Product Datasheet

Laminin Antibody
NB300-144

Unit Size: 0.1 ml
Aliquot and store at -20°C or -80°C. Avoid freeze-thaw cycles.

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Updated 6/21/2021 v.20.1

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# Product Information

<table>
<thead>
<tr>
<th><strong>Unit Size</strong></th>
<th>0.1 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>1 mg/ml</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Aliquot and store at -20°C or -80°C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>5mM Sodium Azide</td>
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<tr>
<td><strong>Isotype</strong></td>
<td>IgG</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>IgG purified</td>
</tr>
<tr>
<td><strong>Buffer</strong></td>
<td>50% PBS, 50% Glycerol</td>
</tr>
<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>337 kDa</td>
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</tbody>
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## Product Description

**Host** | Rabbit  
**Gene ID** | 284217  
**Gene Symbol** | LAMA1  
**Species** | Human, Mouse, Rat, Chinese Hamster, Invertebrate, Mammal, Rabbit, Sheep  
**Reactivity Notes** | Rabbit, Fruit Bat, Chinese Hamster, and S. mansoni reactivity reported in scientific literature (PMID: 18214989, 31877588, 29251349, and 28114363 respectively). Human, Mouse, Rat, and Sheep reported in multiple pieces of scientific literature.  
**Marker** | Basement Membrane Marker  
**Specificity/Sensitivity** | Laminin Antibody is pan-specific and reacts well with all Laminin isoforms tested: Laminin-1 (alpha-1, beta-1, and gamma-1) and Laminin-2 (alpha-2, beta-1, and gamma-1).  
**Immunogen** | Laminin Antibody was made to Laminin 111 isolated from mouse Engelbreth-Holm-Swarm (EHS) sarcoma cells. [UniProt# P19137]  

## Product Application Details

**Applications** | Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunohistochemistry Free-Floating  
**Application Notes** | This Laminin antibody detects bands at around 440, 220, and 158 kDa in Western Blot. Use in flow cytometry (PMID: 31819166) reported in scientific literature. Use in ICC/IF, IHC, IHC-Frozen, IHC-Paraffin, and Western Blot reported in multiple pieces of scientific literature. Immunostaining is enhanced by antigen retrieval with pepsin, especially paraffin tissue. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.
Immunohistochemistry Free-Floating: Laminin Antibody [NB300-144] - Immunohistological analysis of brain stem section stained with rabbit polyclonal Laminin Antibody [NB300-144], dilution 1:1,000 in red, and costained with chicken pAb to Myelin Basic Protein (MBP), dilution 1:5,000 in green. The blue is DAPI staining of nuclear DNA. Following transcardial perfusion of rat with 4% paraformaldehyde, brain was post fixed for 24 hours, cut to 45 uM, and free-floating sections were stained with the above antibodies. The laminin antibody is an excellent marker of basement membranes surrounding blood vessels, while the MBP antibody stains the myelin sheathes around axons.

Immunohistochemistry: Laminin Antibody [NB300-144] - MxIF analysis of mouse submandibular salivary gland morphogenesis. MxIF of a developmental TMA including embryonic stages (E14, E16, E18) and postnatal stages (P5 and P20) was performed using sequential application of directly conjugated antibodies to detect multiple markers of tissue structures and cell types on the same tissue sections. Tissue compartments. The epithelium, mesenchyme, neurons, and basement membranes was detected using antibodies directed towards E-cadherin (ECAD, red), platelet-derived growth factor (PDGFR, green), beta III tubulin (bIII, magenta), and Laminin Antibody [NB300-144] (LMN, cyan), respectively. Image collected and cropped by CiteAb from the following publication (http://bio.biologists.org/cgi/doi/10.1242/bio.20134309), licensed under a CC-BY licence.

Immunohistochemistry Free-Floating: Laminin Antibody [NB300-144] - Staining of mouse section of cortex stained with Laminin Antibody [NB300-144] (red). Blue is DAPI staining of DNA. This antibody reveals strong staining in the basement membranes of blood vessels.

