

# 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

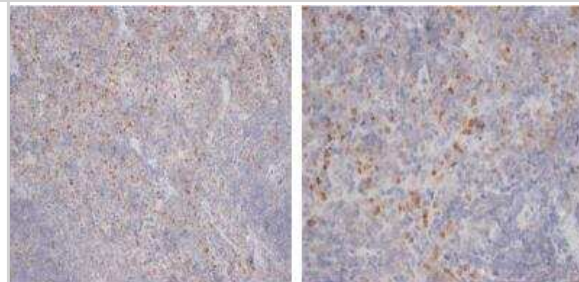
Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	FA-11
Preservative	0.02% Sodium Azide
Isotype	IgG2a
Purity	Protein G purified
Buffer	PBS
Product Description	
Description	Novus Biologicals Rat CD68/SR-D1 Antibody (FA-11) - BSA Free (NBP2-33337) is a monoclonal antibody validated for use in IHC, WB, Flow, ICC/IF and IP. Anti-CD68/SR-D1 Antibody: Cited in 38 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
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, Immunohistochemistry-Paraffin, Flow Cytometry, Flow (Intracellular), Functional, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, 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 <sup>6</sup> 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.



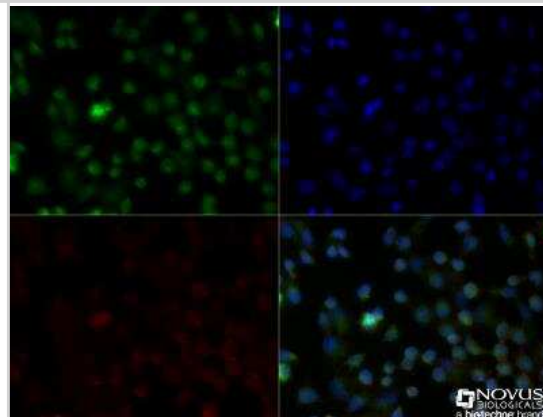
## Images

Immunohistochemistry-Paraffin: CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - Mouse spleen cryosection. 

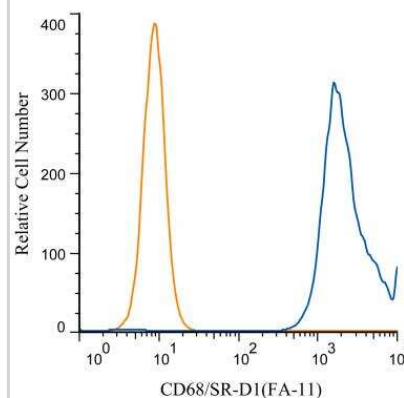
Immunohistochemistry-Paraffin: CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - Mouse spleen.



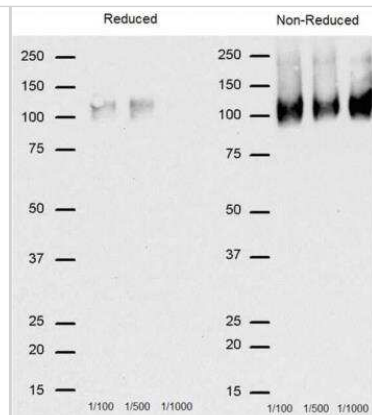
Immunocytochemistry/Immunofluorescence: CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - Wehi-3 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti CD68 (FA-11) [NBP2-33337] at a 1:100 dilution overnight 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.



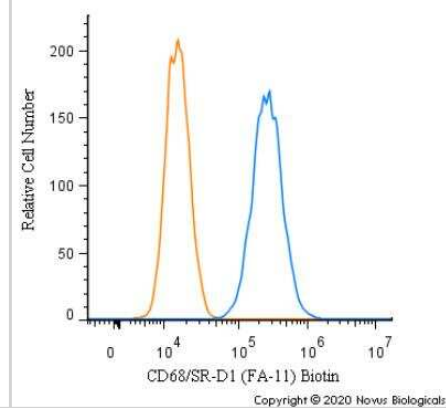
Flow (Intracellular): CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - An intracellular stain was performed on Raw 246.7 cells with CD68/SR-D1 (FA-11) antibody NBP2-33337 (blue) and a matched isotype control MAB006 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by DyLight488-conjugated anti-rat secondary antibody.



