Product Datasheet

Laminin Antibody - BSA Free
NB300-144

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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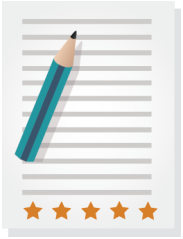
Updated 2/21/2025 v.20.1



NB300-144

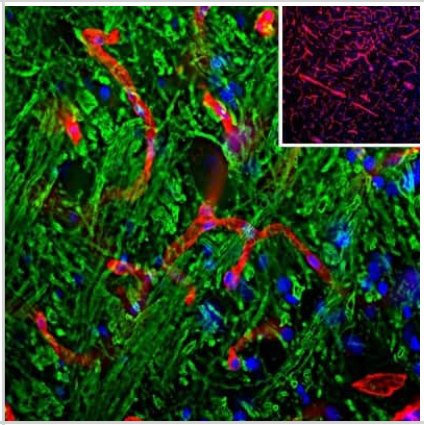
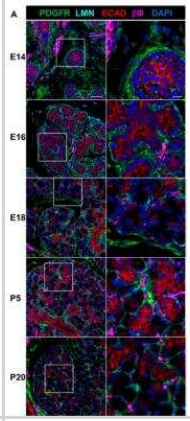
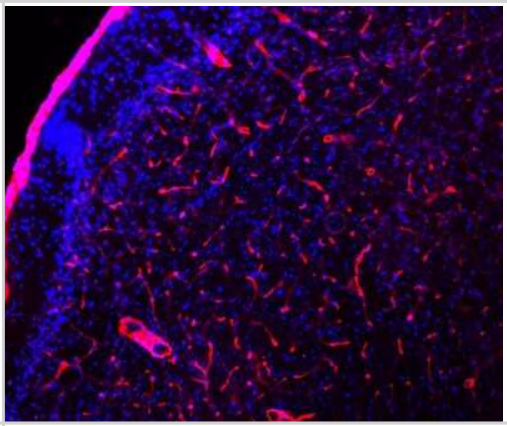
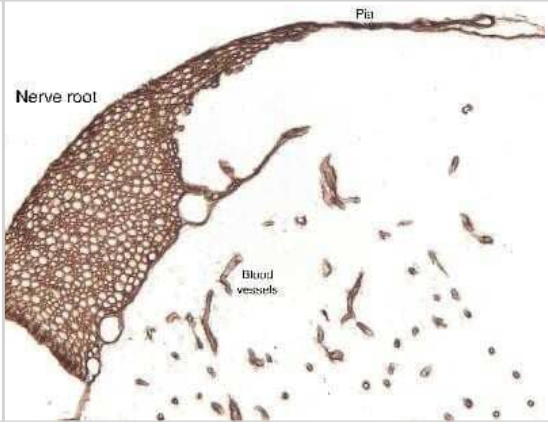
Laminin Antibody - BSA Free

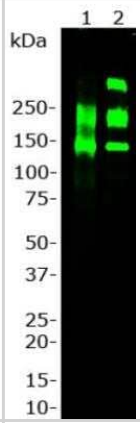
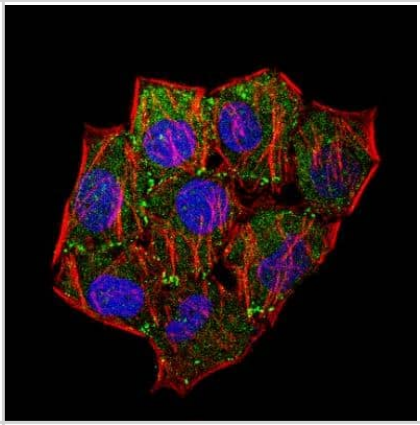
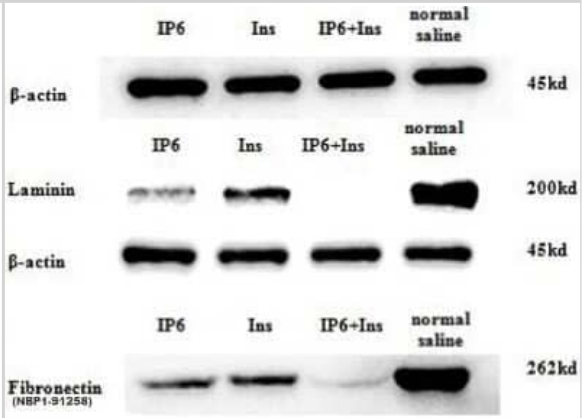
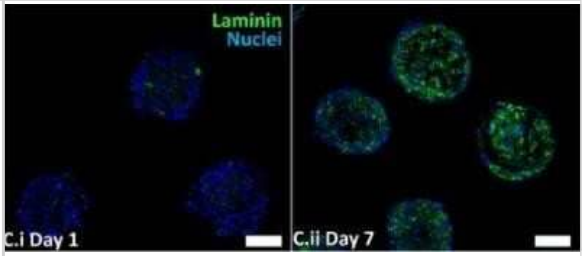
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.035% Sodium Azide
Isotype	IgG
Purity	IgG purified
Buffer	50% PBS, 50% glycerol
Target Molecular Weight	337 kDa

