

Detection for Peanut Agglutinin (PNA) in Frozen Mouse Tissue

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Antibody Information

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Primary antibody: Peanut Agglutinin

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog# B-1075

Label antibody: StriAviGen Super Sensitive Predilute Label Antibody

Biogenex Laboratories

San Ramon, CA 94583

www.biogenex.com

1-800-421-4149

Catalog# HK330-5K

Staining Procedure

-Positive Control Tissue: Mouse spleen and kidney

-Stain localization: Cytoplasmic

For Frozen Tissue Sections

Two sequential 6 micron sections were cut per slide (Probe-On Plus by Diagger). Sections are cut and immediately fixed in Rapid Fix (Shandon-Lipshaw) for 7 seconds. Place section in 1X AB. After the last section is cut, wash in 1X AB for 5 minutes. Repeat buffer wash.

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

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

3. Apply Avidin/Biotin block

Lot# _____ Exp Date _____ New Kit yes / no

Apply avidin block - 15 min @ RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min @ RT.

Wipe excess block.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

4. Apply PNA at a 1:1000 dilution made in 1mM CaCl₂, MgCl₂, MnCl₂ and incubate for one hour at room temperature.

Lot# _____ Reconstitution Date _____

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

6. Apply Biogenex Streptavidin Label and incubate for 30 minutes at room temperature.

Lot# _____ Exp Date _____

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

8. 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

9. Rinse in tap water for 3 minutes.

10. Counterstain with Modified Harris Hematoxylin for 30 seconds.

11. Rinse in tap water until water is clear.

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

13. Dehydrate through the following solutions.

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

14. Coverslip