Product Datasheet

Complement C3 Antibody (11H9) - BSA Free
NB200-540

Unit Size: 0.1 ml

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

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Reviews: 2 Publications: 31

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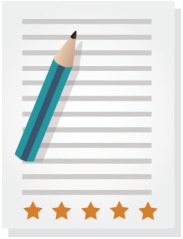
Updated 2/6/2024 v.20.1



NB200-540

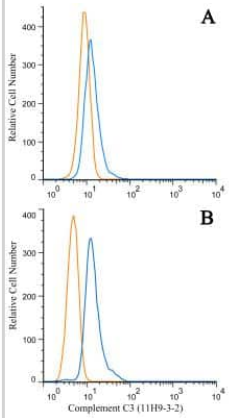
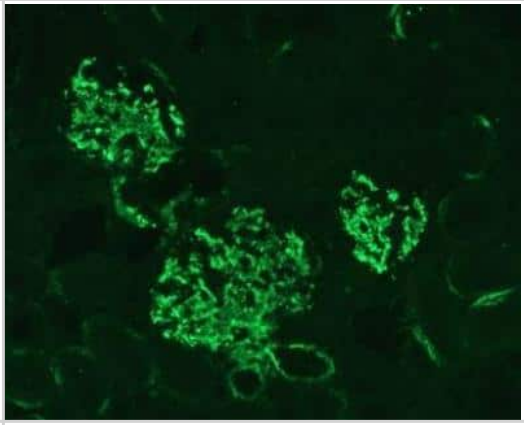
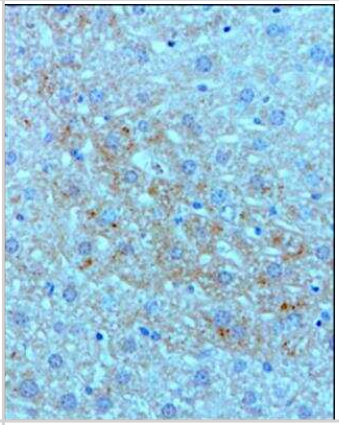
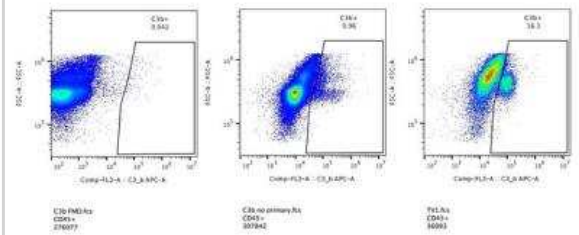
Complement C3 Antibody (11H9) - BSA Free

Product Information	
Unit Size	0.1 ml
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	11H9
Preservative	0.02% Sodium Azide
Isotype	IgG2a
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	187 kDa

Product Description	
Host	Rat
Gene ID	718
Gene Symbol	C3
Species	Mouse, E. coli
Reactivity Notes	Use in Mouse reported in scientific literature (PMID:34433493). Use in E. coli reported in scientific literature (PMID:32422907).
Specificity/Sensitivity	Mouse Complement C3 and its activation products, C3b, iC3b, C3d and C3dg
Immunogen	This Complement C3 Antibody (11H9) was developed against C57BL/6 thymocytes saturated with rat anti-Thy-1 monoclonal antibody of IgG2b subclass (RmT1).

Product Application Details	
Applications	Flow Cytometry, Flow (Intracellular), Immunoassay, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, CyTOF-ready
Recommended Dilutions	Flow Cytometry 1 ug/ml, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen 1:10-1:500, Immunoassay 0.5 ug/well in PBS, Flow (Intracellular) 1 ug/ml, CyTOF-ready

Images

Flow (Intracellular): Complement C3 Antibody (11H9) [NB200-540] - An intracellular stain was performed on RAW 246.7 cells with Complement C3 (11H9-3-2) antibody NB200-540 (blue) and a matched isotype control NBP2-31382 (orange). Cells were either treated with 3uM Monensin for 3 hours to block the secretion of Complement C3 (B) or grown in normal media (A). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2 ug/mL for 30 minutes at room temperature, followed by mouse F(ab)2 IgG (H+L) PE-conjugated secondary antibody (F0102B, R&D Systems).	
Immunocytochemistry/Immunofluorescence: Complement C3 Antibody (11H9) [NB200-540] - C3 protein fragments deposited on kidney cells of MPL-lpr mouse. Staining with antibody 11H9. Glomerular staining pattern. Fixation in 4% paraformaldehyde in PBS pH 7.4. Vibratome sections of 4 um. Pretreated with 3% hydrogen peroxide for 20 min to quench endogenous peroxidases. Microwaved in antigen unmasking solution for 2-5 minutes as antigen retrieval.	
Immunohistochemistry-Paraffin: Complement C3 Antibody (11H9) [NB200-540] - Complement C3 protein in a FFPE tissue section of mouse liver using 1:100 dilution of Complement C3 antibody (clone 11H9) NB200-540. Weak but distinct membrane-cytoplasmic immunopositivity was observed in hepatocytes and few cells developed punctate membrane staining.	
Flow Cytometry: Complement C3 Antibody (11H9) [NB200-540] - Left panel: FMO, Middle panel: No primary antibody control, Right panel: sample. Day 6 murine mammary tumors processed and stained for analysis with flow cytometry. The C3b+ population of CD45+ cells is what the gate in each sample is exhibiting. WB image submitted by a verified customer review.	