Immunohistochemistry: Laminin Antibody [NB300-144] - Staining of rat spinal cord and dorsal root paraformaldehyde/paraffin-embedded tissue using Laminin Antibody [NB300-144]. Pepsin antigen retrieval was performed on this tissue sample.
Western Blot: Laminin Antibody [NB300-144] - Analysis of rat heart cell lysates (lane 1) and 0.2 ug of purified laminin protein from mouse EHS sarcoma (lane 2) Laminin Antibody [NB300-144] recognizes 3 laminin isotypes: alpha 1 (440 kDa), beta 1 (220 kDa) and gamma 1 (220 kDa). Also recognized is a laminin binding protein at 120 kDa in both rat heart lysates and purified laminin protein. Since this protein always coexpresses with laminin this crossreactivity is irrelevant. Theoretical molecular weight of LAMA1 is 337 kDa.

Immunocytochemistry/Immunofluorescence: Laminin Antibody [NB300-144] - IF Confocal analysis of HeLa cells using Laminin Antibody [NB300-144] (1:5). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green, A). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red, B). DAPI was used to stain the cell nuclei (blue, C).

Western Blot: Laminin Antibody [NB300-144] - Western blot analysis of the effects of IP6, Ins, IP6 + Ins and normal saline on the levels of collagen IV, Laminin and Fibronectin, with Laminin Antibody [NB300-144]. IP6 or Ins treatment decreased the protein expression of LN and FN, and the combined IP6 + Ins treatment resulted in significantly greater effects compared with treatment with either compound alone. The Western blot membranes were stripped and reprobed for beta-actin as an internal control to confirm equal loading. (A) representative blots from one of three separate experiments; (B) relative band intensities based on densitometry. The results are expressed as the mean +/- standard deviation from three independent experiments. * p < 0.05 compared to the IP6 + Ins group; #p < 0.05 compared to the normal saline group. Image collected and cropped by CiteAb from the following publication (http://www.mdpi.com/2072-6643/8/5/286), licensed under a CC-BY licence.

Immunocytochemistry/Immunofluorescence: Laminin Antibody [NB300-144] - Immunofluorescence staining for extracellular matrix proteins in day 1 and day 7 HWJSC spheroids. HWJSC spheroids were cultured for either 1 day or 7 days (with 6 days of osteogenic differentiation), fixed, and stained for extracellular matrix protein: laminin (C) and counterstaining with Hoechst. Scale bars represent 100 um. Image collected and cropped by CiteAb from the following publication (journals.plos.org/plosone/article?id=10.1371/journal.pone.0184155), licensed under a CC-BY licence. Using the Alexa Fluor 488 format of this antibody.
Immunohistochemistry-Frozen: Laminin Antibody [NB300-144] - Laminin staining on an E12.5 mouse Right Ventricle. IHC-Fr image submitted by a verified customer review.

Flow Cytometry: Laminin Antibody [NB300-144] - A surface stain was performed on HeLa cells with the Alexa Fluor 647 conjugate of Laminin Antibody [NB300-144AF647] (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. Both antibodies were conjugated to Alexa Fluor 647.

Western Blot: Laminin Antibody [NB300-144] - Analysis of Laminin-1 expression in mouse EHS tumor crude extracts (left) and Laminin-2 expression in rat heart crude extracts (right), using Laminin Antibody [NB300-144]. The Laminin polyclonal antibody was used at 1 ug/mL. Theoretical Molecular Weight of NB300-144 is 337 kDa.

