Product Datasheet

MyD88 Antibody (4D6) - BSA Free
NBP2-27369

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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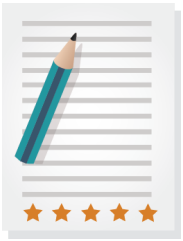
Updated 10/23/2024 v.20.1



NBP2-27369

MyD88 Antibody (4D6) - BSA Free

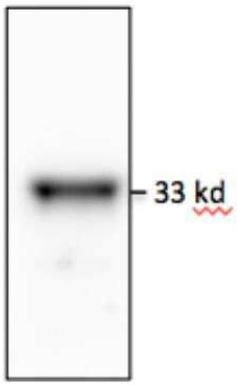
Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	4D6
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	4615
Gene Symbol	MYD88
Species	Human, Mouse, Primate, Rhesus Macaque
Reactivity Notes	Use in Mouse reported in scientific literature (PMID:33763070).
Specificity/Sensitivity	The immunogen is 100% homologous to isoform 3: 264 aa, isoform 4: 204 aa; isoform 5: 159 aa, CRA_a: 304 aa, CRA_b: 309 aa),
Immunogen	A portion of amino acids 50-100 of human MyD88 was used as the immunogen for this antibody.

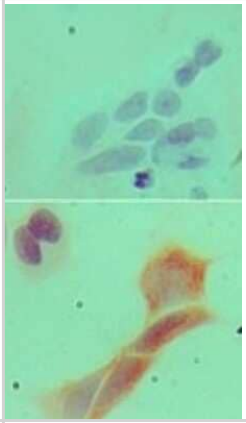
Product Application Details	
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, CyTOF-ready
Recommended Dilutions	Western Blot 2 ug/ml, Flow Cytometry 0.5ug/10^6cells~, Immunocytochemistry/ Immunofluorescence 5ug/ml, Flow (Intracellular), CyTOF-ready
Application Notes	This antibody is Cytof ready.

Images

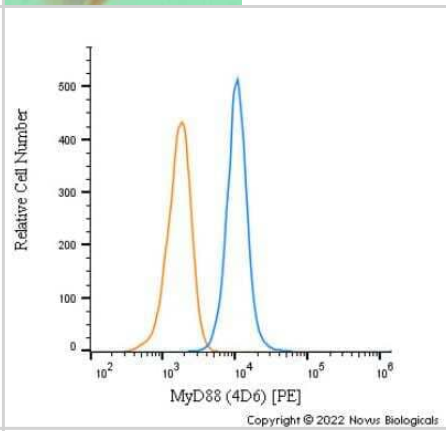
Western Blot: MyD88 Antibody (4D6) [NBP2-27369] - Normal human bronchial epithelial total cell lysate. Image from verified customer review.



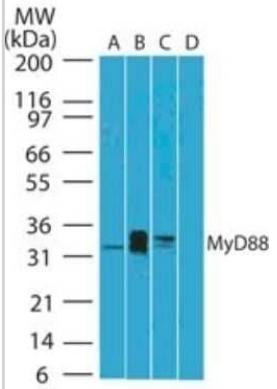
Immunocytochemistry/Immunofluorescence: MyD88 Antibody (4D6) [NBP2-27369] - Analysis of MyD88 antibody in MCF-7 cells using an isotype control (top) and MyD88 Antibody (bottom) at 5 ug/ml.



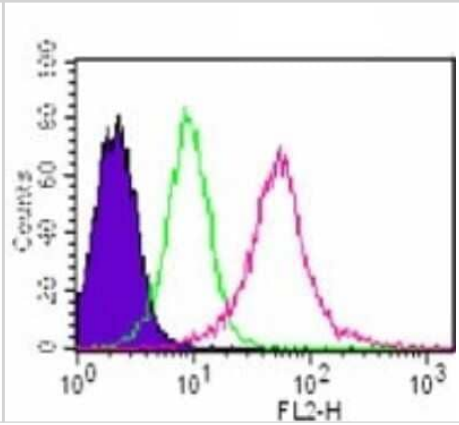
Flow Cytometry: MyD88 Antibody (4D6) - BSA Free [NBP2-27369] - An intracellular stain was performed on Jurkat cells with MyD88 Antibody (4D6) NBP2-27369PE (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.

