

# Product Datasheet

## Peripheral Node Addressin Antibody (MECA-79) - Non-Recombinant Monoclonal NB100-77673

Unit Size: 0.5 mg

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

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[technical@novusbio.com](mailto:technical@novusbio.com)

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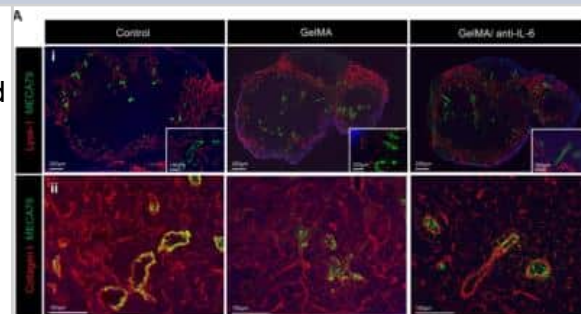


**NB100-77673****Peripheral Node Addressin Antibody (MECA-79) - Non-Recombinant Monoclonal**

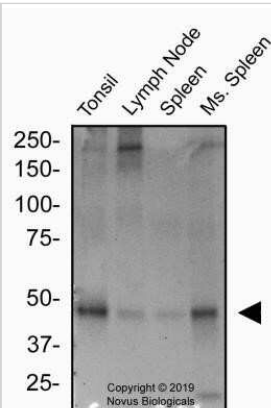
<b>Product Information</b>	
<b>Unit Size</b>	0.5 mg
<b>Concentration</b>	1.0 mg/ml
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Clonality</b>	Monoclonal
<b>Clone</b>	MECA-79
<b>Preservative</b>	0.02% Sodium Azide
<b>Isotype</b>	IgM Kappa
<b>Purity</b>	IgM purified
<b>Buffer</b>	PBS
<b>Product Description</b>	
<b>Host</b>	Rat
<b>Species</b>	Human, Mouse
<b>Immunogen</b>	Mouse lymph node stromal cells
<b>Product Application Details</b>	
<b>Applications</b>	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vivo assay, Immunoprecipitation
<b>Recommended Dilutions</b>	Western Blot 1:100-1:2000, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 31024011), Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:10-1:500, Immunohistochemistry-Frozen 1:10-1:500, In vivo assay reported in scientific literature (PMID 30277476)
<b>Application Notes</b>	Additional reported application of in vitro and in vivo blocking of cell adhesion.

**Images**

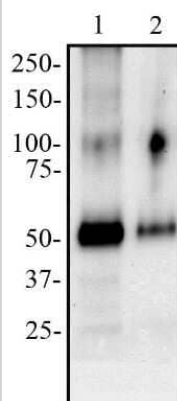
Immunohistochemistry: Peripheral Node Addressin Antibody (MECA-79) - Non-Recombinant Monoclonal [NB100-77673] - Local release of anti-IL-6 from IMB suppresses LN fibrosis. The skin allografts were harvested at 7 days post-transplantation. (A-i) Lymphatic vessel expansion (Lyve-1+) and HEV elongation (MECA79+) were similar between all groups. (A-ii,iii) Dense staining of collagen I was seen in DLNs harvested from control and GelMA group compared to those from GelMA/anti-IL-6 group. (representative images from 4 different mice per group). Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41598-019-42349-w>), licensed under a CC-BY license.



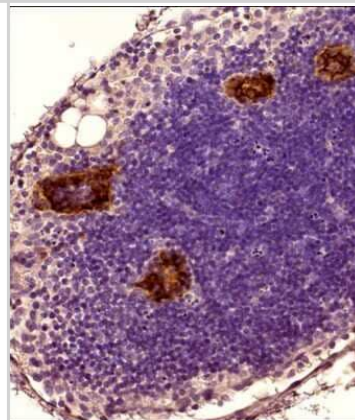
**Western Blot: Peripheral Node Addressin Antibody (MECA-79) - Non-Recombinant Monoclonal [NB100-77673]** - Total protein from human Tonsil, Lymph node, Spleen and mouse Spleen was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-PNAd in blocking buffer and detected with an anti-rat HRP secondary antibody using West Pico PLUS chemiluminescence detection reagent. Image using the standard format of this product.