Immunohistochemistry: Laminin Antibody [NB300-144] - VSOP observed in perivascular-restricted spinal cord lesions with intact BBB. Immunostaining for laminin (brown) using Laminin Antibody [NB300-144] shows vascular endothelium and glia limitans of a perivascular lesion, along with infiltrating cells and VSOP (blue). Image collected and cropped by CiteAb from the following publication (http://asn.sagepub.com/lookup/doi/10.1042/AN20120081), licensed under a CC-BY licence.
Immunohistochemistry: Laminin Antibody [NB300-144] - SOX17 regulates adult muscle regeneration after injury in Pax7CreERT2/+;Sox17fl/fl mutant mice. Representative images of cryosections from regenerating adult TA muscles d28 after injury, showing immunofluorescence for PAX7+ (quiescent, arrows) cells. Scale bar, 25 μm. Image collected and cropped by Citeab from the following publication (SOXF factors regulate murine satellite cell self-renewal and function through inhibition of I2-catenin activity. Elife (2018)) licensed under a CC-BY licence.

Publications

Miwa T, Ito N, Ohta K Tsuchi is essential for the formation of the posterior semicircular canal that detects gait performance Journal of cell communication and signaling Jun 1 2021 12:00AM [PMID: 34061311]

Corsa CAS, Walsh CM, Bagchi DP et al. Adipocyte-specific deletion of lamin A/C largely models human familial partial lipodystrophy type 2 Diabetes Jun 4 2021 12:00AM [PMID: 34088712] (WB)


Mori H, Dugan CE, Nishii A et al. The molecular and metabolic program by which white adipocytes adapt to cool physiologic temperatures PLoS biology May 1 2021 12:00AM [PMID: 33979328] (WB, Mouse)

Reinoso Jacome E, Ling Z, Xing Y et al. Bioorthogonal Labeling and Chemoselective Functionalization of Lung Extracellular Matrix BIO-PROTOCOL Feb 20 2021 12:00AM [PMID: 33732809]


More publications at http://www.novusbio.com/NB300-144
Procedures

Immunohistochemistry-Paraffin protocol for Laminin Antibody (NB300-144)

Laminin Antibody: https://www.novusbio.com/products/laminin-antibody_nb300-144

Laminin Immunohistochemistry ABC/HRP

The fixation is routine paraformaldehyde or formalin fixation of tissue prior to paraffin embedding. However, the staining procedure must include an antigen retrieval step pretreating the deparaffinized sections with pepsin. This is required for all laminin antibodies used on paraffin tissue.

Proteolytic antigen retrieval:

Apply 250 ul of pepsin at 4mg/ml in 0.01M HCL (pH ~2.0).

Incubate for 60 minutes at 37 degrees C in a humid chamber.

Wash x2 in distilled H2O, 5 min. each wash.

Mount paraffin sections on Fisher Plus slides. Bake for >2 hours at 50C.

1. Deparaffinize mounted sections in xylene: 2 changes 5 min each, and a 3rd change for 10 min.

2. Exchange solvent to ethanol with 2 changes of 100% EtOH for 5 min each.

3. Quench endogenous peroxidase for 30 min in 100% methanol + 1% H2O2.


5. 5 min in 95% EtOH; 5 min in 70% EtOH; 5 min in running H2O. Rinse with dH2O.

Circumscribe the sections with PAP Pen.

5. Unmask antigen by proteolysis. Cover sections with 100ul of 4mg/ml of pepsin (Sigma #P6887) dissolved in 0.01M HCL. Treat for 1 hr at 37C in humidified chamber. Rinse in running H2O.

6. Blocking: Block background staining by covering sections with 100ul of PBS containing 10% normal swine serum (Blocking Buffer) for 1 hr at ambient temperature in humidified chamber.

7. Apply 1 degrees antibody. Aspirate Blocking Buffer and immediately apply rabbit anti-EHS laminin (MuirLab prep) diluted to 1 ug/ml in Blocking Buffer. Apply 100ul to sections, and incubate overnight at 4C in humidified chamber.

