Product Datasheet

Dectin-2/CLEC6A Antibody (3D1) - BSA Free
NBP2-27159

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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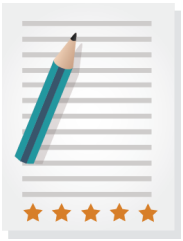
Updated 10/23/2024 v.20.1



NBP2-27159

Dectin-2/CLEC6A Antibody (3D1) - 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	3D1
Preservative	0.02% Sodium Azide
Isotype	IgG3 Kappa
Purity	Protein G purified
Buffer	PBS

Product Description

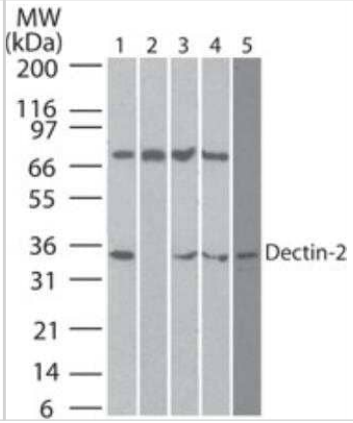
Host	Mouse
Gene ID	93978
Gene Symbol	CLEC6A
Species	Human, Primate
Reactivity Notes	Bovine, Sheep (83%), Mouse (65%), Rat (52%).
Immunogen	A blend of two peptides made to amino acids 44-63 and 90-106 of human Dectin-2.

Product Application Details

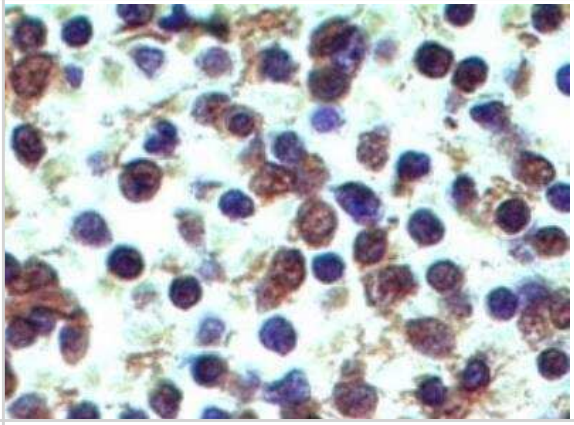
Applications	Western Blot, Flow Cytometry, Flow (Cell Surface), Immunohistochemistry, Immunohistochemistry-Paraffin, CyTOF-ready
Recommended Dilutions	Western Blot 2 - 5ug/mL, Flow Cytometry 0.5 ug/5x10^5 cells, Immunohistochemistry 1:200, Immunohistochemistry-Paraffin 1:200, Flow (Cell Surface), CyTOF-ready

Images

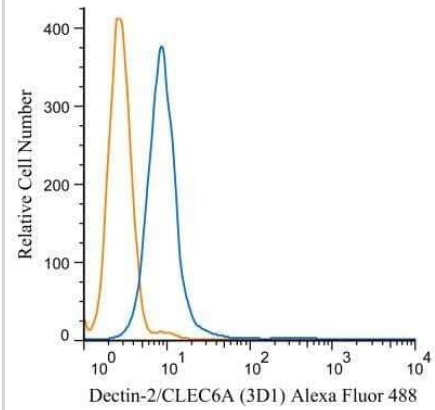
Western Blot: Dectin-2/CLEC6A Antibody (3D1) [NBP2-27159] - Analysis of Dectin-2 in human heart lysate in the 1) absence and 2) presence of immunizing peptide, 3) mouse heart lysate, 4) rat heart lysate and 5) human Ramos cell lysate using Dectin-2 antibody at 2 ug/mL. Goat anti-mouse Ig HRP secondary antibody and PicoTect ECL substrate solution were used for this test.



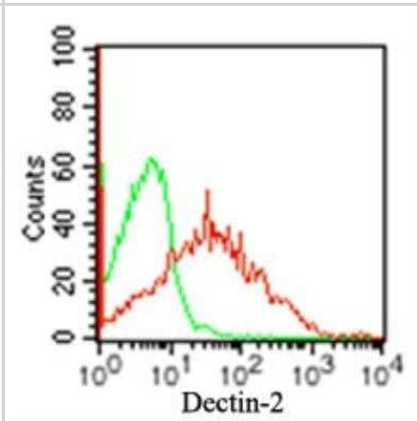
Immunohistochemistry-Paraffin: Dectin-2/CLEC6A Antibody (3D1) [NBP2-27159] - Normal human spleen tissue stained with Dectin-2 antibody (5 ug/mL), peroxidase-conjugate and DAB chromogen. Human tissue TMA was used for this test.



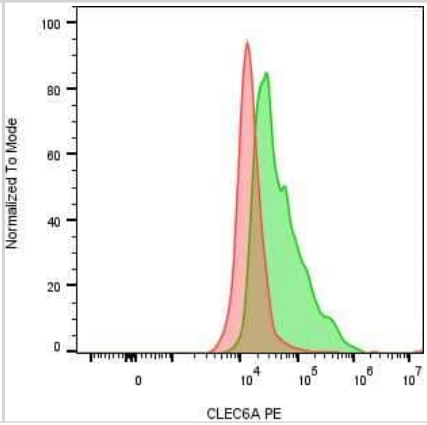
Flow Cytometry: Dectin-2/CLEC6A Antibody (3D1) [NBP2-27159] - An intracellular stain was performed on THP-1 cells with Dectin-2/CLEC6A (3D1) antibody NBP2-27159AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



Flow Cytometry: Dectin-2/CLEC6A Antibody (3D1) [NBP2-27159] - Surface staining of Dectin-2 on 5x10^5 human PBMCs using 0.5 ug of Dectin-2 antibody (red) and 0.5 ug of isotype control antibody (green).



Flow Cytometry: Mouse Monoclonal Dectin-2/CLEC6A Antibody (3D1) [IMGENEX: IMG-6682A] [NBP2-27159] - Dectin-2/CLEC6A expression on human monocyte-derived macrophages. Macrophages were treated with 10 µg/ml of the Dectin-2/CLEC6A Antibody (3D1) [PE] (catalog # NBP2-27159PE) (green) or mouse IgG3 PE isotype control antibody (red) for 30 minutes on ice. Image from a verified customer review.



Procedures

Immunocytochemistry/Immunofluorescence Protocol for Dectin-2/CLEC6A Antibody (NBP2-27159)
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.

Western Blot Protocol for Dectin-2/CLEC6A Antibody (NBP2-27159)
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 manufacturer's instructions.

Flow (Cell Surface) Protocol for Dectin-2/CLEC6A Antibody (NBP2-27159)

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.

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Products Related to NBP2-27159

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-96978	Mouse IgG3 Kappa Light Chain Isotype Control (MG3K)
NBP2-27159PE	Dectin-2/CLEC6A Antibody (3D1) [PE]

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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