Product Datasheet

L1CAM Antibody (UJ127.11) - BSA Free
NB100-2682

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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Reviews: 2 Publications: 4

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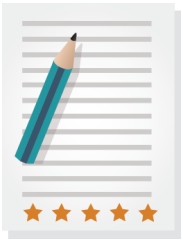
Updated 8/21/2023 v.20.1



NB100-2682

L1CAM Antibody (UJ127.11) - BSA Free

Product Information

Unit Size	0.1 mg
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	UJ127.11
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein A or G purified
Buffer	PBS

Product Description

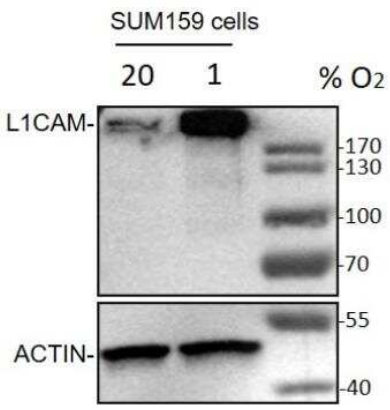
Host	Mouse
Gene ID	3897
Gene Symbol	L1CAM
Species	Human, Mouse
Marker	Axon Marker
Immunogen	Homogenous suspension of 16 week human foetal brain.

Product Application Details

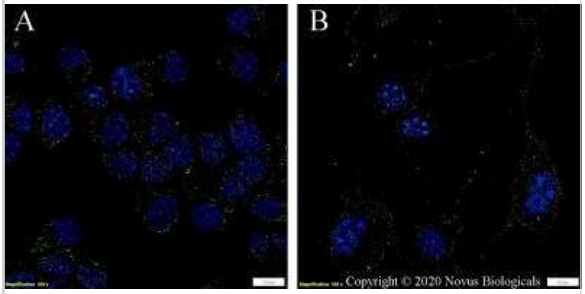
Applications	Western Blot, ELISA, Flow Cytometry, Flow (Cell Surface), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, CyTOF-ready, Immunofluorescence
Recommended Dilutions	Western Blot 2 ug/mL, Flow Cytometry 2.5 ug/mL, ELISA 1:100 - 1:2000, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 2 - 5 ug/mL, Immunoprecipitation 1:10 - 1:500, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen, Flow (Cell Surface) 2.5 ug/mL, Immunofluorescence 1 - 2 ug/mL, CyTOF-ready
Application Notes	Positive control(s): Frozen mouse E16.5 lung sections (IHC), Cell lines TR 14, LAN-1, CHP 100, CHP212, CHP126 (Neuroblastomas or Schwannomas) (IF), M5 melanoma cells or human foetal brain (WB). This antibody is CyTOF ready.

Images

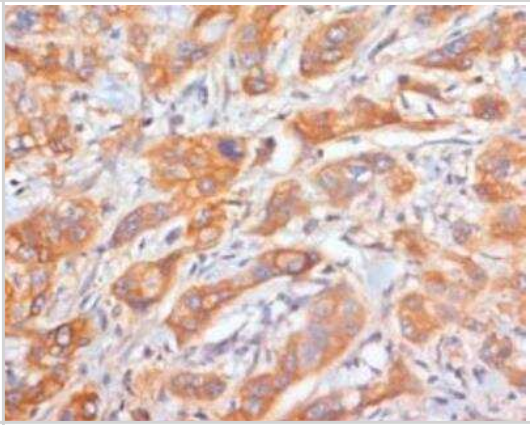
Western Blot: L1CAM Antibody (UJ127.11) [NB100-2682] - SUM159 cells were exposed to 20% or 1% O2 for 48 hours, whole cell lysates were loaded with 50 ug/lane. 10% SDS-PAGE. L1CAM Antibody (NB100 -2682) primary antibody: 1:1000, 4C, overnight. Western blot image submitted by a verified customer review.



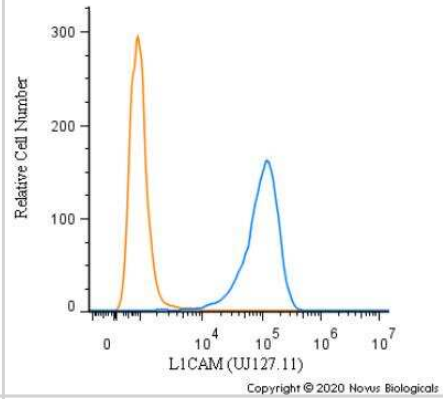
Immunocytochemistry/Immunofluorescence: L1CAM Antibody (UJ127.11) [NB100-2682] - The left panel (A) shows untreated Neuro2a cells and the right panel (B) shows Neuro2a cells that were serum starved then treated with 1mM cAMP overnight to induce axon growth. Cells were fixed in 4% paraformaldehyde for 10 minutes and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton-X100. The cells were incubated with anti- NB100-2682 at 5 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 100X objective and digitally deconvolved.