8. Aspirate 1 degrees antibody and wash slides in a rack by immersion in PBS with 3 changes over >=15 min.

9. Apply biotinylated 2 degrees antibody. Dilute biotinylated swine anti-rabbit 1/500 in Blocking Buffer. Apply 100ul to sections, and incubate for 2 hr. at ambient temperature in humidified chamber. (Before end of incubation, prepare ABC reagents as stated in step 10.)

10. Aspirate 2 degrees antibody and wash by immersion in PBS with 3 changes over >=15 min.

11. Apply ABC complex. Dilute Reagent A (Avidin) 1:50 in PBS + 0.1% Triton X100, mix well. In a separate tube, dilute Reagent B (Biotin-HRP) 1:50 in PBS + 0.1% Triton X100, mix well. Mix equal parts of solutions A and B. Vortex to mix well, and preincubate for at least 30 min. Immediately prior to use, dilute the 1:50 ABCComplex stock an additional 1/5 (i.e., 1:250 final) with PBS + 0.1% BSA. Apply 100ul to sections, and incubate for 2 hr at ambient temperature in humidified chamber.

12. Aspirate ABC solution and Wash by immersion in PBS with 3 changes over >=30 min.

13. Develop with chromogenic substrate. Immediately before use, mix in 3 ml of PBS, 1.5 mg DAB (diaminobenzidine-[HCl]4; Sigma #D5637) and 2ul H2O2 (30%). Filter with a 0.2um syringe filter. Apply 100ul to sections and let develop for 12 min at ambient temperature. Stop chromogenic reaction by submerge slides in running H2O.


5 min in 70% EtOH; 5 min in 95% EtOH; 5 min in 100% EtOH; 5 min in 100% EtOH


Additional

Immunohistochemistry Protocol De-paraffinize: xylene x2 5 min (to remove paraffin) xylene x1 10 min 100% ethanol x2 5 min (to remove xylene) Quench endogenous peroxidase: Quench with 1% H2O2 in 100% methanol (v/v) for 30 min at RT. Rehydrate: 95% ethanol x1 5 min 70% ethanol x1 5 min distilled H2O x2 5 min Circumscribe tissue sections with PAP pen. Proteolytic antigen retrieval: Apply 250 ul of pepsin at 4 mg/ml in 0.01M HCl (pH ~2.0). Incubate for 60 min at 37C in a humid chamber. Wash x2 in distilled H2O, 5 min each wash. Block background: Apply 250 ul of 10% normal goat serum in PBS. Incubate for 30 min at 37C. Pour off excess blocking solution from slides, do not allow tissue to dry. Immunostaining - primary antibody: A. Apply 250 ul of anti-laminin 1 degrees Ab at 1:1000 in PBS containing 10% goat serum. B. Apply 250 ul of 10% goat serum in PBS as negative control. Incubate overnight at 37C in a humid chamber. Immunostaining - secondary antibody: Wash x2 in PBS, 5 min each wash. Apply 250 ul of 2 degrees Ab at 1:500 in PBS. Incubate for 30 min at 37C in a humid chamber. Wash x2 in PBS, 5 min each wash. DAB substrate: Apply 250 ul DAB solution and allow brown color to develop for 30 min at RT. DAB is carcinogenic therefore dispose of it as hazardous chemical waste. Rinse briefly in running distilled H2O to stop reaction. Wash x2 in distilled H2O, 5 min each wash. Mount: Air dry slides for a few minutes. Apply 3-4 drops of Crystal/Mount to tissue sections. Spread evenly by rotation. Dry slides in a 37C oven for 1-2 hours.
FOR LAMININ STAINING PROTOCOL 10X PBS Stock Solution 1X PBS - Working Solution 1.37M NaCl 80.06 g 137 mM NaCl 0.027M KCl 2.01 g 2.7 mM KCl 0.043M Na2HPO4 6.11 g 4.3 mM Na2HPO4 0.014M KH2PO4 1.92 g 1.4 mM KH2PO4 Dissolve in 800 ml distilled H2O. 100 ml stock: 900 ml distilled H2O. pH to 7.4 with 5N NaOH. QS to 1L with distilled H2O. The following volumes are for 20 tissue sections (18 test and 2 controls). Pepsin Solution Dissolve 20 mg of pepsin in 5 ml of 0.01M HCl (pH ~2.0). Pepsin: Roche 03 117 901 001 (from porcine stomach) (EC 3.4.23.1)). Endogenous Peroxidase Block 1% (v/v) = 2.5 ml of 30% H2O2 in 250 ml of 100% methanol (where 30% H2O2 is treated as 100%). Non-specific Protein Block Prepare a 10% solution by diluting 1 ml of normal goat serum in 9 ml of PBS. Goat serum: Sigma G-9023. 1 degrees and 2 degrees Antibodies 1 degrees Ab: rabbit anti-rat laminin PAb (Novus Biologicals NB 300-144). Prepare at 1:1000 by adding 4.5 ul 1 degrees Ab to 4.5 ml of 10% goat serum in PBS. 2 degrees Ab: goat anti-rabbit IgG-HRP (Santa Cruz sc-2030). Prepare at 1:500 by adding 10 ul 2 degrees Ab to 5 ml of PBS. DAB substrate: DAB: Sigma D-4293. DAB (3,3’ diaminobenzidine) is carcinogenic. Prepare by dissolving one DAB tablet and one H2O2 tablet in 5 ml of distilled H2O. Counterstain: Counterstain is not recommended for laminin IHC. Mount: Crystal/Mount, an aqueous based, mounting medium, is from Biomeda (catalog no. M02). LAMININ IMMUNOHISTOCHEMISTRY-HRP PROTOCOL (formalin-fixed paraffin-embedded rat liver sections) (Novus Biologicals NB 300-144). This last protocol is from the lab of: Thomas F. Tracy, Jr., M.D. Professor of Surgery and Pediatrics Vice Chairman, Department of Surgery Brown Medical School Pediatric Surgeon-in-Chief Hasbro Children's Hospital Room 147 593 Eddy Street Providence, RI 02903

