

# Detection of Peanut Agglutinin (PNA) in Frozen Mouse Tissue

## **Reagent and Antibody Information**

[Rapid Fixx](#)

[1X Wash Buffer](#)

[0.3% Hydrogen Peroxide](#)

[1mM CaCl<sub>2</sub>-MgCl<sub>2</sub>-MnCl<sub>2</sub> Diluent](#)

[DAB Chromogen](#)

[Hematoxylin](#)

### Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # SP-2001

### Primary Antibody: Biotinylated Peanut Agglutinin

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # B-1075

### Label Complex: R.T.U. Vectastain Elite ABC Reagent

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # PK-7100

## **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.  
(*Cut an additional slide for an H&E stain.*)  
Immediately fix the section in Rapid Fixx solution for 7 seconds.  
Rinse the slide thoroughly in tap water to remove excess fixative, and then place it 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 BLOCK.
6. Apply the PNA at a 1:1000 dilution. Incubate for 1 hour at room temperature.  
(The diluent for this reagent is the 1mM CaCl<sub>2</sub>-MgCl<sub>2</sub>-MnCl<sub>2</sub>.)  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_
7. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each
8. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.  
Exp. Date \_\_\_\_\_ New Kit: yes / no
9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
10. Apply the DAB chromogen. 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 hematoxylin for 20 seconds.
13. Rinse the slides in tap water until water is clear.
14. Gently agitate slides in 1X wash buffer until the tissues turn blue.

15. Dehydrate through the following solutions:

|              |         |           |
|--------------|---------|-----------|
| 95% Ethanol  | 1 time  | 3 minutes |
| 100% Ethanol | 3 times | 3 minutes |
| Xylene       | 2 times | 5 minutes |

16. Coverslip

*Updated 10/05/05*