astrocyte marker & CD68 (red) & CD11b (green) were used as markers for microglia; (O) HFA from one 17-week- old foetus (passaged once) - GFAP (Novocastra) merged with DAPI & CD11b (Novus); (P) HFA from an 18-week- old foetus (passaged once) - GFAP (DAKO) merged with DAPI & CD68 (zoomed: 2.1× magnification); (O & P) GFAP (green & red) was used as an astrocyte marker & CD68 (red) & CD11b (green) were used as markers for microglia. Nuclei indicated by DAPI (blue) in all images. Image collected & cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0112945>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Garcia-Hernandez ML, Rangel-Moreno J, Garcia-Castaneda M et al. Dendritic cell-specific transmembrane protein is required for synovitis and bone resorption in inflammatory arthritis *Frontiers in Immunology* 2022-11-07 [PMID: 36420272]

Dejanovic B, Huntley MA, De Mazière A, Meilandt WJ et Al. Changes in the Synaptic Proteome in Tauopathy and Rescue of Tau-Induced Synapse Loss by C1q Antibodies *Neuron* 2018-11-06 [PMID: 30392797]

Christoffer Gebhardt, Astrid Riehl, Moritz Durchdewald, Julia Németh, Gerhard Fürstenberger, Karin Müller-Decker, Alexander Enk, Bernd Arnold, Angelika Bierhaus, Peter P. Nawroth, Jochen Hess, Peter Angel RAGE signaling sustains inflammation and promotes tumor development *The Journal of Experimental Medicine* 2008-02-18 [PMID: 18208974]

Liu J, Zhou L, Zhao F et al. Therapeutic effect of adipose stromal vascular fraction spheroids for partial bladder outlet obstruction induced underactive bladder *Stem cell research & therapy* 2022-02-09 [PMID: 35139904] (FLOW, Rat)

### Details:

Adipose tissue around epididymis was collected and ad-SVF were isolated; conjugate with Allophycocyanin (APC)

Salman S, Meyers DJ, Wicks EE Et al. HIF inhibitor 32-134D eradicates murine hepatocellular carcinoma in combination with anti-PD1 therapy *J Clin Invest* 2022-05-02 [PMID: 35499076] (FLOW, Mouse)

### Details:

Citation using the Alexa Fluor 488 version of this antibody.

Pouso-Vazquez E, Bai X, Batalle G et al. Effects of heme oxygenase 1 in the molecular changes and neuropathy associated with type 2 diabetes in mice *Biochemical pharmacology* 2022-03-08 [PMID: 35276215] (WB, Mouse)

Li H, Xiao Y, Li Q Et al. The allergy mediator histamine confers resistance to immunotherapy in cancer patients via activation of the macrophage histamine receptor H1 *Cancer cell* 2021-11-18 [PMID: 34822775]

Liu X, Zheng H Modulation of Sirt1 and FoxO1 on Hypothalamic Leptin-Mediated Sympathetic Activation and Inflammation in Diet-Induced Obese Rats *Journal of the American Heart Association* 2021-07-20 [PMID: 34259031] (IHC-Fr)

Redondo A, Riego G, Pol O The Antinociceptive, Antioxidant and Anti-Inflammatory Effects of 5-Fluoro-2-Oxindole during Inflammatory Pain Antioxidants (Basel, Switzerland) 2020-12-09 [PMID: 33316895] (WB, Mouse)

Li H, Xiao Y, Yao J et al. Allergic Mediator Histamine Confers Immunotherapy Resistance in Cancer Patients via Histamine Receptor 1 on Macrophage SSRN Electronic Journal 2020-12-04 (WB)

Samanta D, Huang T, Shah R et al. BIRC2 Expression Impairs Anti-Cancer Immunity and Immunotherapy Efficacy *Cell Rep* [PMID: 32846130] (FLOW, Mouse)

### Details:

Citation using the PE format of this antibody.

Alvarez-Carbonell D, Ye F, Ramanath N et al. Cross-talk between microglia and neurons regulates HIV latency *PLoS Pathog.* 2019-12-01 [PMID: 31887215] (ICC/IF, Human)

More publications at <http://www.novusbio.com/NB110-40766>



## Procedures

### Western Blot protocol for CD11b/c Antibody (NB110-40766)

#### 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/c Antibody (NB110-40766)

#### Immunohistochemistry

1. Prepare tissue with formalin fixation and by embedding it in paraffin wax.
  2. Make 4-mm sections and place on pre-cleaned and charged microscope slides.
  3. Heat in a tissue-drying oven for 45 minutes @ 60 degrees Celcius.
  4. Deparaffinize the tissues by wash drying the slides in 3 changes of xylene for 5 minutes each @ RT.
  5. Rehydrate the tissues by washing the slides in 3 changes of 100% alcohol for 3 minutes each @ RT.
  6. Wash the slides in 2 changes of 95% alcohol for 3 minutes each @ RT.
  7. Wash the slides in 1 change of 80% alcohol for 3 minutes @ RT.
  8. Rinse the slides in gentle running distilled water for 5 minutes @ RT.
  9. Perform antigen retrieval by steaming the slides in 0.01M sodium citrate buffer (pH 6.0) @ 99-100 degrees Celcius for 20 minutes.
  10. Remove the slides from the heat and let stand in buffer @ RT for 20 minutes.
  11. Rinse the slides in 1X TBS-T for 1 minute @ RT.
- \*\*Do not allow the tissues to dry at any time during the staining procedure\*\***
12. Begin the immunostaining by applying a universal protein block for 20 minutes @ RT.
  13. Drain protein block from the slides and apply the diluted primary antibody for 45 minutes @ RT.
  14. Rinse the slide in 1X TBS-T for 1 minute @ RT.
  15. Apply a biotinylated anti-rabbit IgG (H+L) secondary for 30 minutes @ RT.
  16. Rinse the slide in 1X TBS-T for 1 minute @ RT.
  17. Apply an alkaline phosphatase streptavidin for 30 minutes @ RT.
  18. Rinse the slide in 1X TBS-T for 1 minute @ RT.
  19. Apply an alkaline phosphatase chromagen substrate for 30 minutes @ RT.
  20. Rinse the slide in distilled water for 1 minute @ RT.
- \*\*This method should only be used if the chromagen substrate is alcohol insoluble (ie: Vector Red, DAB)\*\***
21. Dehydrate the tissue by washing the slides in 2 changes of 80% alcohol for 1 minute each @ RT.
  22. Wash the slides in 2 changes of 95% alcohol for 1 minute each @ RT.
  23. Wash the slides in 3 changes of 100% alcohol for 1 minute each @ RT.
  24. Wash the slides in 3 changes of xylene for 1 minute each @ RT.
  25. Apply cover slip.

**Immunocytochemistry/Immunofluorescence protocol for CD11b/c Antibody (NB110-40766)**

## 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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General: novus@novusbio.com

### **Products Related to NB110-40766**

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NBP1-30157	Raw 264.7 Whole Cell Lysate
NB110-40766PEP	CD11b/c Antibody Blocking Peptide
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
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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