Product Datasheet

CD47 Antibody (B6H12.2) - Azide and BSA Free
NBP2-31106

Unit Size: 0.1 mg

Store at -20C. Avoid freeze-thaw cycles.

www.novusbio.com technical@novusbio.com

Reviews: 3 Publications: 6

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NBP2-31106

Updated 9/10/2024 v.20.1

NBP2-31106

CD47 Antibody (B6H12.2) - Azide and BSA Free

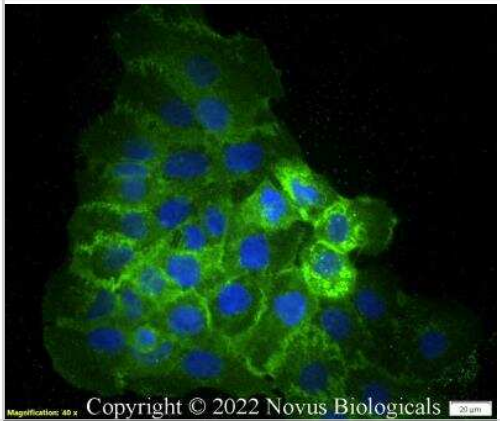
Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	B6H12.2
Preservative	No Preservative
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS

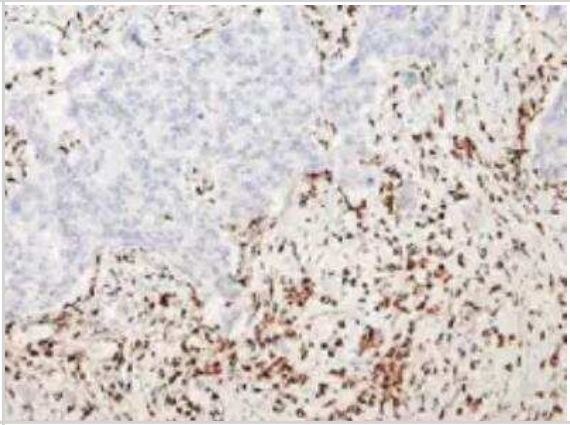
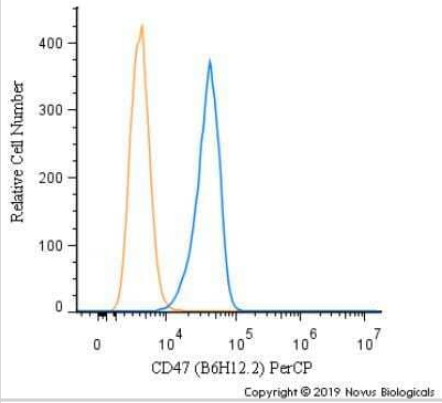
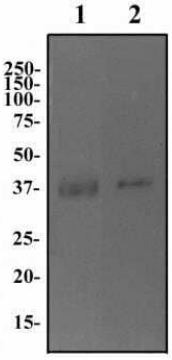
Product Description	
Host	Mouse
Gene ID	961
Gene Symbol	CD47
Species	Human, Mouse
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 30833751).
Immunogen	This antibody was raised against CD47.

Product Application Details	
Applications	Western Blot, Flow Cytometry, Flow (Cell Surface), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, CyTOF-ready
Recommended Dilutions	Western Blot 1:1000, Flow Cytometry 1 ug/mL, Immunohistochemistry, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation, Immunohistochemistry-Paraffin 0.5 ug/mL, Flow (Cell Surface), CyTOF-ready
Application Notes	This antibody is CyTOF ready.

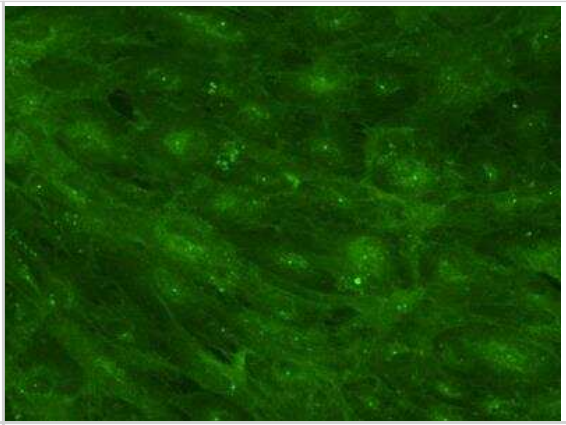
Images

Immunocytochemistry/Immunofluorescence: CD47 Antibody (B6H12.2) - Azide Free [NBP2-31106] - A431 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD47 Antibody [B6H12.2] (NBP2-31106) at 1 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

