Detection of Myosin Heavy Chain in Formalin-Fixed, Paraffin-Embedded Rodent Tissue

Reagents:

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

Antibody Information:

Kit: Vector M.O.M. Kit
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-2200

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

Primary antibody: Mouse anti-Cardiac Myosin Heavy Chain
Abcam Inc.
Cambridge, MA 02139
www.abcam.com
1-888-772-2226
Catalog# ab15

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

Label antibody: Vector Elite Label
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6100

Staining Procedure

-Positive Control Tissue: heart
-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	3 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 each.

5. Incubate sections for 1 HOUR in M.O.M. specific IgG blocking reagent
Made via 2.5 mls 1x PBS plus 2 drops of Mouse IgG blocking reagent
Kit Lot#_____ Exp Date_____New Kit: yes / no

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.
Wipe excess block

DO NOT RINSE SECTIONS WITH BUFFER.

Prepare Vector M.O.M .diluent: 600ul of protein concentrate stock in 7.5 mls of 1X PBS.
Make primary secondary, and label antibody dilution in Vector M.O.M. diluent.

7. Apply myosin primary antibody at 1:40 and incubate for one hour:
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody and use this to make the 1:40 dilution. Apply to 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 M.O.M. biotinylated anti-mouse IgG and incubate for 10 minutes
Made via 10ul of antibody in 2.5mls of Vector M.O.M. diluent.

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

11. Apply Vector Elite label for 5 minutes. (Prepare 30 minutes before use)
Made via 2 drops of Reagent A plus 2 drops of Reagent B in 2.5mls M.O.M. diluent
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)
Lot #_____ Exp Date_____ New Kit: yes / no

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.Rinse slides in 1x automation buffer for 1 min.

18. Dehydrate through the following solutions.

95% EtOH	1 times	3 minutes
100% EtOH	3 times	3 minutes
Xylene	2 times	5 minutes

19. Coverslip
updated 10/25/04