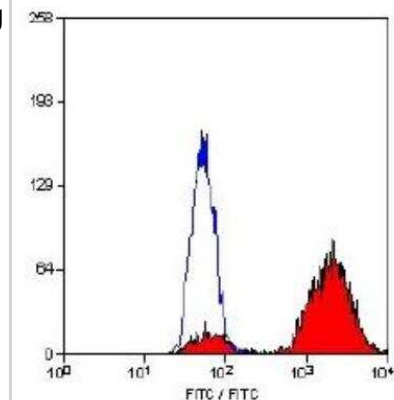
Western Blot: CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - Expression on J774 cells.



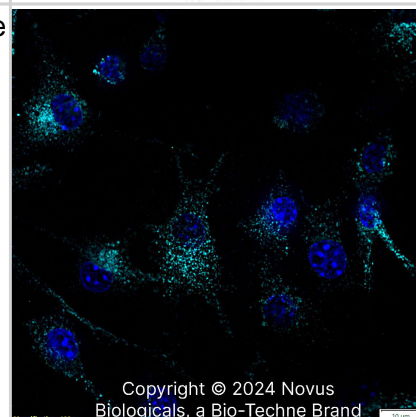
Flow Cytometry: CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - An intracellular stain was performed on Raw264.7 cells with CD68/SR-D1 Antibody (FA-11) NBP2-33337B (blue) and a matched isotype control (orange). Both antibodies were conjugated to Biotin. Cells were fixed with 4% PFA and then permeabilized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



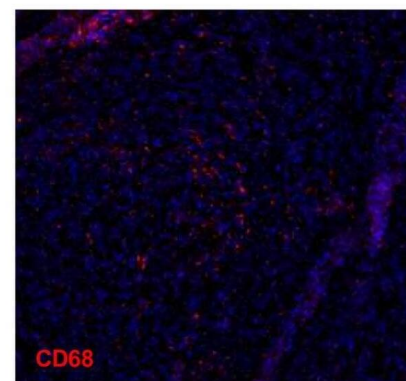
Flow Cytometry: CD68/SR-D1 Antibody (FA-11) [NBP2-33337] - Staining of permeabilised Mouse peritoneal Macrophages cells with Rat anti Mouse CD68 Antibody (Clone FA-11) visualised with F(ab')<sub>2</sub> Goat Anti Rat IgG:FITC (Mouse Adsorbed).



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  $\mu\text{g}/\text{mL}$  overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



Detection of CD68 + cells within the TIM(E) (a) Representative image of tissue sections labeled anti-CD68 for macrophages. (b) Comparison between total macrophages among the tumors obtained from the BL/6nA group compared to those obtained from the BL/6nB group. (c) Comparison between macrophages populations present in each tumor regions for the BL/6nA and BL/6nB groups. Spleen. Macrophage staining control in spleen cut. \*\*\*P < 0.001. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/38956203>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Ferreira N, Richner M, van der Laan A et al. Prodromal neuroinvasion of pathological  $\alpha$ -synuclein in brainstem reticular nuclei and white matter lesions in a model of  $\alpha$ -synucleinopathy Brain Communications 2021-05-14 [PMID: 34136810] (Flow Cytometry, Mouse)

Fan W, Liu P, Tan L et al. Tet2 modulates M2 macrophage polarization via mRNA 5-methylcytosine in allergic rhinitis. International immunopharmacology 2024-10-31 [PMID: 39486186]

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]

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)

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  $2 \times 10^5$  and  $1 \times 10^6$  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  $\mu$ L 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  $1 \times 10^6$  cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100  $\mu$ L 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  $\mu$ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100  $\mu$ L of a permeabilization buffer to every  $1 \times 10^6$  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  $\mu$ L of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1  $\mu$ g 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  $\mu$ L 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  $\mu$ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500  $\mu$ L per sample) and proceed with analysis on your flow cytometer.



**Immunocytochemistry/ Immunofluorescence Protocol for CD68/SR-D1 Antibody (NBP2-33337)****Immunocytochemistry Protocol**

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

1. Remove culture medium and wash the cells briefly in PBS. Add 4% paraformaldehyde to the dish and fix at room temperature for 10 minutes.
2. Remove the paraformaldehyde and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 2 min.
4. Remove the permeabilization buffer and wash three times for 5 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 5 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 5 minutes each.
10. Counter stain DNA with DAPI if required.

**Immunohistochemistry-Paraffin Protocol for CD68/SR-D1 Antibody (NBP2-33337)****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 (keep slides in the sodium citrate buffer at all times).

**Staining:**

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





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### **Products Related to NBP2-33337**

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NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF005	Goat anti-Rat IgG Secondary Antibody [HRP]
NB7115	Goat anti-Rat IgG (H+L) Secondary Antibody [HRP]
NBP2-21947-0.1mg	Rat IgG2a Isotype Control (2A3)

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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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