Product Datasheet

CD11b/c Antibody
NB110-40766SS

Unit Size: 0.025 ml

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

Reviews: 2 Publications: 12

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Updated 6/15/2014 v.20.1

NB110-40766SS

CD11b/c Antibody

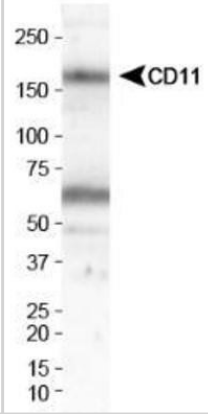
Product Information	
Unit Size	0.025 ml
Concentration	1.13 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS, 30% glycerol.
Target Molecular Weight	160 kDa

Product Description	
Host	Rabbit
Gene ID	3684
Gene Symbol	ITGAM
Species	Human, Mouse, Rat
Species Reactivity	Human and mouse. Rat reactivity reported in scientific literature (PMID: 25150592)
Marker	Microglia Marker, Macrophages Marker
Immunogen	A synthetic peptide made to an internal region (within residues 500-600) of the mouse CD11 (b/c) protein. [Swiss-Prot# P05555]

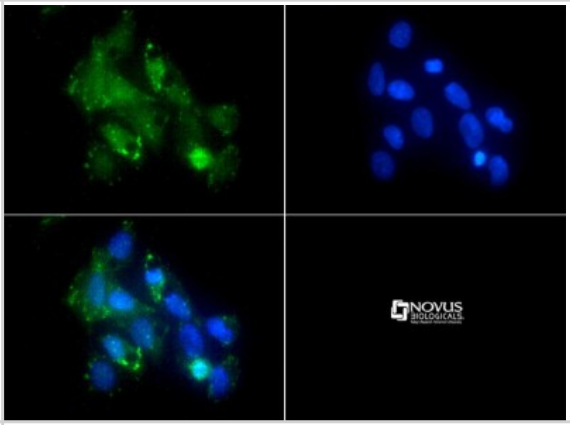
Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Flow Cytometry 1:50-1:200, Immunocytochemistry/Immunofluorescence 1:50-1:200, Immunohistochemistry 5-10 ug/ml, Immunohistochemistry-Paraffin 5-10 ug/ml, Western Blot 2-4 ug/ml
Application Notes	This CD11b/c antibody is useful for Immunocytochemistry/Immunofluorescence, Western Blot, Immunohistochemistry paraffin embedded sections and Flow Cytometry. In Western blot a specific band is observed ~ 160 kDa and an apparant non-specific band is observed ~ 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

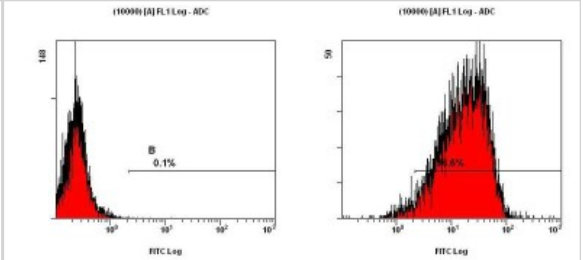
Western Blot: CD11b/c Antibody [NB110-40766] - WB analysis of CD11b/c in Raw 264.7 whole cell lysate.



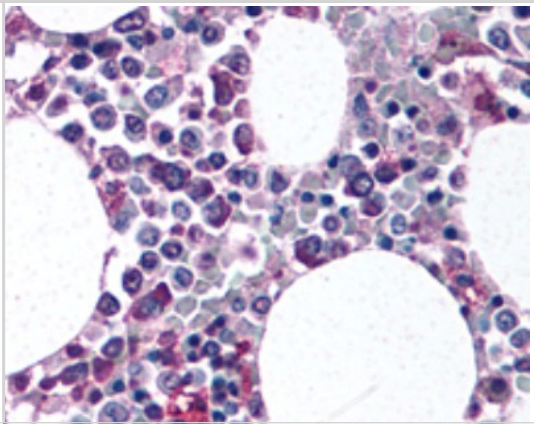
Immunocytochemistry/Immunofluorescence: CD11b/c Antibody [NB110-40766] - CD11 antibody was tested in Raw264.7 cells with FITC (green). Nuclei were counterstained with Dapi (blue).



