Product Datasheet

LYVE-1 Antibody - BSA Free
NB100-725

Unit Size: 0.1 ml

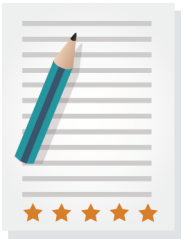
Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

www.novusbio.com technical@novusbio.com

Reviews: 2 Publications: 12

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB100-725

Updated 10/23/2024 v.20.1



NB100-725

LYVE-1 Antibody - BSA Free

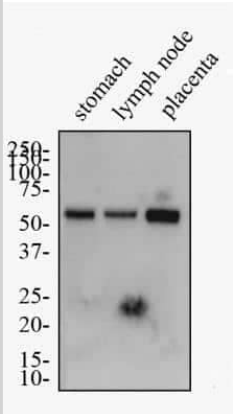
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	35 kDa

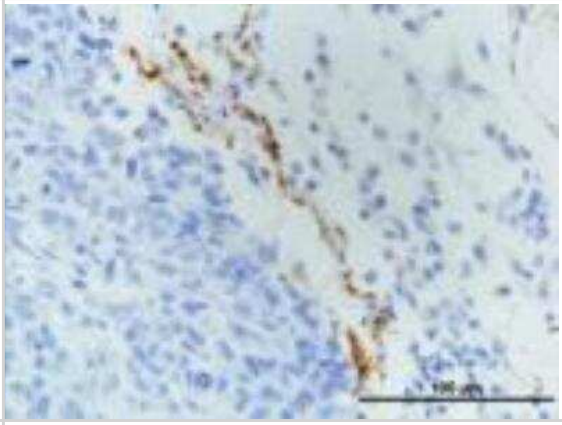
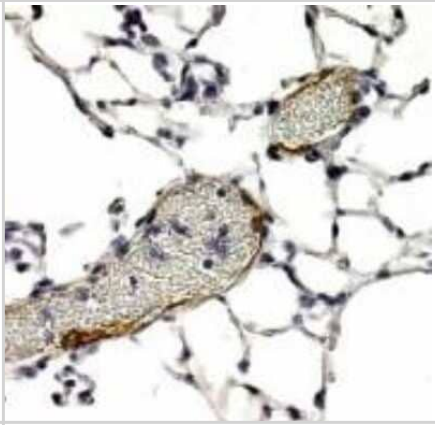
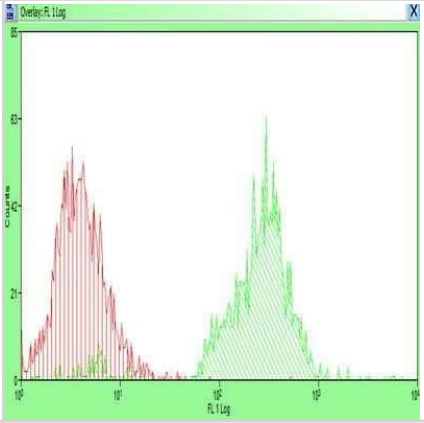
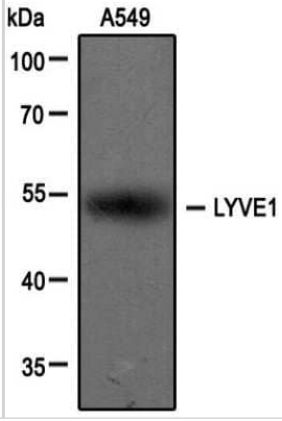
Product Description	
Host	Rabbit
Gene ID	10894
Gene Symbol	LYVE1
Species	Human, Mouse, Rat
Marker	Lymphatic Vessel Marker
Immunogen	This LYVE-1 Antibody was developed against a synthetic peptide made to a C-terminal portion of the mouse LYVE1 protein sequence (between residues 250-318). [UniProt# Q8BHC0]