Immunohistochemistry-Paraffin: Complement C3 Antibody (11H9) [NB200-540] - Complement C3 protein in a FFPE tissue section of mouse lymph node using 1:100 dilution of Complement C3 antibody (clone 11H9) NB200-540. This representative photomicrograph shows a membrane-cytoplasmic immunopositivity in non-germinal center cells, and few cells developed an intense staining for this target protein.	

Publications

Gustavo Satoru Kajitani, Lear Brace, Jose Humberto Trevino-Villarreal, Kaspar Trocha, Michael Robert MacArthur, Sarah Vose, Dorathy Vargas, Roderick Bronson, Sarah Jayne Mitchell, Carlos Frederico Martins Menck, James Robert Mitchell Neurovascular dysfunction and neuroinflammation in a Cockayne syndrome mouse model Aging (Albany NY) 2021-10-15 [PMID: 34628368]

Engavale MB Determining the impact of macrophage-derived Dnase1L3 in lupus-like phenotypes in mice and its implications for treatment Thesis 2023-01-01

Liu XL, Sun DD, Zheng MT et al. Maraviroc promotes recovery from traumatic brain injury in mice by suppression of neuroinflammation and activation of neurotoxic reactive astrocytes Neural Regeneration Research 2023-01-01 [PMID: 35799534] (ICC/IF, WB)

Chen XC, Wu D, Wu HL et al. Metformin improves renal injury of MRL/lpr lupus-prone mice via the AMPK/STAT3 pathway Lupus Science & Medicine 2022-04-11 [PMID: 35414608] (In Vivo)

Uapinyoying P, Hogarth M, Battacharya S et al. Single-cell transcriptomic analysis of the identity and function of fibro/adipogenic progenitors in healthy and dystrophic muscle iScience 2023-08-18 [PMID: 37599828]

Stym-Popper G, Matta K, Chaigneau T et al. Regulatory T cells decrease C3-positive reactive astrocytes in Alzheimer-like pathology Journal of neuroinflammation 2023-03-08 [PMID: 36890536] (IHC-Fr, Mouse)

Khazaei S, Chen CCL, Andrade AF et al. Single substitution in H3.3G34 alters DNMT3A recruitment to cause progressive neurodegeneration Cell 2023-03-16 [PMID: 36931244]

Engavale M, Hernandez CJ, Infante A et al. Deficiency of macrophage-derived Dnase1L3 causes lupus-like phenotypes in mice bioRxiv : the preprint server for biology 2023-04-18 [PMID: 37131692] (IHC-Fr, Mouse)

Linde IL, Prestwood TR, Qiu J et al. Neutrophil-activating therapy for the treatment of cancer Cancer cell 2023-01-19 [PMID: 36706760] (FLOW, IHC-Fr, Mouse)

Salarian M, Ghim M, Toczek J et al. Homeostatic, Non-Canonical Role of Macrophage Elastase in Vascular Integrity Circulation research 2023-01-24 [PMID: 36691905] (ELISA, Mouse)

Details:
Mouse plasma

Aung A, Cui A, Maiorino L et al. Low protease activity in B cell follicles promotes retention of intact antigens after immunization Science (New York, N.Y.) 2023-01-27 [PMID: 36701450] (ELISA, Mouse)

Peng L, Liu S, Xu J et al. Metformin Alleviates Prolonged Isoflurane Inhalation Induced Cognitive Decline Via Reducing Neuroinflammation in Adult Mice Int Immunopharmacol 2022-06-16 [PMID: 35709590]

More publications at http://www.novusbio.com/NB200-540

Procedures

Flow (Intracellular) Protocol for Complement C3 Antibody (NB200-540)
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.

Immunohistochemistry-Paraffin Protocol for Complement C3 Antibody (NB200-540)
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 NB200-540

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)
P3343-10ug	Recombinant Mouse Complement C3 GST (N-Term) Protein

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