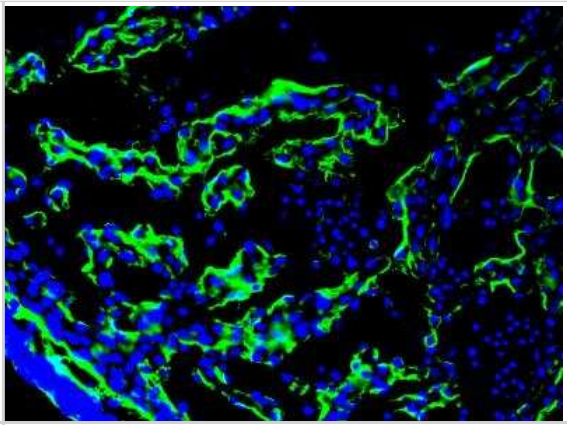
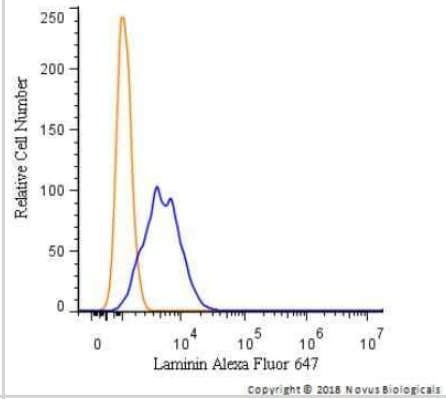
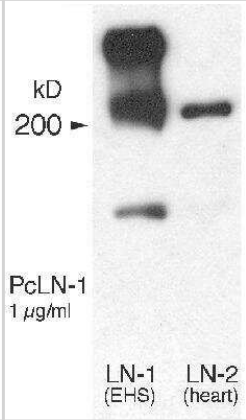
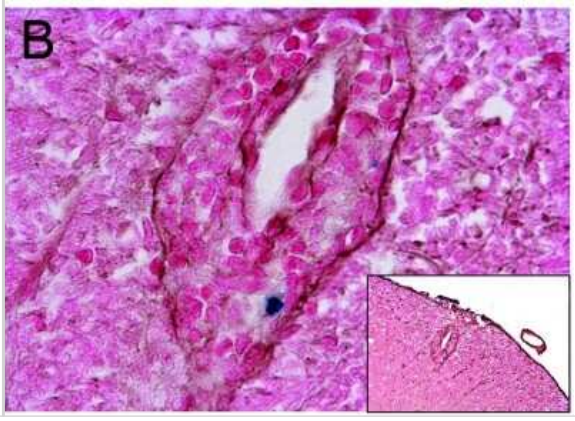
Product Description	
Host	Rabbit
Gene ID	284217
Gene Symbol	LAMA1
Species	Human, Mouse, Rat, Chinese Hamster, Invertebrate, Mammal, Rabbit, Sheep
Reactivity Notes	Rabbit, Fruit Bat, Chinese Hamster, and S. mansoni reactivity reported in scientific literature (PMID: 18214989, 31877588, 29251349, and 28114363 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
Recommended Dilutions	Western Blot 1:100-1:5000, Flow Cytometry, Immunohistochemistry 1:500-1:2000, Immunocytochemistry/ Immunofluorescence 1:50-1:200, Immunohistochemistry-Paraffin 1:500-1:2000, Immunohistochemistry-Frozen 1:500-1:2000, Immunohistochemistry Free-Floating 1:1000-1:5000
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. br/> 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.

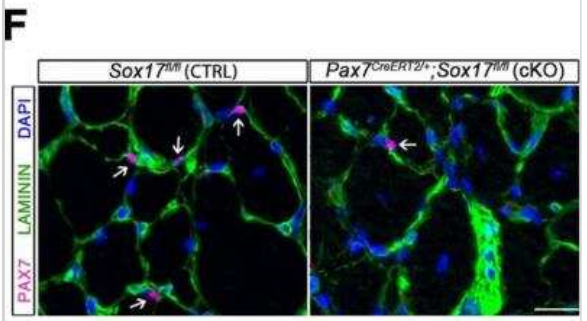
Images

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.	
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 (https://bio.biologists.org/cgi/doi/10.1242/bio.20134309), licensed under a CC-BY license.	
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.	
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.	

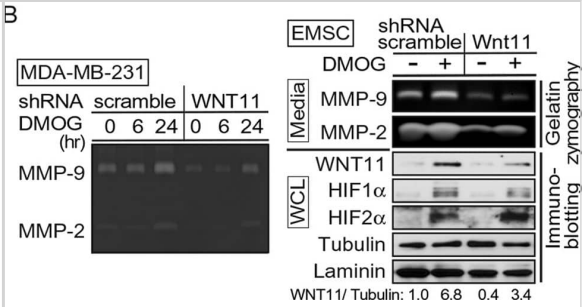
Analysis of rat heart cells lysates (lane 1) and 0.2 ug of purified laminin protein from mouse EHS sarcoma (lane 2) Laminin Antibody [NB300-144] recognizes 3 laminin isotypes: alpha 1 (440 kDa), beta 1 (220 kDa) and gamma 1 (220 kDa). Also recognized is a laminin binding protein at 120 kDa in both rat 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.	
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 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 (https://www.mdpi.com/2072-6643/8/5/286), licensed under a CC-BY license.	
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 counter-staining 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 license. Using the Alexa Fluor 488 format of this antibody.	

Laminin staining on an E12.5 mouse Right Ventricle. IHC-Fr image submitted by a verified customer review.	
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.	
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.	
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 (https://asn.sagepub.com/lookup/doi/10.1042/AN20120081), licensed under a CC-BY license.	

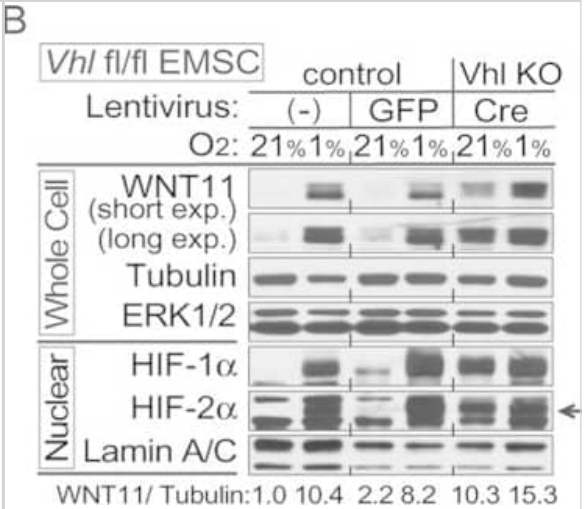
SOX17 regulates adult muscle regeneration after injury in Pax7CreERT2/+;Sox17fl/fl mutant mice. Representative images of cryosections from regenerating adult TA muscles d28 after injury, showing immunofluorescence for PAX7+ (quiescent, arrows) cells. Scale bar, 25 Aum. Image collected and cropped by Citeab from the following publication (SOXF factors regulate murine satellite cell self-renewal and function through inhibition ofl2-catenin activity. Elife (2018)) licensed under a CC-BY license.



WNT11 regulates MMPs activities.(A–C) (Top panels): Serum-free medium was conditioned for 24 hrs by the indicated cells, concentrated 20-fold & assayed by gelatin zymography. Gelatinolytic activity is indicated by clear zones against a dark background of stained substrate. (Bottom): Whole cell extracts were immunoblotted with indicated antibodies. (A) Overexpression of Wnt11 in EMSC or BT473 cells enhances activity of MMP-9 & MMP-2. (B) Impaired activity of MMP-9 & MMP-2 in MDA-MB-231 cells (left) or EMSCs (right) stably expressing Wnt11 shRNAs & treated with DMOG. (C) WNT11 is required for MMP-9 & MMP-2 activity in MDA-MB-231 cells (left) or EMSCs (right) under normoxic & hypoxic culture conditions. (D) WNT11 regulates MMP2 protein in media. (Top): conditioned media from indicated cells & treatments. (Bottom): whole cell lysates were immunoblotted with indicated antibodies. (E) Recombinant WNT11 induces both MMP-2 protein & MMP-2 activity in media. (Top panels): Gelatin zymography & immunoblot of serum-free medium conditioned for the indicated times after recombinant WNT11 (r-WNT11) treatment. (Bottom): Whole cell lysates were immunoblotted with indicated antibodies. (F) MMP-2 inhibitor attenuated induced migration by WNT11. MDA-MB-231 cells infected with lentiviruses for stable expression of Wnt11 or GFP (n = 4) were incubated with either vehicle or 1 μM of ARP100. Media in the lower compartment had same concentration of DMSO or inhibitor. Values are mean ± s.e.m. *p < 0.05, **p < 0.01. For panels (A–D), HIF-1α & HIF-2α were shown as a marker of hypoxia, WNT11 normalized to α-Tubulin was shown. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep21520), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

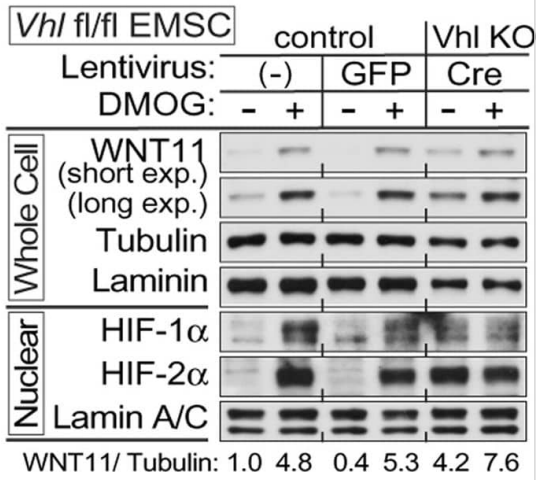


Hypoxia induces expression of WNT11 through VHL.(A,B) Higher basal levels of WNT11 protein in Vhl-deleted cells (lenti-Cre infected Vhlf/f). EMSCs isolated from Vhlf/f mouse were infected with lentivirus carrying either GFP gene (for control) or Cre recombinase (for knockout). Non-infected cells were also used as a control. Immunoblot analysis of control or Vhl KO EMSCs treated with 0.1 mM DMOG (A), & EMSCs exposed to air (21% O2) or hypoxia (1% O2) for 24 hrs (B). Laminin, α-tubulin, & lamin A/C were used as loading controls, WNT11 normalized to α-Tubulin was shown. (C,D) Inactivation of the Vhl gene results in increased Wnt11 mRNA. Wnt11 & Vegf mRNA levels in liver (C) or duodenum (D) were measured by qPCR in Liver-VhlcKO or duodenum-VhlcKO & control mice (n = 5 per group). Values normalized to Tbp mRNA are expressed relative to tissues from control mice. For panels (C,D), values are mean ± s.e.m. *p < 0.05, **p < 0.01. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep21520), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



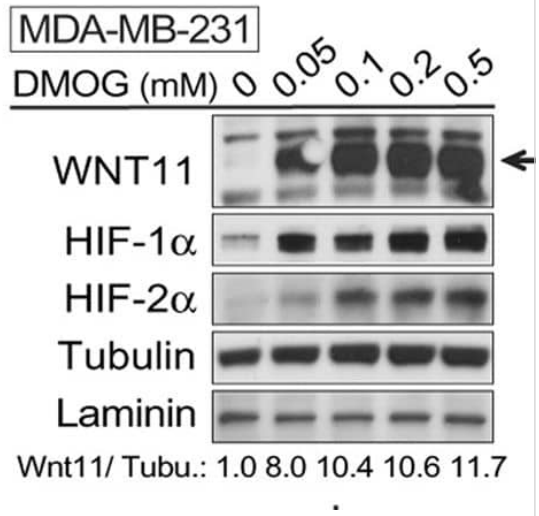
Hypoxia induces expression of WNT11 through VHL.(A,B) Higher basal levels of WNT11 protein in Vhl-deleted cells (lenti-Cre infected Vhlf/f). EMSCs isolated from Vhlf/f mouse were infected with lentivirus carrying either GFP gene (for control) or Cre recombinase (for knockout). Non-infected cells were also used as a control. Immunoblot analysis of control or Vhl KO EMSCs treated with 0.1 mM DMOG (A), & EMSCs exposed to air (21% O2) or hypoxia (1% O2) for 24 hrs (B). Laminin, α-tubulin, & lamin A/C were used as loading controls, WNT11 normalized to α-Tubulin was shown. (C,D) Inactivation of the Vhl gene results in increased Wnt11 mRNA. Wnt11 & Vegf mRNA levels in liver (C) or duodenum (D) were measured by qPCR in Liver-VhlcKO or duodenum-VhlcKO & control mice (n = 5 per group). Values normalized to Tbp mRNA are expressed relative to tissues from control mice. For panels (C,D), values are mean ± s.e.m. *p < 0.05, **p < 0.01. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep21520), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

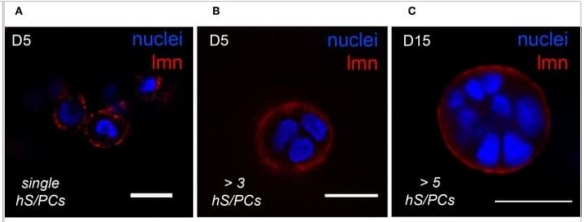
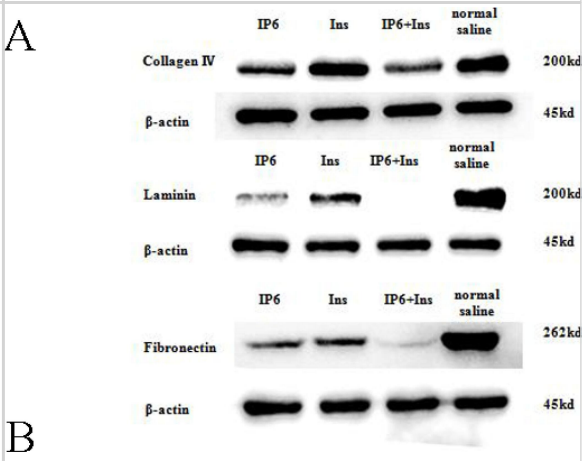
A



WNT11 is induced by hypoxia or hypoxic mimetics in different cell types. (A) Increased Wnt11 mRNA in EMSC adipocytes (Day 12) after hypoxia-mimetic treatments. EMSC adipocytes were treated with CoCl2 (0.1 mM), DFO (0.1 mM) or DMOG (0.1 mM) for 24 hrs. Values were normalized to Tbp mRNA & are expressed relative to control (n = 3). (B,C) Increased Wnt11 mRNA by hypoxia in EMSC preadipocytes & adipocytes (Day 0–12 after differentiation) (B), & C2C12 myoblast & myocyte (Day 0 & 8 after differentiation) (C). Wnt11 mRNA was assessed by quantitative PCR in cells exposed to air (21% O2) or hypoxia (1% O2) for 24 hrs. (n = 4). Values were normalized to Tbp mRNA & are expressed relative to 21% O2 samples (left panel). (D) Immunoblot analyses of HeLa cells under normal air or hypoxia for 24 hrs. (E,F) Induction of Wnt11 by increasing concentrations of DMOG in MDA-MB-231 cells (E) & 4T1 cells (F). (G) EMSCs treated with 0.1 mM DMOG for the indicated times. Wnt11 & Vegf mRNA expression was measured by qPCR & normalized to Tbp mRNA (n = 4). (H) WNT11 protein levels after DMOG treatment normalized to α-Tubulin (upper panel; n = 4). Representative immunoblots of EMSCs treated with 0.1 mM DMOG for the indicated times (Lower panel). (I) Protein expression in MDA-MB-231 cells treated with 0.1 mM DMOG. (J) Induction of Wnt11 promoter activity by hypoxia or hypoxia mimetics. pGL3-Wnt11 promoter plasmid was transfected into C2C12 cells. Cells were incubated with DMOG (left panel, n = 4) or under 21% O2 or 1% O2 (right panel, n = 8) for 24 hrs. For panels (A–C,G,H,J), values are mean ± s.e.m. *p < 0.05, **p < 0.01. For panels of immunoblotting, laminin, α-tubulin, & ERK were used as loading controls, WNT11 normalized to α-Tubulin was shown. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep21520), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

E



Immunocytochemistry/ Immunofluorescence: Laminin Antibody [NB300-144] - hS/PCs sequentially secrete basement membrane proteins during microstructure reorganization & function. Encapsulated hS/PCs in HA-PEGDA hydrogels initially produce laminin (red) (A–C) & collagen IV (green) (D–F) even at the single-cell state (A,D). Perlecan (green) (G–I) secretion follows to stabilize the laminin & collagen IV networks. Scalebar = 20 μm in all confocal micrographs. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31750298), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: Laminin Antibody [NB300-144] - Western blot analysis of the effects of IP6, Ins, IP6 + Ins & normal saline on the levels of collagen IV, Lamininand Fibronectin. IP6 or Ins treatment decreased the protein expression of collagen IV, LN & FN, & the combined IP6 + Ins treatment resulted in significantly greater effects compared with treatment with either compound alone. The samples were probed with antibodies against p-collagen IV, p-LN, & p-FN. The Western blot membranes were stripped & reprobed for β-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 & cropped by CiteAb from the following publication (http://www.mdpi.com/2072-6643/8/5/286), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Wilbourne J, Jia S, Fogarty A, Takaku M et Al. Crucial Roles of the Mesenchymal Androgen Receptor in Wolffian Duct Development Endocrinology 2023-12-26 [PMID: 38146640]

Matrongolo MJ, Ho-Nguyen KT, Jain M, Ang PS et Al. Loss of Twist1 and balanced retinoic acid signaling from the meninges causes cortical folding in mice Development 2023-08-17 [PMID: 37590085]

Schluga, PHC;Larangote, D;de Melo, AM;Lobermayer, GK;Torrejón, D;de Oliveira, LS;Alvarenga, VG;Vivas-Ruiz, DE;Veiga, SS;Sanchez, EF;Gremski, LH; A Novel P-III Metalloproteinase from Bothrops barnetti Venom Degrades Extracellular Matrix Proteins, Inhibits Platelet Aggregation, and Disrupts Endothelial Cell Adhesion via ?5?1 Integrin Receptors to Arginine-Glycine-Aspartic Acid (RGD)-Containing Molecules Toxins 2024-11-09 [PMID: 39591241]

Han X, Burger LL, Garcia-Galiano D et Al. Hypothalamic and Cell-Specific Transcriptomes Unravel a Dynamic Neuropil Remodeling in Leptin-Induced and Typical Pubertal Transition in Female Mice iScience 2020-09-16 [PMID: 33083731]

Hansen CE, Vacondio D, van der Molen L et Al. Endothelial-Ercc1 DNA repair deficiency provokes blood-brain barrier dysfunction Cell Death Dis 2025-01-03 [PMID: 39753531]

Chen HH, Yeo HT, Huang YH et Al. AAV-NRIP gene therapy ameliorates motor neuron degeneration and muscle atrophy in ALS model mice Skelet Muscle 2024-07-24 [PMID: 39044305]

Taylor X, Noristani HN, Fitzgerald GJ et al. Amyloid-β (Aβ) immunotherapy induced microhemorrhages are linked to vascular inflammation and cerebrovascular damage in a mouse model of Alzheimer's disease Molecular Neurodegeneration 2024-10-21 [PMID: 39434125]

Jelle C. B. C. de Jong, Martien P. M. Caspers, Nicole Worms, Nanda Keijzer, Robert Kleemann, Aswin L. Menke, Arie G. Nieuwenhuizen, Jaap Keijer, Lars Verschuren, Anita M. van den Hoek Translatability of mouse muscle-aging for humans: the role of sex GeroScience 2024-01-24 [PMID: 38265577]

Nhi T. Tran, Nadia Hale, Anawar Aung Win Maung, Manon Wiersma, David W. Walker, Graeme Polglase, Margie Castillo-Melendez, Flora Y. Wong Intrauterine inflammation and postnatal intravenous dopamine alter the neurovascular unit in preterm newborn lambs Journal of Neuroinflammation 2024-05-28 [PMID: 38807204]

Hiroyuki Mori, Sydney K. Peterson, Rachel C. Simmermon, Katherine A. Overmyer, Akira Nishii, Emma Paulsson, Ziru Li, Annie Jen, Romina M. Uranga, Jessica N. Maung, Warren T. Yacawych, Kenneth T. Lewis, Rebecca L. Schill, Taryn Hetrick, Ryo Seino, Ken Inoki, Joshua J. Coon, Ormond A. MacDougald Scd1 and monounsaturated lipids are required for autophagy and survival of adipocytes Molecular Metabolism 2024-03-14 [PMID: 38492843]

Rickelt S, Hynes RO. Antibodies and methods for immunohistochemistry of extracellular matrix proteins Matrix Biol. 2018-05-03 [PMID: 29730502]

Juan F. Zapata-Acevedo, Mónica Losada-Barragán, Johann F. Osma, Juan C. Cruz, Andreas Reiber, Klaus G. Petry, Amael Caillard, Audrey Sauldubois, Daniel Llamosa Pérez, Aníbal José Morillo Zárate, Sonia Bermúdez Muñoz, Agustín Daza Moreno, Rafaela V. Silva, Carmen Infante-Duarte, William Chamorro-Coral, Rodrigo E. González-Reyes, Karina Vargas-Sánchez, Alexander V. Ljubimov Specific nanoprobe design for MRI: Targeting laminin in the blood-brain barrier to follow alteration due to neuroinflammation PLOS ONE 2024-04-11 [PMID: 38603692]

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

Procedures

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

Laminin Immunohistohemistry 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 H20, 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.
4. Hydrate to H2O in graded ethanol series.
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 ABComplex 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.
14. Dehydrate through graded alcohol series.
5 min in 70% EtOH; 5 min in 95% EtOH; 5 min in 100% EtOH; 5 min in 100% EtOH
16. 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 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)

Western Blot
1. SDS-PAGE on 5% mini-gel under reducing conditions
2. Electroblot to nitrocellulose by Towbin methods
3. Remove nitrocellulose sheet from electroblotting 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.
3. Remove the blot from fixative and wash in a large volume changes of dH2O for > 10 min.
4. Place blot in plastic tray with lid. Equilibrate >10 min. with Washing Buffer. Pour off.
5. 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.
6. 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.
7. Pour off primary antibody and wash X3 with Washing Buffer over 20-30 min (or more).
8. 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 37C.
9. Wash blot thoroughly (30 min and up to hours) in Washing Buffer and then with a final wash in Washing buffer without Triton.
10. Chemiluminescent Detection: According to manufacturers instructions.

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)

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Products Related to NB300-144

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NB300-144AF647	Laminin Antibody [Alexa Fluor® 647]

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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