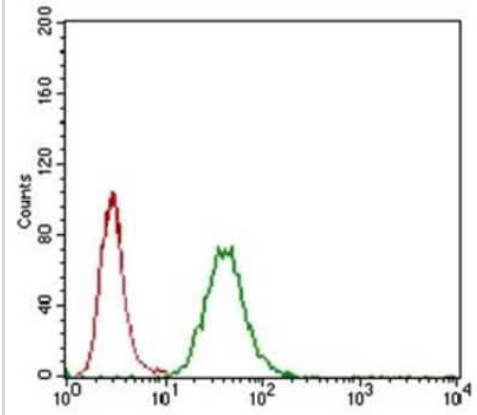
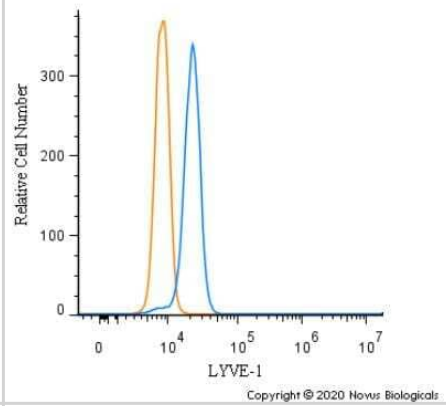
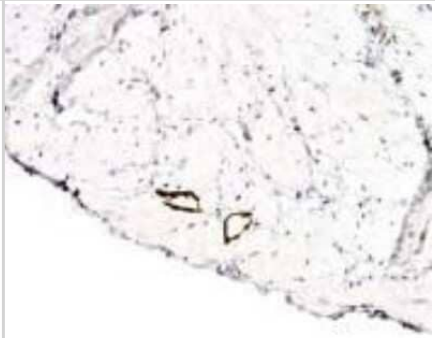
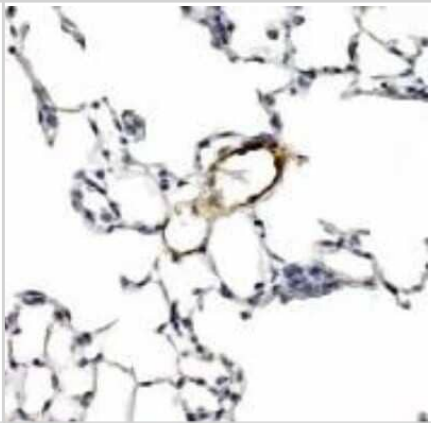
Product Application Details	
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:500-1:2000, Flow Cytometry 1:50-1:500, Immunohistochemistry 1:100-1:200, Immunohistochemistry-Paraffin 1:100-1:200, Immunohistochemistry-Frozen 1:100-1:200, Flow (Intracellular)
Application Notes	In Western Blot, a band at ~52 kDa is seen. For Immunohistochemistry citrate buffer antigen retrieval is recommended.

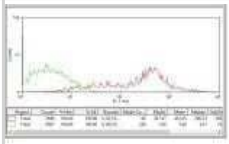
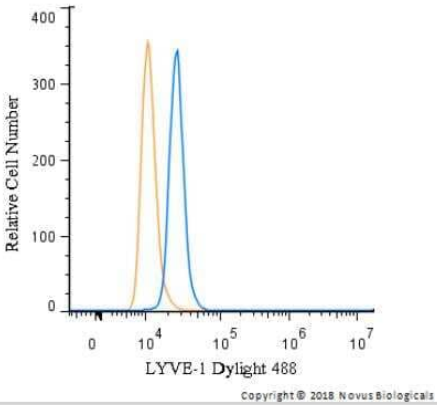
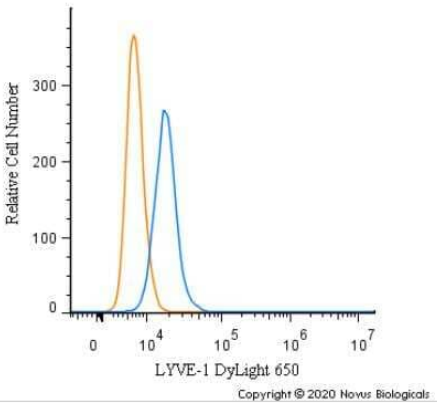
Images

Western Blot: LYVE-1 Antibody [NB100-725] - Western Blot: [NB100-725] - Total protein from human stomach, lymph node and placenta 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 1.0 ug/ml anti-LYVE1 in 1% milk, and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.



Immunohistochemistry-Paraffin: LYVE-1 Antibody [NB100-725] - Analysis using the DyLight 488 conjugate of NB100-725. Staining of LYVE1 in paraffin embedded MDA-MB-231 breast cancer orthotopic transplantation tissue. Image courtesy of product review submitted by Luana Schito.	
Immunohistochemistry: LYVE-1 Antibody [NB100-725] - Detection of LYVE1 in endothelial cells of human lung blood vessels using NB100-725. Note presence of RBCs within vessel lumen.	
Flow Cytometry: LYVE-1 Antibody [NB100-725] - Analysis using the DyLight 488 conjugate of NB100-725. Unlabeled control with labeled samples of cells dissociated mechanically and enzymatically from human skin. Image provided via product review by Patricia Redondo.	
Western Blot: LYVE-1 Antibody [NB100-725] - Analysis of extracts from A549 cells using LYVE1 antibody (NB100-725, 1:100). Image from verified customer review.	

