Detection of Insulin in Frozen Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
DAB Chromagen
Hematoxylin

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

Note: This kit contains all reagents necessary to make the blocking reagent, secondary antibody and label complex.

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 Antibody
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

1. Cut each frozen section at 6µm and mount on a positively charged slide.
   Immediately fix the section in Rapid Fix Solution for 7 seconds.
   Rinse the slide thoroughly in tap water to remove excess fixative and then place in 1X Wash Buffer.
   Once all the slides have undergone this process, proceed to step 2.

2. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

3. Quench endogenous peroxidase by placing the slides in 0.3% hydrogen peroxide for 30 minutes.

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

   M.O.M Peroxidase Kit
   Exp. Date_____ New Kit: yes / no

5. Apply the blocking reagent from the M.O.M. Kit, and incubate for 1 hour at room temperature.
   (Add 2 drops of the Mouse IgG Blocking Reagent to 2.5 ml of 1X PBS.)

   DO NOT RINSE THE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Avidin / Biotin Blocking Kit
   Lot #_____ Exp. Date_____ New Kit: yes / no
   Apply avidin block for 15 minutes at room temperature.
   Quick rinse in 1X Wash Buffer.
   Apply biotin block for 15 minutes at room temperature.

   DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
   ONLY WIPE EXCESS BUFFER.

   M.O.M. Diluent: Add 600ul of the Protein Concentrate stock solution to 7.5 ml of 1X PBS. Use this as the diluent for the primary, negative, and secondary antibodies.

7. Apply the primary antibody at a 1:16,000 dilution, and incubate for 15 minutes at room temperature.
   Lot #_____ Exp. Date_____

   For negative control slides, dilute the protein concentration of the normal mouse serum to match that of the primary antibody. Make a 1:16,000 dilution from this normalized serum, and apply to the slides.
   Incubate for 15 minutes at room temperature.
   Lot #_____ Date Reconstituted_____

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

9. Apply the secondary antibody from the M.O.M. Kit, and incubate for 10 minutes at room temperature.
   (Add 10ul of the Biotinylated anti-Mouse IgG Reagent to 2.5 ml of the M.O.M. Dilutent).

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

11. Apply the label complex from the M.O.M. Kit, and incubate for 5 minutes at room temperature. (Add 2 drops of Reagent A to 2.5 ml of 1X PBS. Mix. Then add 2 drops of Reagent B and mix. Prepare at least 30 minutes prior to use.)

12. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

13. Apply the DAB chromagen, and incubate in the dark for 6 minutes at room temperature. (Add 1 drop of DAB per ml of substrate)
Lot #_____ Exp. Date_____ New Kit: yes / no

14. Rinse the slides in tap water 3 minutes.

15. Counterstain with Harris Hematoxylin for 20 seconds.

16. Rinse the slides in tap water until water is clear.

17. Gently agitate slides in 1X Wash Buffer until they turn blue.

18. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

19. Coverslip

Updated 08/23/07