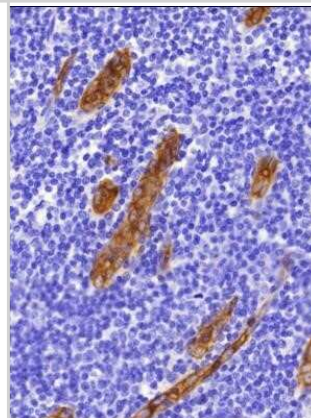
**Western Blot: Peripheral Node Addressin Antibody (MECA-79) - Non-Recombinant Monoclonal [NB100-77673]** - Total protein from Human tonsil (lane 1) and human lymph node (lane 2) was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2 ug/mL anti-PNAd in 1% milk, and detected with an anti-rat IgM HRP conjugated secondary antibody using chemiluminescence.



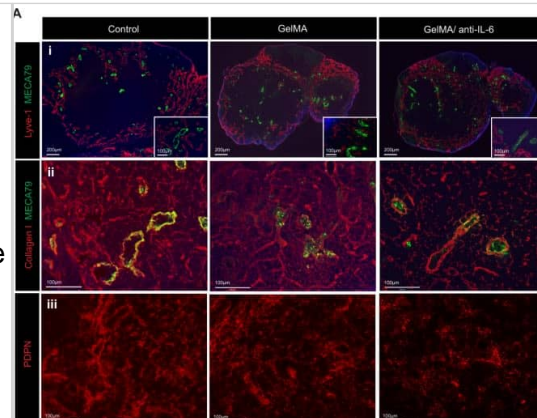
**Immunohistochemistry-Paraffin: Peripheral Node Addressin Antibody (MECA-79) - Non-Recombinant Monoclonal [NB100-77673]** - Analysis of FFPE mouse adipose tissue section (with lymph node areas) using Peripheral Node Addressin antibody (clone MECA-79) at 1:100. The staining was developed with HRP-DAB detection method and the counterstaining was performed using hematoxylin. This Peripheral Node Addressin antibody generated a strong and specific staining of MECA-79 antigen in the the cytoplasm and the membranes of high endothelial venules (HEVs) aka peripheral lymph node addressin (PNAd) in lymph node areas of tested adipose tissue section.



**Immunohistochemistry: Peripheral Node Addressin Antibody (MECA-79) - Non-Recombinant Monoclonal [NB100-77673]** - Analysis of FFPE human tonsil tissue section using Peripheral Node Addressin antibody (clone MECA-79R) at 1:100. The staining was developed with HRP-DAB detection method and the counterstaining was performed using hematoxylin. Image using the standard format of this product.



Immunocytochemistry/ Immunofluorescence: Peripheral Node Addressin Antibody (MECA-79) - Non-Recombinant Monoclonal [NB100-77673] - Local release of anti-IL-6 from IMB suppresses LN fibrosis. The skin allografts were harvested at 7 days post-transplantation. (A-i) Lymphatic vessel expansion (Lyve-1+) & HEV elongation (MECA79+) were similar between all groups. (A-ii,iii) Dense staining of collagen I & PDPN was seen in DLNs harvested from control & GelMA group compared to those from GelMA/anti-IL-6 group. (representative images from 4 different mice per group). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31024011>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Zhao J, Jung S, Li X et al. Delivery of costimulatory blockade to lymph nodes promotes transplant acceptance in mice *Journal of Clinical Investigation* 2022-12-15 [PMID: 36519543]

Gavish A, Tyler M, Greenwald AC et al. Hallmarks of transcriptional intratumour heterogeneity across a thousand tumours *Nature* 2023-06-01 [PMID: 37258682]

