Identification of CC10 using LSAB Kit in Formalin-Fixed, Paraffin Embedded Rodent Tissue

Reagents:
1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #SP-2001

Primary antibody: Goat anti-CC10 (T-18) Polyclonal Antibody
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog #sc-9772
Concentration: 200ug/ml

Dako Protein Block Serum-Free
DAKO Corporation
Carpinteria CA 93013
www.dakousa.com
Code no. X0909

DAKO LSAB+ System HRP
DAKO Corporation
Carpinteria CA 93013
www.dakousa.com
Code No. K06901

Staining Procedure

-Positive Control Tissue: Mouse lung; bronchiolar airpways. Identifies clara cells
-Stain Localization: cytoplasm

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

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

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Unmasking Techniques using the decloaker.
Add 500 ml D/W to the pan of the decloaker.
Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.
Decloak for 5 minutes. Pressure_____
Depressurize for 10 minutes.
Remove pan top and cool for 10 min.Temp_____
Rinse in D/W, 2x for 3 min each

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes.

5. Use Dako Serum-free Protein Block for 10 mins
Lot#_____ Exp. Date_____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Apply Avidin/Biotin block
Lot#_____ Exp. Date_____ New Kit: yes / no
Apply avidin block - 15 min at RT.
Quick rinse in 1X AB.
Apply biotin block - 15 min at RT.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Goat anti-CC-10) at 1:100 dilution and incubate for 30 mins.
Lot#_____ Aliquoted yes / no Date Aliquoted_____

For negative control slides, normalize the protein concentration of normal goat serum to the protein concentration of the primary antibody (anti-CC10) and use this to make the 1:100 dilution. Apply normal goat serum to the slides and incubate for one hour.
Lot#_____ Reconstituted Date_____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply Link (yellow bottle) from LSAB+ Kit for 15 mins
Lot#_____ Exp_____

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply label (red bottle) from LSAB+ kit and incubate for 15 minutes.

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Exp.Date_____ New Kit: yes / no Lot#_____

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

17. Place slides in 1X Automation Buffer for 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip.
updated 10/25/04