Detection of FLAG in Formalin-Fixed, Paraffin Embedded RodentTissue

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: Mouse Anti-FLAG M2 peroxidase conjugate
Sigma Corporation
St. Loius, MO 63178
www.sigma-aldrich.com
1-800-325-3010
Catalog #A-8592

Negative control serum: Normal Mouse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #015-000-001

Staining Procedure

-Positive Control Tissue: Tissue with FLAG insert.
-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 1X Automation Buffer for 5 minutes.

5. 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.
Wipe excess block.

6. Apply primary antibody (Mouse anti-FLAG) at 1:10 dilution and incubate for one hour.
Lot #_____ Aliquoted yes / no Date Aliquoted_____

For the negative control slides, match the protein concentration of the normal mouse serum to the protein concentration of the primary antibody (FLAG) and use this to make the 1:10 dilution. Apply to the slides and incubate for one hour.
Lot#_____ Reconstituted Date_____

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

8. 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

9. Rinse in tap water 3 minutes.

10. Counterstain with Modified Harris Hematoxylin for 30 seconds.

11. Rinse in tap water until water is clear.

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

13. Dehydrate through the following solutions.

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

14. Coverslip

updated 2/13/04