Western Blot protocol for Laminin Antibody (NB300-144)

Laminin Antibody: https://www.novusbio.com/products/laminin-antibody_nb300-144

Western Blot
1. SDS-PAGE on 5% mini-gel under reducing conditions
2. Electroblot to nitrocellulose by Towbin methods
3. Remove nitrocellulose sheet from electrobobting sandwich and rinse briefly in dH2O.
4. Fixation: In a glass dish immerse the blot in 25% isopropanol/10% acetic acid/65% dH2O. Cover and shake gently for at least 30 min. at room temperature.
5. Remove the blot from fixative and wash in a large volume changes of dH2O for > 10 min.
6. Place blot in plastic tray with lid. Equilibrate >10 min. with Washing Buffer. Pour off.
7. Blocking: Place the blot in Blocking Buffer (just enough to cover). Incubate with gentle shaking for at least 1 h (overnight if background is a big problem). Pour off.
8. Primary Antibody: Dilute PcAbLN antibody (3/4 1 ug/ml) in Blocking Buffer. Add just enough to cover blot and incubate with shaking for 2 h at 37°C or overnight at room temp.
9. Pour off primary antibody and wash X3 with Washing Buffer over 20-30 min (or more).
10. Peroxidase-conjugated Secondary Antibody: Dilute peroxidase conjugated 2 degrees IgG (Dako, affinity purified) diluted 1:2000 in Blocking Buffer. Add just enough to cover the blot and incubate with shaking for 2 h at 37°C.
11. Wash blot thoroughly (30 min and up to hours) in Washing Buffer and then with a final wash in Washing buffer without Triton.

Washing Buffer
0.05 Tris-HCl, pH 7.4
1.5% NaCl
0.1% Triton X100

Blocking Buffer
Washing Buffer
5% powered milk
(dissolve for hours, filter)
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

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