Wang Z, Shi X, Zhao Y et al. DC101, an anti-VEGFR2 agent, promotes high-endothelial venule formation and immune infiltration versus SAR131675 and fruquintinib *Biochemical and biophysical research communications* 2023-06-18 [PMID: 37084488] (IHC, ICC/IF)

Quigley LT, Pang L, Tavanche E et al. Protocol for investigating tertiary lymphoid structures in human and murine fixed tissue sections using Opal<sup>®</sup>-TSA multiplex immunohistochemistry STAR protocols 2023-01-10 [PMID: 36633948] (IHC-P, Human)

Details:

Dilution used in IHC-P 1:100

Smith KJ, Minns D, McHugh BJ Et al. The antimicrobial peptide cathelicidin drives development of experimental autoimmune encephalomyelitis in mice by affecting Th17 differentiation *PLoS Biol* 2022-08-26 [PMID: 36026478]

Details:

Citation using the Non-Recombinant Monoclonal version of this antibody.

Li X, Zhao J, Kasinath V et al. Lymph node fibroblastic reticular cells deposit fibrosis-associated collagen following organ transplantation *J. Clin. Invest.* 2020-06-29 [PMID: 32597832] (IHC-Fr, Human)

Details:

Citation using the Non-Recombinant Monoclonal format of this antibody.

He B, Johansson-Percival A, Backhouse J et al. Remodeling of Metastatic Vasculature Reduces Lung Colonization and Sensitizes Overt Metastases to Immunotherapy *Cell Rep.* [PMID: 31968248] (ICC/IF, Mouse, Human)

Matsubara S, Seki M, Suzuki S et al. Tertiary lymphoid organs in the inflammatory myopathy associated with PD-1 inhibitors *J Immunother Cancer.* 2019-09-18 [PMID: 31533865] (IF/IHC, Human)

Details:

Citation used the Non-Recombinant Monoclonal format of this antibody.

Uehara M, Li X, Sheikhi A et al. Anti-IL-6 eluting immunomodulatory biomaterials prolong skin allograft survival. *Sci Rep.* 2019-04-25 [PMID: 31024011] (ICC/IF, Mouse)

Details:

Citation using the Non-Recombinant Monoclonal version of this antibody.

Bahmani B, Uehara M, Ordikhani F et al Ectopic high endothelial venules in pancreatic ductal adenocarcinoma: A unique site for targeted delivery. EBioMedicine. 2018-12-01 [PMID: 30497977] (IHC-Fr, Human)

Details:

Citation using the Non-Recombinant Monoclonal version of this antibody.

Bahmani B, Uehara M, Jiang L et al Targeted delivery of immune therapeutics to lymph nodes prolongs cardiac allograft survival. J Clin Invest. 2018-11-01 [PMID: 30277476] (In Vivo, Mouse)

Details:

Citation using the Non-Recombinant Monoclonal version of this antibody.

Drayton DL, Ying X, Lee J et al Ectopic LT alpha beta directs lymphoid organ neogenesis with concomitant expression of peripheral node addressin and a HEV-restricted sulfotransferase. J Exp Med. 2003-05-05 [PMID: 12732657] (IF/IHC, Mouse)

Details:

Citation using the Non-Recombinant Monoclonal version of this antibody.

More publications at <http://www.novusbio.com/NB100-77673>



## Procedures

### Western Blot Protocol for Peripheral Node Addressin Antibody (NB100-77673)

#### 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 Peripheral Node Addressin Antibody (NB100-77673)

#### 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.







### **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 NB100-77673**

HAF005	Goat anti-Rat IgG Secondary Antibody [HRP]
NBP1-75398	Goat anti-Rat IgG (H+L) Secondary Antibody (Pre-adsorbed)
NBP1-43320-0.5mg	Rat IgM Kappa Light Chain Isotype Control (eBRM)

### **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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