Product Datasheet

Laminin Antibody
NB300-144

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

Reviews: 7  Publications: 52

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www.novusbio.com/NB300-144

Updated 8/16/2019 v.20.1
**NB300-144**  
Laminin Antibody

### Product Information

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
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<tbody>
<tr>
<td>Host</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Gene ID</td>
<td>284217</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>LAMA1</td>
</tr>
<tr>
<td>Species</td>
<td>Human, Mouse, Rat, Chinese Hamster, Invertebrate, Rabbit, Sheep</td>
</tr>
<tr>
<td>Reactivity Notes</td>
<td>Rabbit, Sheep, Chinese Hamster, and Invertebrate reactivity reported in scientific literature (PMID: 18214989, 25639519, 29251349, and 28114363 respectively).</td>
</tr>
<tr>
<td>Marker</td>
<td>Basement Membrane Marker</td>
</tr>
<tr>
<td>Specificity/Sensitivity</td>
<td>This 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).</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Laminin 111 isolated from mouse Engelbreth-Holm-Swarm (EHS) sarcoma cells. [UniProt# P19137]</td>
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### Product Application Details

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applications</td>
<td>Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunohistochemistry Free-Floating</td>
</tr>
<tr>
<td>Application Notes</td>
<td>This Laminin antibody can be used for Immunocytochemistry/Immunofluorescence, Immunohistochemistry, and Western blotting where it detects bands at around 440, 220, and 158kDa. The antibody functionally inhibits Laminin in mouse and rat. It binds to Laminin and inhibits most, if not all, of its cell adhesion and growth promotive properties. Immunostaining is enhanced by antigen retrieval with pepsin, especially paraffin tissue. Use in IHC-P and IHC-Fr reported in scientific literature (PMID: 31153978 and 30367035 respectively). 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.</td>
</tr>
</tbody>
</table>
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). The Laminin polyclonal antibody was used at 1 ug/ml.

Immunocytochemistry/Immunofluorescence: Laminin Antibody [NB300-144] - Detection of Laminin (Green) in Hela cells using NB300-144 at a 1:50 dilution. Nuclei (Blue) were counterstained using Hoechst 33258.

Immunohistochemistry: Laminin Antibody [NB300-144] - Staining of rat spinal cord and dorsal root paraformaldehyde/paraffin-embedded tissue using NB 300-144. Pepsin antigen retrieval was performed on this tissue sample.

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).

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

Flow Cytometry: Laminin Antibody [NB300-144] - A surface stain was performed on HeLa cells with 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 rat heart cells lysates (lane 1) and 0.2 ug of purified laminin protein from mouse EHS sarcoma (lane 2) This antibody recognizes 3 laminin isotypes: alpha 1 (440kDa), beta 1 (220kD) and gamma 1 (220kDa). Also recognized is a laminin binding protein at 120kDa in both rat heart lysates and purified laminin protein. Since this protein always coexpresses with laminin this crossreactivity is irrelevant.
Immunohistochemistry Free-Floating: Laminin Antibody [NB300-144] - Immunohistological analysis of brain stem section stained with rabbit pAb to laminin, 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 45uM, 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.

Publications


MacDougall MS, Clarke R, Merrill BJ Intracellular Ca2+ Homeostasis and Nuclear Export Mediate Exit from Naive Pluripotency Cell Stem Cell May 15 2019 12:00AM [PMID: 31104942] (Mouse)


Miska J, Lee-Chang C, Rashidi A et al. HIF-1 alpha Is a Metabolic Switch between Glycolytic-Driven Migration and Oxidative Phosphorylation-Driven Immunosuppression of Tregs in Glioblastoma Cell Rep Apr 2 2019 12:00AM [PMID: 30943404] (ICC/IF, Mouse)


Freitas-De-Sousa Luciana Aparecida, Colombini Monica, Lopes-Ferreira Monica et al. Insights into the Mechanisms Involved in Strong Hemorrhage and Dermonecrosis Induced by Atroxlysin-1a, a PI-Class Snake Venom Metalloproteinase. Toxins 2017 [PMID: 28767072] (IHC, Mouse)


Guo H, Adah D, James PB et al. Xueshuantong Injection (Lyophilized) Attenuates Cerebral Ischemia/Reperfusion Injury by the Activation of Nrf2-VEGF Pathway. Neurochem. Res. Apr 9 2018 12:00AM [PMID: 29633164] (IHC, Rat)


Details:
This citation used the Alexa Fluor 488 form of this antibody

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

Immunohistochemistry-Paraffin protocol for 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. De-paraffinize 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 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-HP) 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 chromagenic 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 chromagenic 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
15. Coverslip in Permount.

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.  
RECIPES
FOR LAMININ STAINING PROTOCOL 10X PBS Stock Solution 1X PBS - Working Solution 1.37M NaCl 80.06 g
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

**WB Protocol specific for Laminin Antibody (NB300-144)**

Western Blot

1. SDS-PAGE on 5% mini-gel under reducing conditions
2. Electrobolt to nitrocellulose by Towbin methods
3. Remove nitrocellulose sheet from electroblootting 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 37C or overnight at room temp.
9. 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

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NB300-144

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