

# Detection of Pax-5 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

## Reagents

[1X Automation Buffer](#)  
[3% Hydrogen Peroxide](#)  
[Antibody Diluent](#)  
[Citrate Buffer](#)  
[DAB Chromagen](#)  
[Hematoxylin](#)

## Antibody Information

Blocking Serum: Normal Donkey Serum  
Jackson ImmunoResearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog # 017-000-011

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: Goat Polyclonal Pax-5 Antibody (C-20)  
Santa Cruz Biotechnology  
Santa Cruz, CA 95060  
[www.scbt.com](http://www.scbt.com)  
1-800-457-3801  
Catalog # sc-1974

Negative Serum Control: Normal Goat Serum  
Jackson ImmunoResearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog # 005-000-121

Secondary Antibody: Biotin-SP-conjugated Donkey anti-Goat  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog # 705-065-147

Label Antibody: Peroxidase-conjugated Streptavidin SS Label  
Biogenex  
San Ramon, CA 94583  
[www.biogenex.com](http://www.biogenex.com)  
1-800-421-4149  
Catalog # HK330-9K

### **Staining Procedure**

Positive Control Tissue: Spleen (B-cells)  
Stain Localization: Nuclear

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. Unmasking technique using the decloaker.  
Add 500 ml distilled water to the pan of the decloaker.  
Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.  
Decloak for 5 minutes. Pressure \_\_\_\_\_  
Depressurize for 10 minutes.  
Remove pan top and cool for 10 minutes. Temperature before cooling \_\_\_\_\_  
Rinse in distilled water twice for 3 minutes each.
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
5. Block with 10% Normal Donkey Serum for 20 minutes at room temperature.  
Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

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

6. Apply the Avidin Biotin Blocking Kit

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 temperature.

No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Pax-5) at a 1:1000 dilution and incubate for one hour at room temperature.

Lot# \_\_\_\_\_ Exp Date \_\_\_\_\_

For negative control slides, normalize the protein concentration of the normal goat serum to match the protein concentration of the primary antibody (Pax-5) and use this to make a 1:1000 dilution. Apply to slides and incubate for one hour at room temperature.

Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

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

9. Apply the donkey anti-goat secondary antibody at a 1:500 dilution and incubate for 30 minutes at room temperature.

Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

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

11. Apply Biogenex SS Label and incubate for 30 minutes at room temperature.

Lot# \_\_\_\_\_ Exp Date \_\_\_\_\_

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

13. 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

14. Rinse in tap water for 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation buffer until they turn blue.

18. Dehydrate through the following solutions.

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

19. Coverslip

*Updated 01/05/07*