Flow Cytometry: CD11b/c Antibody [NB110-40766] - Detection of CD11 b/c in fixed Hela cells.



Western Blot: CD11b/c Antibody [NB110-40766] - Staining of human bone marrow, myeloid precursors.



Publications

Jin D, Yang J, Hu J et al. MCP-1 stimulates spinal microglia via PI3K/Akt pathway in bone cancer pain. Brain Res. 2014 Dec 31 [PMID: 25555372] (WB, ICC/IF, Human)

Details:
CD11b/c antibody used for WB and ICC/IF to show the activation level of microglia.

Adams S, Teo C, Mcdonald Kl et al. Involvement of the kynurenine pathway in human glioma pathophysiology. PLoS OnE. 2014 Nov 22 [PMID: 25415278] (ICC/IF, Human)

Malmevik J, Rogers ML, Nilsson M et al. Selective transfection of microglia in the brain using an antibody-based non-viral vector. Brain Res. 2014 Aug 21 [PMID: 25150592] (IHC, Rat)

Berbegall AP, Villamon E, Tadeo I et al. Neuroblastoma after Childhood: Prognostic Relevance of Segmental Chromosome Aberrations, ATRX Protein Status, and Immune Cell Infiltration. Neoplasia 2014 Jun 01 [PMID: 25077701] (IHC-P, Human)

Details:
CD11b antibody used for IHC of formalin-fixed paraffin-embedded tissue sections of human neuroblastoma - 1/100 primary dilution, autostainer setup. Staining images not shown but Table 5 shows expression levels using grades of staining in stroma-rich region and neuroblast-rich region

Kutsukake M, Matsutani T, Tamura K, Matsuda A. Pioglitazone attenuates lung injury by modulating adipose inflammation. Journal of Surgical Research 3/12/2014 [PMID: 24713471] (IHC-P, Mouse)

Shi X, Chen X, Li X et al. Gambogic acid induces apoptosis in imatinib-resistant chronic myeloid leukemia cells via inducing proteasome inhibition and caspase-dependent Bcr-Abl downregulation. Clin. Cancer Res. 2014 Jan 1 [PMID: 24334603] (IHC-P, Human)

Hervera A, Leanez S, Motterlini R, Pol O. Treatment with Carbon Monoxide-Releasing Molecules and an HO-1 Inducer Enhances the Effects and Expression of mu-opioid Receptors during Neuropathic Pain Anesthesiology 2013 Jan 25 [PMID: 23358127]

Yen YT, Yang HR, Lo HC et al. Enhancing autophagy with activated protein C and rapamycin protects against sepsis-induced acute lung injury. Surgery 2013 Feb 19 [PMID: 23434181] (IHC-P, Mouse)

Hervera A, LeAnez S, Negrete R et al. Carbon monoxide reduces neuropathic pain and spinal microglial activation by inhibiting nitric oxide synthesis in mice PLoS One 2012 [PMID: 22928017] (WB, Mouse)

Chakrabarty P, Jansen-West K, Beccard A et al. Massive gliosis induced by interleukin-6 suppresses A{beta} deposition in vivo: evidence against inflammation as a driving force for amyloid deposition. FASEB J :fj09-141754. 2009 [PMID: 19825975] (WB, FLOW, Mouse)

Chakrabarty P, Ceballos-Diaz C, Beccard A et al. IFN-gamma promotes complement expression and attenuates amyloid plaque deposition in amyloid beta precursor protein transgenic mice. J Immunol 184(9):5333-43. 2010 May 1. [PMID: 20368278] (WB, Mouse)

Wong CC, Gilkes DM, Zhang H et al. Hypoxia-inducible factor 1 is a master regulator of breast cancer metastatic niche formation. Proc Natl Acad Sci U S A. 2011 Sep 12. [PMID: 21911388] (IHC, Mouse)

Procedures

Western Blot protocol specific 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 Embedded Sections (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 steptavidin 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 1:25,0000 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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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 guarantee, please visit www.novusbio.com/guarantee.