Product Datasheet

Integrin alpha M/CD11b Antibody
NB110-89474SS

Unit Size: 0.025 ml

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

Reviews: 2 Publications: 14

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Integrin alpha M/CD11b Antibody

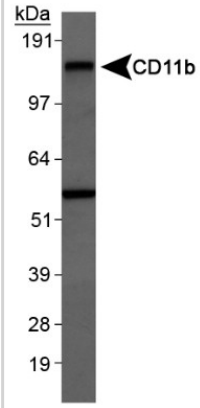
Product Information	
Unit Size	0.025 ml
Concentration	1.00 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS, 30% glycerol
Target Molecular Weight	160 kDa

Product Description	
Host	Rabbit
Gene ID	3684
Gene Symbol	ITGAM
Species	Human, Mouse, Rat
Species Reactivity	Mouse, rat and human. We have feedback that this antibody does not work in human samples with Western blot. Immunogen has 100% homology to bovine and 88% homology with sheep.
Marker	Microglia Marker, Myeloid Marker
Immunogen	A synthetic peptide made to an internal region (within residues 250-350) of the mouse CD11b protein. [Swiss-Prot# P05555]

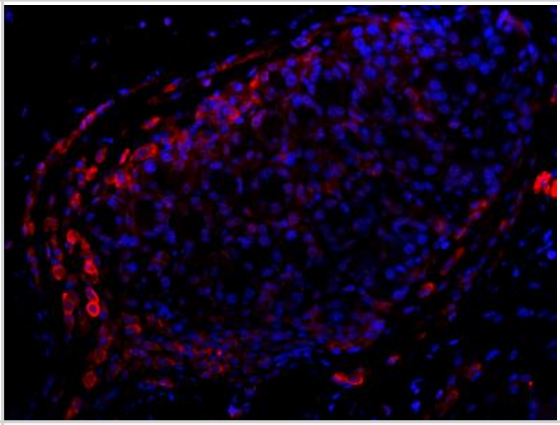
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Flow Cytometry 1:10-1:1000, Immunocytochemistry/Immunofluorescence 1:200, Immunohistochemistry 1:400, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin 1:400, Simple Western 1:50, Western Blot 2 ug/ml
Application Notes	This CD11b antibody is useful for Western blot analysis, Immunocytochemistry, Flow Cytometry (PMID 21422470) and IHC-paraffin embedded sections. In Western blot 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 with Western blot. Use in Immunohistochemistry-Frozen reported in scientific literature (PMID 23980916)

Images

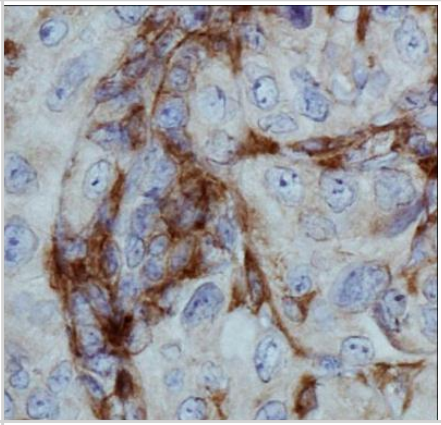
Western Blot: CD11b Antibody [NB110-89474] - Detection of CD11b in RAW 264.7 whole cell lysates using NB110-89474.



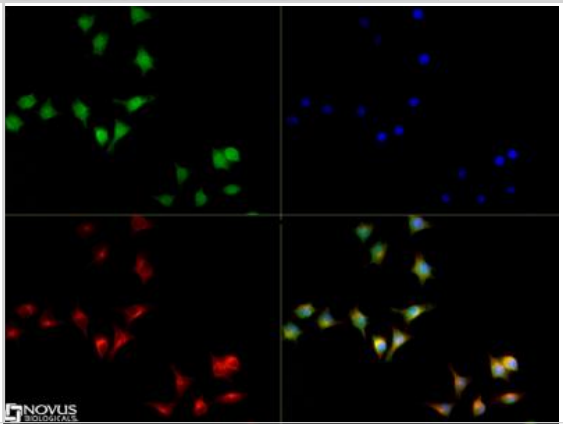
Immunocytochemistry/Immunofluorescence: CD11b Antibody [NB110-89474] - Staining of mouse pancreas using CD11b antibody at 1:400 dilution. Nuclei counterstained with DAPI (blue). Image provided by product review by verified customer.



Immunohistochemistry: CD11b Antibody [NB110-89474] - Analysis of CD11b in human renal cancer using DAB with hematoxylin counterstain.