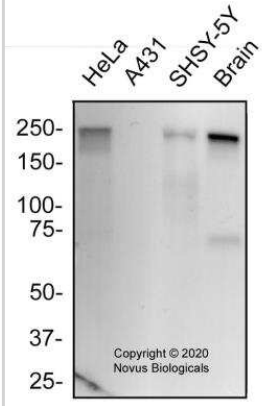
Immunohistochemistry-Frozen: L1CAM Antibody (UJ127.11) [NB100-2682] - Human pancreatic ductal adenocarcinoma (PDAC) tissue section stained with L1CAM antibody. IHC-Fr image submitted by a verified customer review.



Flow Cytometry: L1CAM Antibody (UJ127.11) [NB100-2682] - A surface stain was performed on HeLa cells with L1CAM Antibody (UJ127.11) NB100-2682 (blue) and a matched isotype control (orange). Cells were incubated in an antibody dilution of 2.5 ug/mL for 20 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).



Western Blot: L1CAM Antibody (UJ127.11) [NB100-2682] - Total protein from human HeLa, A431 and SHSY-5Y cell lines and human Brain was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-L1CAM (NB100-2682) in blocking buffer and detected with an anti-mouse HRP secondary antibody using NovaLume chemiluminescence detection reagent (NPB2-61915).



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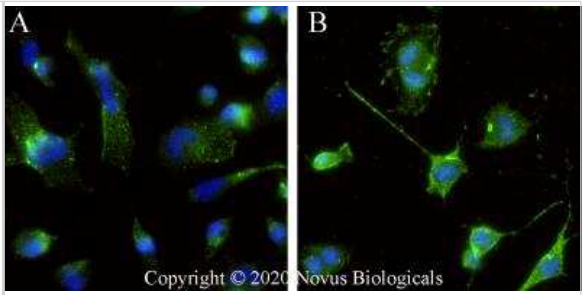
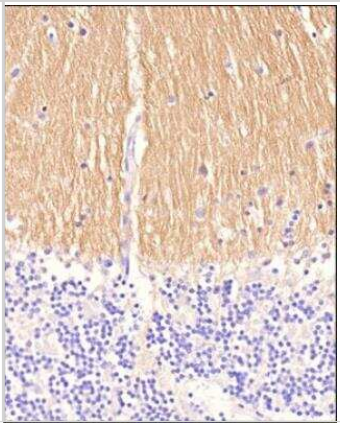


Immunohistochemistry-Paraffin: L1CAM Antibody (UJ127.11) [NB100-2682] - Analysis of a FFPE tissue section of human brain using 1:200 dilution of L1CAM [UJ127.11] antibody. The staining was developed using HRP labeled anti-mouse secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin. Cytoplasmic and membrane staining as observed in the cerebral cortex



Publications

Gajendran N, Rajasekaran S, Witt SN Knocking out alpha-synuclein in melanoma cells downregulates L1CAM and decreases motility Scientific reports 2023-06-07 [PMID: 37286800] (WB, Human)

Liu Q, Adhikari E, Lester D et al. Androgen Drives Melanoma Invasiveness and Metastatic Capacity by Promoting Tumorigenic Fucosylation Research Square 2023-03-10 (WB, IHC-P)

Tomomasa R, Arai Y, Kawabata-Iwakawa R et al. Ependymoma-like tumor with mesenchymal differentiation harboring C11orf95-NCOA1/2 or -RELA fusion: A hitherto unclassified tumor related to ependymoma Brain pathology (Zurich, Switzerland) 2021-02-12 [PMID: 33576087] (IHC-P, Human)

Sasaki A, Hirato J, Hirose T et al. Review of ependymomas: assessment of consensus in pathological diagnosis and correlations with genetic profiles and outcome. Brain Tumor Pathol. 2019-03-30 [PMID: 30929114] (IF/IHC, Human)

Procedures

Western Blot Protocol for L1CAM Antibody (NB100-2682)
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

Immunohistochemistry-Paraffin Protocol for L1CAM Antibody (NB100-2682)
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.

Flow (Cell Surface) Protocol for L1CAM Antibody (NB100-2682)

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 NB100-2682

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)
NBP2-59904-50ug	Recombinant Human L1CAM 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.

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