Product Datasheet

CD68/SR-D1 Antibody (FA-11) - BSA Free
NBP2-33337

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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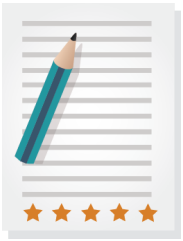


NBP2-33337

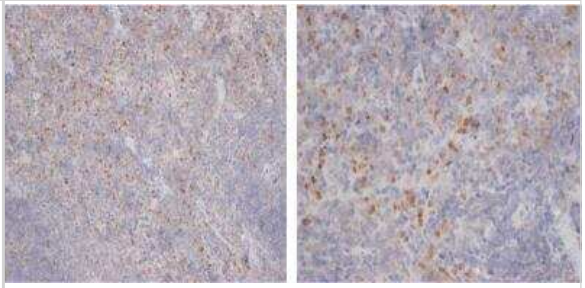
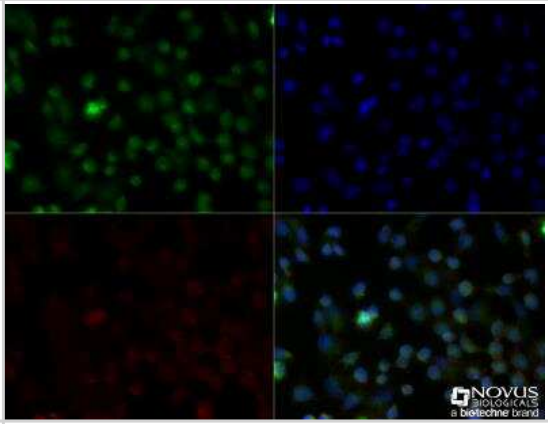
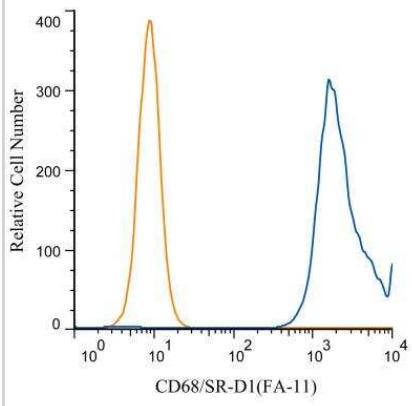
CD68/SR-D1 Antibody (FA-11) - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	FA-11
Preservative	0.02% Sodium Azide
Isotype	IgG2a
Purity	Protein G purified
Buffer	PBS

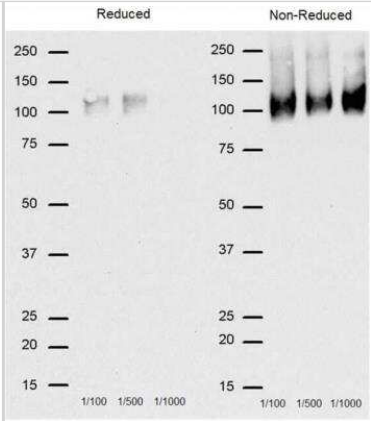
Product Description	
Host	Rat
Gene ID	968
Gene Symbol	CD68
Species	Mouse
Immunogen	This CD68/SR-D1 Antibody (FA-11) was developed against purified ConA acceptor glycoproteins from the P815 cell line.

Product Application Details	
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Functional, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Single Cell Western
Recommended Dilutions	Western Blot 1:100-1:2000, Flow Cytometry 1:50-1:100, Immunohistochemistry 1:10-1:500. Use reported in scientific literature (PMID 34478932), Immunocytochemistry/ Immunofluorescence 1:10-1:500. Use reported in scientific literature (PMID 34478932), Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:10-1:500, Immunohistochemistry-Frozen 1:10-1:500, Functional reported in scientific literature (PMID 11085350), Flow (Intracellular), Single Cell Western
Application Notes	For Flow Cytometry: Use 10 ul of suggested dilution to label 10^6 cells in 100 ul. IHC requires antigen retrieval using heat treatment prior to staining of paraffin sections. Sodium citrate buffer pH 6.0 is recommended for this purpose.

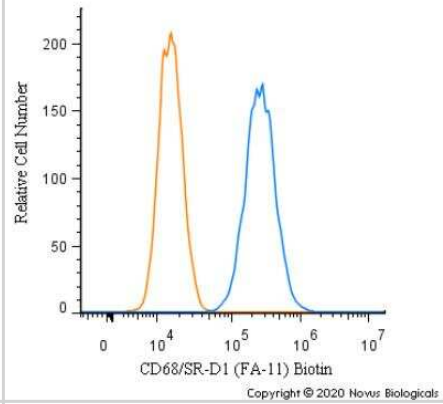
Images

Immunohistochemistry-Paraffin: CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - Mouse spleen cryosection.	
Immunohistochemistry-Paraffin: CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - Mouse spleen.	
Immunocytochemistry/Immunofluorescence: CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - Wehi-3 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti CD68 (FA-11) [NBP2-33337] at a 1:100 dilution overnight at 4C and detected with an anti-rat DyLight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	
Flow (Intracellular): CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - An intracellular stain was performed on Raw 246.7 cells with CD68/SR-D1 (FA-11) antibody NBP2-33337 (blue) and a matched isotype control MAB006 (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by DyLight488-conjugated anti-rat secondary antibody.	

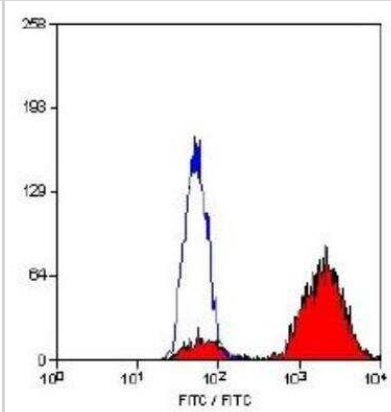
Western Blot: CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - Expression on J774 cells.



