Detection of Marck's Protein in Formalin-Fixed, Paraffin Embedded Rodent Tissue

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

Antibody Information:
Blocking Serum: Normal Horse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #008-000-001

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

Primary antibody : Marck's Protein
(2F12)
Provided by Dr. Perry Blackshear
NIEHS
Suggested dilution: 1:100

Negative control serum: Pre-immune Mouse Serum
(6F6)
Supplied by Dr. Perry Blackshear
National Institute of Environmental Health Sciences
Research Triangle Park, NC
Suggested dilution 1:100

Secondary antibody: Biotinylated anti-Mouse IgG (made in Horse)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Suggested dilution: 1:800
Catalog #BA-2001

Label antibody: Vector EliteVectastain® ABC

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6101

Staining Procedure

-Positive Control Tissue: Bovine Retina

-Stain Localization: Membrane

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. Perform Heat Induced Epitope Retrieval using Microwave Oven
   Place a full rack of slides in distilled water and place in the microwave oven.
   When using a tissue tek™ container, add 250 mls of distilled water.
   Microwave slides at 80% power for 2 minutes
   Rest for one minute.
   Repeat microwave/rest cycle at 80% power 2 times

   Following the third microwave cycle, transfer slides to fresh distilled water and allow slides to cool for 15 minutes. Temp._____

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

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

6. Apply Avidin/Biotin block
   New kit yes / no Lot#_____ Exp Date_____
   Apply avidin block - 15 min @ RT.
   Quick rinse in 1X AB.
   Apply biotin block - 15 min @ RT.
   No wash, wipe excess block and apply primary antibody

7. Apply primary antibody (Marck's protein) at 1:100 dilution and incubate for one hour.
   Lot#_____ Aliquoted yes / no Date Aliquoted_____

For the negative control slides, normalize the protein concentration of the Pre-immune mouse serum to the protein concentration of the primary antibody (Marck's protein) and use this to make the 1:100 dilution. Apply to the slides and incubate for one hour.

   Lot#_____

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

9. Apply secondary antibody (Biotinylated Horse anti-mouse) @ 1:800 dilution and incubate for 30 minutes.
   Lot#_____ Reconstituted Date_____

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

11. Apply Label antibody and incubate for 30 minutes. (Prepare 30 minutes prior to use)
   2 drops Reagent A + 5 ml diluent -> Mix and then add 2 drops Reagent B
   Exp. Date _____ New kit yes / no

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 30-45 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 1/22/2003