Detection of p15 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagents:

1X Automation Buffer

3% Hydrogen Peroxide

Antibody Diluent

Citrate Buffer

DAB Chromagen

Hematoxylin

Antibody Information

Block: Normal Donkey Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #017-000-001

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

Primary antibody: Goat anti-p15
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog #sc-1429

Negative Serum Control: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #005-000-121

Secondary antibody: Biotinylated Donkey Anti-Goat

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #705-065-147

Label antibody: StriAviGen Super Sensitive Predilute Label Antibody

Biogenex Laboratories

San Ramon, CA 94583

www.biogenex.com

1-800-421-4149

Catalog #HK330-5K

Staining Procedure
-Positive Control Tissue: 7 day post-natal mouse lung
-Stain Localization: Nuclear

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. Block in 5% Normal Donkey Serum for 20 minutes.
Lot#_____ Reconstituted Date_____

6. Apply Avidin/Biotin block
Lot#_____ Exp Date_____New Kit yes / no
Apply avidin block - 15 min @ RT.
Quick rinse in 1X AB.
Apply biotin block - 15 min @ RT.
Wipe excess block

7. Apply primary antibody (p15) at a 1:100 dilution and incubate for one hour.
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of the normal goat serum to the protein concentration of the primary antibody (p15) and use this to make the 1:100 dilution and incubate for one hour.
Lot#_____ Reconstituted Date_____

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

9. Apply secondary antibody (biotinylated donkey anti-goat) at a 1:1000 and incubate for 30 minutes.
Lot#_____ Reconstituted Date_____

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

11. 9. Apply Label (Biogenex) antibody and incubate for 30 minutes.
Lot# _____ Exp. Date _____

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)
Lot#_____ Exp. Date_____ New kit yes / no

14. Rinse slides in tap water 3 minutes.

15. Place slides in 1% copper sulfate for 15 seconds.

16. Rinse slides in tap water for 3 minutes.

17. Counterstain with Modified Harris Hematoxylin for 30 seconds.

18. Rinse in tap water until water is clear.

19. Place slides in 1X Automation buffer for one minute with gentle agitation to blue slides.

20. Dehydrate through the following solutions.

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

21. Coverslip.
updated 6/7/04