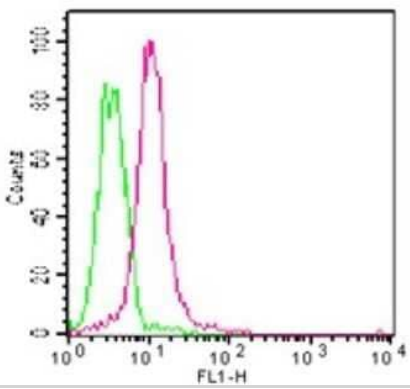
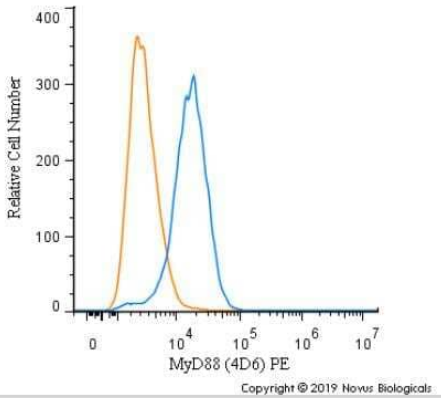
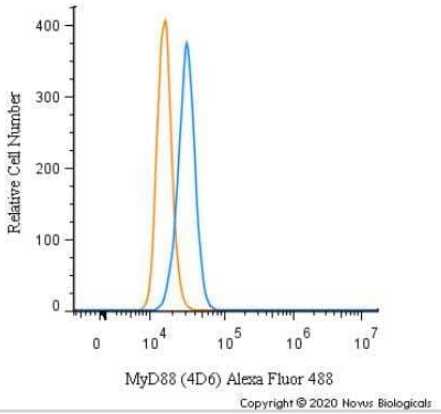
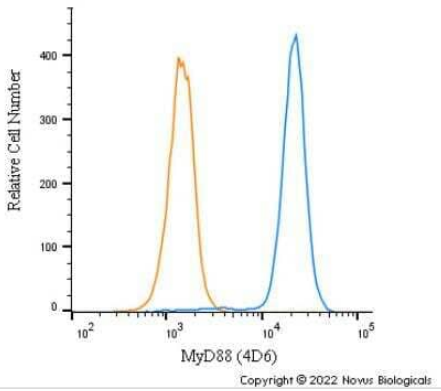


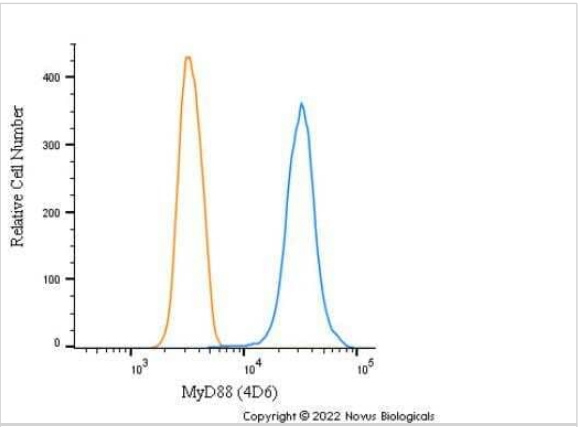
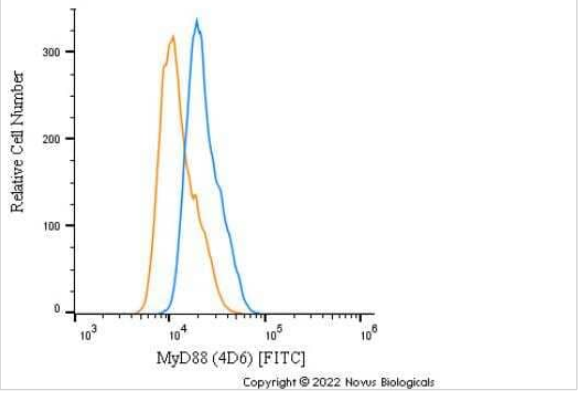
Western Blot: MyD88 Antibody (4D6) [NBP2-27369] - Analysis of MyD88 in A) human ovary, B) human prostate and Jurkat cell lysate in the C) absence and D) presence of immunizing peptide using this antibody. Goat anti-mouse Ig HRP secondary antibody and PicoTect ECL substrate solution were used for this test.



Flow Cytometry: MyD88 Antibody (4D6) [NBP2-27369] - Intracellular analysis of MyD88 antibody in Jurkat cells using 0.5 ug. Shaded histogram represents cells without antibody; green represents isotype control; red represents MyD88 antibody. This antibody was used for this test, and an anti-mouse IgG PE conjugated secondary antibody.



