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

Reagents:

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

Antibody Information

Kit: M.O.M. Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog: PK-2200
Note: The Vector M.O.M. Kit contains solutions needed to make the block, secondary and label antibodies.

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

Primary antibody: Monoclonal anti-Insulin
Sigma-Aldrich
St. Louis, MO
www.sigmaaldrich.com
1-800-325-3010
Catalog # I2018

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: Pancreas (Islets of Langerhans)
Stain Localization: Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	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. Incubate sections for 1 HOUR in M.O.M. specific IgG blocking reagent (made with 2 drops of Mouse IgG blocking reagent in 2.5ml 1X PBS).
   Kit Lot#_____ Exp Date_____New Kit: yes / no

4. Apply Avidin/Biotin block
   Lot#_____ Exp Date_____ New Kit: yes / no
   Apply avidin block - 15 minutes at room temperature.
   Quick rinse in 1X AB.
   Apply biotin block - 15 minutes at room temperature.
   Wipe excess block

   DO NOT RINSE SECTIONS WITH BUFFER.

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

5. Apply primary antibody (Insulin) at a 1:8000 dilution and incubate for 15 minutes at room temperature.
   Lot#_____ Exp Date_____

   For negative control slides, normalize the protein concentration of normal mouse serum to match the protein concentration of the primary antibody (Insulin), and use this to make a 1:8000 dilution. Apply to slides and incubate for 15 minutes at room temperature.
   Lot#_____ Reconstituted Date_____

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

7. Apply M.O.M. biotinylated anti-mouse IgG and incubate for 10 minutes at room temperature (made with 10ul of antibody in 2.5 ml of M.O.M. diluent).

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

9. Apply M.O.M label for 5 minutes at room temperature. (Prepare at least 30 minutes before use - made with 2 drops of Reagent A plus 2 drops of Reagent B in 2.5 ml M.O.M. diluent).

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

11. 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

12. Rinse in tap water 3 minutes.

13. Counterstain with Modified Harris Hematoxylin for 20 seconds.

14. Rinse in tap water until water is clear.

15. Gently agitate slides in 1X Automation buffer until they turn blue.

16. Dehydrate through the following solutions.

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

17. Coverslip

Updated 10/17/06