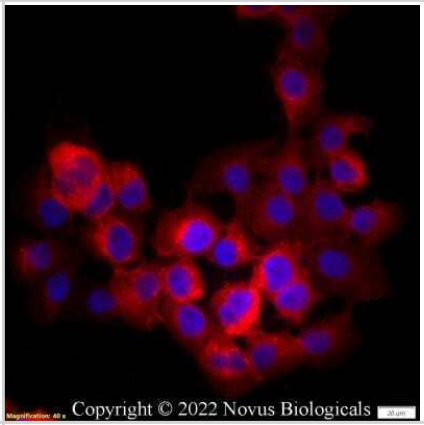


Western Blot: CD47 Antibody (B6H12.2) - Azide Free [NBP2-31106] - Detection of CD47 expression on two hematological cancer cell lines, Jurkat and Raji. WB image submitted by a verified customer review.	
Immunohistochemistry-Paraffin: CD47 Antibody (B6H12.2) - Azide Free [NBP2-31106] - Human breast cancer tissue stained with CD47 Antibody (B6H12.2). IHC-P image submitted by a verified customer review.	
Flow Cytometry: CD47 Antibody (B6H12.2) - Azide Free [NBP2-31106] - A surface stain was performed on A431 cells with CD47 Antibody [B6H12.2] NBP2-31106PCP (blue) and a matched isotype control (orange). Cells were incubated in an antibody dilution of 5 ug/mL for 20 minutes at room temperature. Both antibodies were conjugated to PerCP.	
Western Blot: CD47 Antibody (B6H12.2) [Azide Free] [NBP2-31106] - Human brain (lane 1) and testis (lane 2) protein was separated on a 12% gel by SDS-PAGE. Protein was transferred to PVDF membrane, blocked and then probed with 2 ug/ml of anti-CD47. CD47 protein was detected using an anti-mouse HRP secondary antibody.	

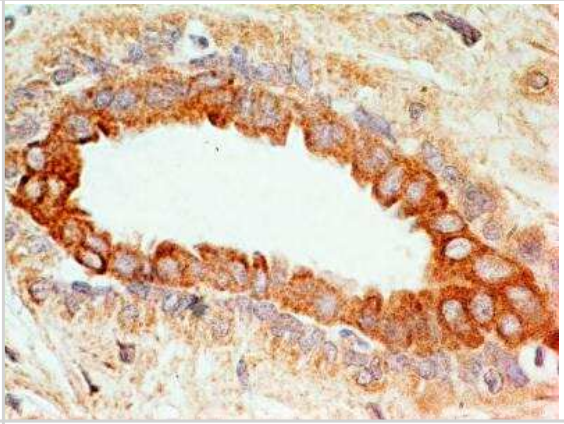
Immunocytochemistry: CD47 Antibody (B6H12.2) - Azide Free [NBP2-31106] - Human trabecular meshwork (primary ocular cells). Image from verified customer review.



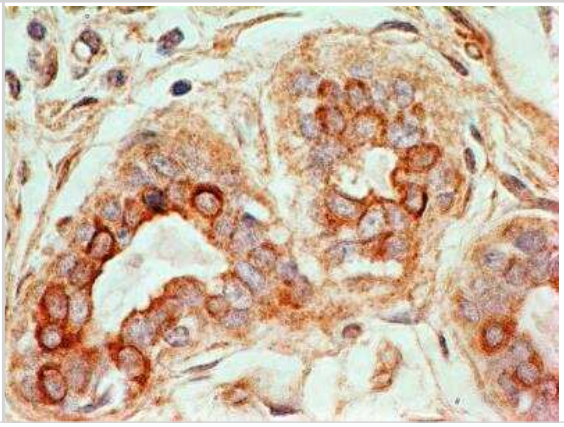
Immunocytochemistry/Immunofluorescence: CD47 Antibody (B6H12.2) - Azide Free [NBP2-31106] - A431 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with CD47 Antibody [B6H12.2] conjugated to Biotin (NBP2-31106B) at 5 ug/ml for 1 hour at room temperature then detected with Streptavidin conjugated to DyLight 550. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



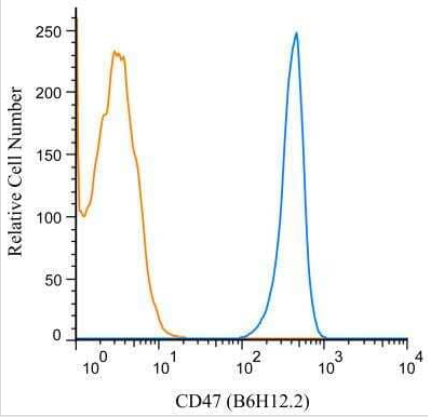
Immunohistochemistry-Paraffin: CD47 Antibody (B6H12.2) - Azide Free [NBP2-31106] - Tissue section of human normal breast using mouse monoclonal CD47 antibody (clone B6H12.2) at 0.5ug/ml concentration. The ductal/acinar epithelial cells in the breast section developed specific membrane-cytoplasmic staining.