Flow (Intracellular): MyD88 Antibody (4D6) [NBP2-27369] - Analysis using the Alexa Fluor (R) 488 conjugate of NBP2-27369. Staining of MyD88 antibody in Jurkat cells using 10 ul (0.5 ug) of MyD88 antibody. Green represents isotype control (20108); red represents anti-MyD88 antibody.	
Flow Cytometry: MyD88 Antibody (4D6) [NBP2-27369] - An intracellular stain was performed on MCF7 cells with MyD88 (4D6) antibody NBP2-27369PE (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.	
Flow Cytometry: MyD88 Antibody (4D6) [NBP2-27369] - An intracellular stain was performed on MCF7 cells with MyD88 [4D6] Antibody NBP2-27369AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 10 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.	
Flow Cytometry: MyD88 Antibody (4D6) - BSA Free [NBP2-27369] - An intracellular stain was performed on Jurkat cells with MyD88 Antibody (4D6) NBP2-27369 (blue) and a matched isotype control MAB002 (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 Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (84540, Thermo Fisher).	

Flow Cytometry: MyD88 Antibody (4D6) - BSA Free [NBP2-27369] - An intracellular stain was performed on U-937 cells with MyD88 Antibody (4D6) NBP2-27369 (blue) and a matched isotype control MAB002 (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 Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (84540, Thermo Fisher).	
Flow Cytometry: MyD88 Antibody (4D6) - BSA Free [NBP2-27369] - An intracellular stain was performed on MCF7 cells with MyD88 Antibody (4D6) NBP2-27369F (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to FITC.	

Publications

Maja Šutić, Antje Motzek, Gordana Bubanović, Matthias Linke, Ivan Sabol, Oliver Vugrek, Petar Ozretić, Luka Brčić, Sven Seiwerth, Željko Debeljak, Antonija Jakovčević, Zoran Janevski, Dinko Stančić-Rokotov, Andrea Vukić-Dugac, Marko Jakopović, Miroslav Samaržija, Ulrich Zechner, Jelena Knežević Promoter methylation status of ASC/TMS1/PYCARD is associated with decreased overall survival and TNM status in patients with early stage non-small cell lung cancer (NSCLC) Translational Lung Cancer Research 2019-12-01 [PMID: 32010578]

Herbst C, Bouteau A, Menyk? E et al. Dendritic Cells Overcome Cre/Lox Induced Gene Deficiency by Siphoning Material From Neighboring Cells Using Intracellular Monitoring a Novel Mechanism of Antigen Acquisition bioRxiv 2023-07-25 [PMID: 37546718] (Flow Cytometry, Mouse)

Yan K, Xu G, Li Z MicroRNA-20b carried by mesenchymal stem cell-derived extracellular vesicles protects alveolar epithelial type II cells from Mycobacterium tuberculosis infection in vitro Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases 2022-04-30 [PMID: 35504589] (WB)

Karmakar J, Mandal C Interplay Between Sialic Acids, Siglec-E, and Neu1 Regulates MyD88- and TRIF-Dependent Pathways for TLR4-Activation During Leishmania donovani Infection Frontiers in immunology 2021-03-03 [PMID: 33763070] (WB, Mouse)

Davis S, Cirone A M et al. Phagocytosis-mediated M1 activation by chitin but not by chitosan. Am J Physiol Cell Physiol 2018-01-07 [PMID: 29719169] (PLA, Mouse)

Im JY, Kim DM, Park H et al. Bacterial Lipoteichoic Acid Attenuates Toll-Like Receptor Dependent Dendritic Cells Activation and Inflammatory Response Biochim Biophys Acta Mol Cell Res 2020-10-08 [PMID: 33069758] (FLOW, Mouse)

Ramirez-Ramirez D, Vadillo E, Arriaga-Pizano LA et al. Early Differentiation of Human CD11c. Journal of Immunology Research 2016-08-12 [PMID: 27847830] (Human)

Bauer JMJ. Interferon-b moduliert die Expression von Toll-like Rezeptoren in mononuklearen Blutzellen und induziert Toll-like Rezeptor 7 in plasmazytoiden dendritischen Zellen von Patienten mit Multipler Sklerose. thesis. 2015-11-12

Derkow K, Kruger C, Dembny P, Lehnardt S. Microglia Induce Neurotoxic IL-17+ gammadelta T Cells Dependent on TLR2, TLR4, and TLR9 Activation. PLoS ONE. 2015-08-20 [PMID: 26288016] (FLOW, Mouse)

Details:
MyD88 antibody (clone 4D6) was used for FLOW analaysis (intracellular staining) of gamma delta T cells from C57BL/6J mice mice. The cells were blocked for Fc gamma-receptors before MyD88 staining and the data was compared with isotype controls (Fig 2A).

Procedures

Western Blot Protocol for MyD88 Antibody (NBP2-27369)
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.

Flow (Intracellular) Protocol for MyD88 Antibody (NBP2-27369)

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 permeablization 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 permeabization 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 NBP2-27369

NBP2-29328	MyD88 Inhibitor Peptide Set
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

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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