Detection of Laminin in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
Carezyme II (Pepsin)
Normal Rabbit IgG – Affinity Purified
DAB Chromogen
Hematoxylin

Blocking Solution: Rodent Block M (Ready-To-Use)
Biocare Medical
Concord, CA 94520
www.biocare.net
1-800-799-9499
Catalog # RBM961

Primary Antibody: Rabbit Polyclonal Antibody To Laminin (Ab-1)
Lab Vision / Thermo Fisher Scientific
Fremont, CA 94539
www.labvision.com
1-800-828-1628
Catalog # RB-082

Polymer Reagent: Rabbit-on-Rodent HRP-Polymer Detection
Biocare Medical
Concord, CA 94520
www.biocare.net
1-800-799-9499
Catalog # RMR622

Staining Procedure

Positive Control Tissue: Skin, lung, and kidney
Stain Localization: Cytoplasmic (Basement membrane)

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

4. Proteolytic-Induced Epitope Retrieval Using Pepsin
   Incubate the slides in Carezyme II: Pepsin (predilute) for 8 minutes at 37ºC.
   (Allow the pepsin to reach room temperature before use.)
   Rinse the slide in distilled water for 1 minute to stop the enzymatic reaction.

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

6. Block with the Rodent Block M reagent for 20 minutes at room temperature.
   Lot #_____ Exp. Date_____

   DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
   ONLY WIPE EXCESS BLOCK.

7. Apply primary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.
   Lot #_____ Exp. Date _____

   For negative control slides, dilute normal rabbit IgG so that it's IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:500 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 30 minutes at room temperature.
   Lot #_____ Exp. Date _____

8. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

9. Apply the Rabbit-on-Rodent HRP-Polymer reagent, and incubate for 30 minutes at room temperature.
   Lot #_____ Exp. Date _____

10. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

11. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.
    (Add 1 drop of DAB per ml of substrate)
    Lot #_____ Exp. Date_____ New Kit: yes / no

12. Rinse the slides in tap water 3 minutes.

13. Counterstain with hematoxylin for 20 seconds.

14. Rinse the slides in tap water until water is clear.

15. Gently agitate slides in 1X wash buffer until the tissues turn blue.

16. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

17. Coverslip

Updated 10/04/12