Detection of Pancreatic Polypeptide in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagents

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

Antibody Information

Block: Dako Protein Block Serum-Free
Dako Corporation
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Catalog # X0909

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

Primary antibody: Rabbit anti-Human Pancreatic Polypeptide
Dako Corporation
Carpintera, CA
www.dakousa.com
1-800-235-5763
Catalog # A0619

Negative control serum: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 011-000-001

Dako LSAB+ System HRP
Dako Corporation
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Catalog# K06901
Note: This kit includes reagents needed for link and label antibodies.

Staining Procedure

Positive Control Tissue: Pancreas (periphery of the islets of Langerhans – alpha cells)
Stain Localization: Cytoplasmic
Staining Procedure

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 slides in Dako Serum-Free Protein Block for 10 minutes at room temperature.
   Lot#_____ Exp. Date_____

   DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN.

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

   DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

5. Apply primary antibody (PP) at a 1:500 dilution and incubate for one hour at room temperature.
   Lot#_____ Exp Date _____

For negative control slides, normalize the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (PP) and use this to make a 1:500 dilution and incubate for one hour at room temperature.
Lot#_____ Reconstituted Date _____

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

   LSAB+ Kit Lot#_____ Exp. Date_____

7. Apply Link – Secondary (yellow bottle) from LSAB+ Kit and incubate for 30 minutes at room temperature.

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

9. Apply Label (red bottle) from LSAB+ Kit and incubate for 30 minutes at room temperature.

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 30 seconds.

14. Rinse in tap water until water is clear.

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

16. Dehydrate through the following solutions.

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

17. Coverslip

Updated 10/12/06