Immunocytochemistry/Immunofluorescence: CD11b Antibody [NB110-89474] - CD11b antibody was tested in Raw264.7 cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



Simple Western: Integrin alpha M/CD11b Antibody [NB110-89474] - Simple Western lane view shows a specific band for Cd11b in 1.0 mg/ml of Dentate Gyrus from Rat Brain at an antibody concentration of 1:50. This experiment was performed under reducing conditions using the 12-230 kDa separation system. Image provided through customer review.

Publications

de la Pisa IG, Cebrian C, Ortega JE et al. Cytokine pathway disruption in a mouse model of schizophrenia induced by Munc18-1a overexpression in the brain. J Neuroinflammation 2014 Jul 29 [PMID: 25069615] (WB, Mouse)

Details:
CD11b antibody used for WB application at 1:1000 dilution in mouse model of schizophrenia induced by Munc18-1a overexpression/OE in the brain (see full text for sample preparation details). Figures 3a and 4a shows the WB of microglia /macrophage marker CD11bas single band of 160 kDa in cerebral cortex and striatum tissues of Munc18-OE and wild-type mice.

Sakaguchi S, Shono JI, Suzuki T et al. Implication of anti-inflammatory macrophages in regenerative moto-neuritogenesis: Promotion of myoblast migration and neural chemorepellent semaphorin 3A expression in injured muscle Int. J. Biochem. Cell Biol. 2014 Jun 02 [PMID: 24886696] (IHC-Fr, ICC/IF, Mouse)

Details:
Antibody used for ICC-IF (as macrophage marke) on macrophages isolated from peritoneal cavity of male C57BL/6 mice (Figure 1A, B). Antibody also used for IHC-Fr on tibialis anterior muscle of mice for detecting the total population of activated monocytes/macrophages (Figure 6B, C)

Shono JI, Sakaguchi S, Suzuki T et al. Preliminary time-course study of antiinflammatory macrophage infiltration in crush-injured skeletal muscle. Anim Sci J. 2013 Aug 25 [PMID: 23980916] (IHC-Fr, ICC/IF, Mouse)

Bray JG, Reyes KC, Roberts AJ et al. Synaptic plasticity in the hippocampus shows resistance to acute ethanol exposure in transgenic mice with astrocyte-targeted enhanced CCL2 expression Neuropharmacology 2012 Nov 16 [PMID: 23164616] (WB, Mouse)

Yin L, Ahmad R, Kosugi M et al. Terminal differentiation of chronic myelogenous leukemia cells is induced by targeting of the MUC1-C oncoprotein Cancer Biol Ther 2010 Sep [PMID: 20592495] (IHC, Human)

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. Sep. 2011 [PMID: 21911388] (IHC-P, Human)

Mei Zhang, Julian A Kim. Effect of molecular size and modification pattern on the internalization of water soluble Beta-(1 to 3)-(1 to 4)-glucan by primary murine macrophages The International Journal of Biochemistry & Cell Biology 2012 Jun [PMID: 22679629] (ICC/IF, Mouse)

Jin H, Li YH, Xu JS et al. Lipoxin A4 analog attenuates morphine antinociceptive tolerance, withdrawal-induced hyperalgesia, and glial reaction and cytokine expression in the spinal cord of rat Neuroscience 2012 April 9 [PMID: 22366510] (WB, Rat)

Nelson TE, Hao C, Manos J et al. Altered hippocampal synaptic transmission in transgenic mice with astrocyte-targeted enhanced CCL2 expression. Brain Behav. Immun. 25 Suppl 1:S106-119. 2011 Jun. [PMID: 21356306] (WB, Mouse)

Yin L, Wu Z, Avigan D et al. MUC1-C oncoprotein suppresses reactive oxygen species-induced terminal differentiation of acute myelogenous leukemia cells. Blood;117(18):4863-4870. 2011 May 5. [PMID: 21422470]

Gruol DL, Puro A, Hao C et al. Neuroadaptive changes in cerebellar neurons induced by chronic exposure to IL-6. J Neuroimmunol. 2011 Sep 2. [PMID: 21890220] (WB, Rat)

Nelson TE, Olde Engberink A, Hernandez R et al. Altered synaptic transmission in the hippocampus of transgenic mice with enhanced central nervous systems expression of interleukin-6. Brain Behav Immun. 2012 May 17. [PMID: 22609298] (WB, Mouse)

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

Procedures

Western Blot protocol specific 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 Embedded Sections (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 1:25,0000 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.

Novus Biologicals USA
8100 Southpark Way, A-8
Littleton, CO 80120
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
novus@novusbio.com

Novus Biologicals Canada
461 North Service Road West, Unit B37
Oakville, ON L6M 2V5
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada@novusbio.com

Novus Biologicals Europe
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: technical@novusbio.com
Orders: orders@novusbio.com
General: novus@novusbio.com

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.