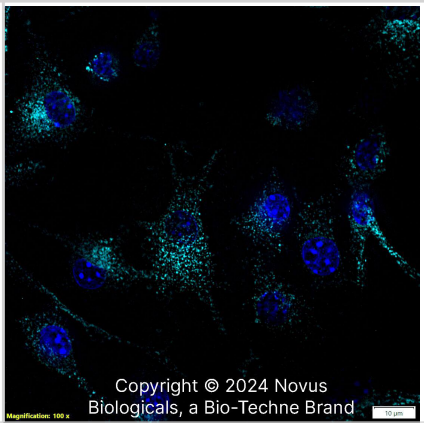
Flow Cytometry: CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - An intracellular stain was performed on Raw264.7 cells with CD68/SR-D1 Antibody (FA-11) NBP2-33337B (blue) and a matched isotype control (orange). Both antibodies were conjugated to Biotin. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Streptavidin - R-Phycoerythrin Protein (2012-1000, Novus Biologicals).



Flow Cytometry: CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - Staining of permeabilised Mouse peritoneal Macrophages cells with Rat anti Mouse CD68 Antibody (Clone FA-11) visualised with F(ab')2 Goat Anti Rat IgG:FITC (Mouse Adsorbed).



CD68/SR-D1 (FA-11) was detected in immersion fixed Raw 264.7 mouse macrophage cell line using Rat anti-CD68/SR-D1 (FA-11) Protein-G purified Monoclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP2-33337AF647) (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

Rong Bao, Shuiyuan Wang, Xiaoxian Liu, Kejun Tu, Jingquan Liu, Xiaohe Huang, Chunsen Liu, Peng Zhou, Shen Liu Neuromorphic electro-stimulation based on atomically thin semiconductor for damage-free inflammation inhibition Nature Communications 2024-02-13 [PMID: 38351088]

Ferreira N, Richner M, van der Laan A et al. Prodromal neuroinvasion of pathological ?-synuclein in brainstem reticular nuclei and white matter lesions in a model of ?-synucleinopathy Brain Communications 2021-05-14 [PMID: 34136810] (Immunohistochemistry, Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence)

Zhang W, Xu M, Chen F et al. Targeting the JAK2-STAT3 pathway to inhibit cGAS-STING activation improves neuronal senescence after ischemic stroke Experimental neurology 2023-07-05 [PMID: 37419174]

Warner WS, Stubben C, Yeoh S et al. Next-generation RNA sequencing elucidates transcriptomic signatures of pathophysiologic nerve regeneration Scientific reports 2023-05-31 [PMID: 37258605] (IHC-Fr, Mouse)

Chung BS, Liao IC, Lin PC et al. PD-L1 Expression in High-Risk Early-Stage Colorectal Cancer-Its Clinical and Biological Significance in Immune Microenvironment International journal of molecular sciences 2022-10-31 [PMID: 36362062] (IHC-P, Human)

Richner M, GonCalves NP, Jensen PH et al. Recombinant adeno-associated virus mediated gene delivery in the extracranial nervous system of adult mice by direct nerve immersion STAR Protocols 2022-03-01 [PMID: 35243373]

Mahmud F, Roy R, Mohamed MF et al. Therapeutic evaluation of immunomodulators in reducing surgical wound infection FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2022-01-01 [PMID: 34907595] (Mouse)

Feng L, Wang Q, Li Y et al. Ablation of Hypoxia-induced mitogenic factor promotes cardiac repair after myocardial infarction by downregulating matrix metalloproteinase-9 expression in macrophage Research Square 2021-09-15 (IHC-P, Mouse)

Feng L, Wang Q, Li Y et al. Ablation of Hypoxia-induced mitogenic factor promotes cardiac repair after myocardial infarction by downregulating matrix metalloproteinase-9 expression in macrophage Research Square Sep 15 2021 12:00AM (IHC-P, Mouse)

Huang J, Fan C, Chen Y Et al. Single-cell RNA-seq reveals functionally distinct biomaterial degradation-related macrophage populations Biomaterials 2021-10-01 [PMID: 34478932] (ICC/IF, IF/IHC, Mouse)

Li Y, Dong M, Wang Q et al. HIMF deletion ameliorates acute myocardial ischemic injury by promoting macrophage transformation to reparative subtype Basic research in cardiology 2021-04-23 [PMID: 33893593] (IF/IHC, Mouse)

Zhai M, Luan P, Shi Y, et al. Identification of Three Significant Genes Associated with Immune Cells Infiltration in Dysfunctional Adipose Tissue-Induced Insulin-Resistance of Obese Patients via Comprehensive Bioinformatics Analysis International Journal of Endocrinology 2021-01-22 [PMID: 33564304] (IHC-P, Mouse)

More publications at http://www.novusbio.com/NBP2-33337

Procedures

Flow (Intracellular) Protocol for CD68/SR-D1 Antibody (NBP2-33337)
Protocol for Flow Cytometry Intracellular Staining
Sample Preparation.
1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 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 uL 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 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL 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 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabilization buffer to every 1 x 106 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 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug 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 uL 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 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

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Products Related to NBP2-33337

HAF005	Goat anti-Rat IgG Secondary Antibody [HRP]
NBP1-75398	Goat anti-Rat IgG (H+L) Secondary Antibody (Pre-adsorbed)
NBP2-21947-0.1mg	Rat IgG2a Isotype Control (2A3)
NBP2-33337AF647	CD68/SR-D1 Antibody (FA-11) [Alexa Fluor® 647]

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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