

Detection of Peanut Agglutinin (PNA) in Frozen Mouse Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[DAB Chromagen](#)

[Hematoxylin](#)

[Rapid Fixx](#)

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 Complex: Peroxidase-Conjugated Streptavidin SS Label

Biogenex Laboratories

San Ramon, CA 94583

www.biogenex.com

1-800-421-4149

Catalog # HK330-9K

Staining Procedure

Positive Control Tissue: Spleen and kidney
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 the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
5. 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 REAGENT.
ONLY WIPE EXCESS BUFFER.
6. Apply the PNA at a 1:1000 dilution, and incubate for 1 hour at room temperature.
(The diluent for this reagent is the 1mM CaCl₂, MgCl₂, MnCl₂.)
Lot # _____ Date Reconstituted _____
7. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each
8. Apply the Streptavidin SS Label, and incubate for 30 minutes at room temperature.
Lot # _____ Exp Date _____
9. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
10. 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
11. Rinse the slides in tap water 3 minutes.
12. Counterstain with Harris Hematoxylin for 20 seconds.
13. Rinse the slides in tap water until water is clear.
14. Gently agitate slides in 1X Wash Buffer until they turn blue.

15. Dehydrate through the following solutions:

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

16. Coverslip

Updated 10/05/05