Flow Cytometry: LYVE-1 Antibody [NB100-725] - LYVE1 antibody was tested at 1:400 in A549 cells using an Alexa Fluor 488 secondary (shown in green) alongside unstained cells (shown in red).	
Flow Cytometry: LYVE-1 Antibody [NB100-725] - An intracellular stain was performed on A549 cells with LYVE-1 Antibody NB100-725 (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 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	
Immunohistochemistry: LYVE-1 Antibody [NB100-725] - Detection of LYVE1 in endothelial cells of human bladder vasculature using NB100-725.	
Immunohistochemistry: LYVE-1 Antibody [NB100-725] - Detection of LYVE1 in endothelial cells of human lung blood vessels using NB100-725.	

Flow Cytometry: LYVE-1 Antibody [NB100-725] - Analysis using the Alexa Fluor (R) 488 conjugate of NB100-725. Staining of LYVE1 in human dissociated skin lyve1+ using AF488 conjugated anti-LYVE1 antibody. Image from verified customer review.	
Flow Cytometry: LYVE-1 Antibody [NB100-725] - An intracellular stain was performed on A549 cells with LYVE-1 Antibody NB100-725G (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 Dylight 488.	
Flow Cytometry: LYVE-1 Antibody [NB100-725] - An intracellular stain was performed on K562 cells with LYVE-1 Antibody NB100-725C (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 DyLight 650.	

Publications

Chaurasiya V, Pham DD, Harju J et al. Human visceral adipose tissue microvascular endothelial cell isolation and establishment of co-culture with white adipocytes to analyze cell-cell communication Experimental cell research 2023-10-16 [PMID: 37852349]

Cheon H, Gelvosa MN, Kim SA et al. Lymphatic channel sheet of polydimethylsiloxane for preventing secondary lymphedema in the rat upper limb model Bioengineering & Translational Medicine 2023-01-01 [PMID: 36684082] (Immunohistochemistry)

Nagahara AI, Homma J, Ryu B et al. Networked lymphatic endothelial cells in a transplanted cell sheet contribute to form functional lymphatic vessels Scientific reports 2022-12-15 [PMID: 36522421] (IHC, Rat)

Pique-Regi R, Romero R, Tarca AL et al. Single Cell Transcriptional Signatures of the Human Placenta in Term and Preterm Parturition Elife 2019-12-13 [PMID: 31829938]

Cheon H, Gelvosa M, Kim S et al. Lymphatic channel sheet of polydimethylsiloxane for preventing secondary lymphedema in the rat upper limb model Bioengineering & Translational Medicine 2022-07-05

Kang MJ, Lee S, Jung U Et al. Inhibition of Hepatic Stellate Cell Activation Suppresses Tumorigenicity of Hepatocellular Carcinoma in Mice The American journal of pathology 2021-08-21 [PMID: 34428424] (IHC-P, Mouse)

Schafflick D, Wolbert J, Heming M Et Al. Single-cell profiling of CNS border compartment leukocytes reveals that B cells and their progenitors reside in non-diseased meninges Nature neuroscience 2021-07-12 [PMID: 34253922] (IHC-Fr)

Esposito E, Ahn BJ, Shi J et al. Brain-to-cervical lymph node signaling after stroke Nat Commun. 2019-11-22 [PMID: 31757960] (Mouse)

Details:
Citation used the Biotin format of this antibody.

Pique-Regi R, Romero R, Tarca AL et al. Single Cell Transcriptional Signatures of the Human Placenta in Term and Preterm Parturition bioRxiv (IF, IHC-Fr, Human)

Azam SH Characterizing a Regulatory Axis of MicroRNA-200b, the RNA-Binding Protein Quaking, and Cyclin D1 in Modulating Tumor Angiogenesis and Metastasis Thesis 1905-07-11

Griveau A, Seano G, Shelton SJ et al. A Glial Signature and Wnt7 Signaling Regulate Glioma-Vascular Interactions and Tumor Microenvironment. Cancer Cell 2018-04-06 [PMID: 29681511] (Human)

Bumb A, Regino CA, Egen JG et al. Trafficking of a dual-modality magnetic resonance and fluorescence imaging superparamagnetic iron oxide-based nanoprobe to lymph nodes Mol Imaging Biol 2011-12-01 [PMID: 21080233] (IHC-Fr, Mouse)

Procedures

Western Blot Protocol for LYVE1 Antibody (NB100-725)
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.

Immunohistochemistry-Paraffin Protocol for LYVE-1 Antibody (NB100-725)
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.

Flow (Intracellular) Protocol for LYVE-1 Antibody (NB100-725)

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.

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 NB100-725

NB820-59670	Mouse Spleen Whole Tissue Lysate (Adult Whole Normal)
NB100-725PEP	LYVE-1 Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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/NB100-725

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