Immunohistochemistry-Paraffin: CD47 Antibody (B6H12.2) - Azide Free [NBP2-31106] - Tissue section of human normal breast using mouse monoclonal CD47 antibody (clone B6H12.2) at 0.5ug/ml concentration. The ductal/acinar epithelial cells in the breast section developed specific membrane-cytoplasmic staining.



Flow (Cell Surface): CD47 Antibody (B6H12.2) - Azide Free [NBP2-31106] - A surface stain was performed on human peripheral blood lymphocytes with CD47 (B6H12.2) antibody NBP2-31106 (blue) and a matched isotype control NBP2-27287 (orange). Cells were incubated in an antibody dilution of 1 ug/mL for 20 minutes at room temperature, followed by mouse F(ab)2 IgG (H+L) APC-conjugated secondary antibody [F0101B, R&D Systems].



Publications

Singla B, Aithbathula RV, Pervaiz N et al. CD47 Activation by Thrombospondin-1 in Lymphatic Endothelial Cells Suppresses Lymphangiogenesis and Promotes Atherosclerosis Arteriosclerosis, thrombosis, and vascular biology 2023-07-01 [PMID: 37259865] (IHC-P, WB, Mouse, Human)

Details:
IHC Dilution: 1:100

Scheepstra KWF, Mizee MR, van Scheppingen J et al. Microglia transcriptional profiling in major depressive disorder shows inhibition of cortical grey matter microglia Biological psychiatry 2023-04-28 [PMID: 37121366] (WB)

Dai S, Liu Y, Zhao F et al. Aqueous extract of Taxus chinensis var. mairei targeting CD47 enhanced antitumor effects in non-small cell lung cancer Biomedicine & Pharmacotherapy 2022-10-01 [PMID: 36058145] (WB, IP, IHC-P, ICC/IF, Mouse, Human)

Davis RM, Kiss B, Trivedi DR el al. Surface-Enhanced Raman Scattering Nanoparticles for Multiplexed Imaging of Bladder Cancer Tissue Permeability and Molecular Phenotype ACS Nano. 2018-10-23 [PMID: 30203645] (IHC-P, Human)

Logtenberg MEW, Jansen JHM, Raaben M et al. Glutaminyl cyclase is an enzymatic modifier of the CD47-SIRP alpha axis and a target for cancer immunotherapy Nat. Med. 2019-04-01 [PMID: 30833751] (IP, Mouse)

Kojima Y, Volkmer JP, McKenna K et al. CD47-blocking antibodies restore phagocytosis and prevent atherosclerosis Nature 2016-07-20 [PMID: 27437576]

Procedures

Immunohistochemistry-Paraffin protocol for CD47 Antibody (NBP2-31106)
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 all the time).

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.

Immunocytochemistry/Immunofluorescence Protocol for CD47 Antibody (NBP2-31106)
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 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 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 10 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 10 minutes each.
10. Counter stain DNA with DAPi if required.

Flow (Cell Surface) Protocol for CD47 Antibody (NBP2-31106)
Protocol for Flow Cytometry Cell Surface 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 15 mL conical tube and centrifuge for 4 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 permeablization steps might reduce the availability of surface antigens.
Cell surface staining
1. Recommended: Block non-specific interactions using 0.5-1 ug of a species specific Fc-blocking reagent such as an anti-mouse CD16/CD32 antibody (NBP1-27946).
2. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined) to 100 uL of staining buffer (NBP2-26247) per sample (eg. use 1 mL of staining buffer for 10 samples).
3. Mix well and incubate at room temperature in dark for 20 minutes.
4. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
5. 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.
6. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
7. Incubate at room temperature in dark for 20 minutes.
8. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
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. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

Western Blot Protocol for CD47 Antibody (NBP2-31106)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST 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.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
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.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

Products Related to NBP2-31106

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)
NBP2-52320-0.05mg	Recombinant Human CD47 hIgG-His Protein

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 100% guarantee, please visit www.novusbio.com/guarantee

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NBP2-31106

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications