

NB300-144 Protocol

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.

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 degree 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 degree antibody and wash slides in a rack by immersion in PBS with 3 changes over >=15 min.

9. Apply biotinylated 2 degree 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 degree 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 ABC complex 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 degree 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 degree 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 degree and 2 degrees

Antibodies
1 degree Ab: rabbit anti-rat laminin PAb (Novus Biologicals NB 300-144). Prepare at 1:1000 by adding 4.5 ul 1 degree Ab to 4.5 ml of 10% goat serum in PBS. 2 degree Ab: goat anti-rabbit IgG-HRP (Santa Cruz sc-2030). Prepare at 1:500 by adding 10 ul 2 degree 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 Biomedica (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